SEASONAL CHANGES IN THE PHYSIOLOGICAL AND HORMONAL ASPECTS OF REPRODUCTION IN THE MALE LONG-FINGERED BAT *MINIOPTERUS SCHREIBERSII* (MAMMALIA : CHIROPTERA)

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CONTENTS

ABSTRACT

The reproductive cycle of Miniopterus schreibersii is modified as a consequence of a period of torpor/hibernation over the winter months. This bat is one of three genera which employ the reproductive strategy of delayed implantation in which the blastocyst remains free in the uterine lumen during the winter months. Spermatogenesis is initiated in February, with spermiogenesis occuring in March. Copulation, ovulation and fertilization takes place between March and May after which the testes regress and remain in an inactive condition until the following summer. Activity in the accessory gland complex is initiated in March and continues until early June. Plasma testosterone concentrations reach a peak in May, decline over the winter months and reach a second, unexplainable peak in October. Leydig cells are secretorily active in February/early March after which they undergo vacuolation and final degeneration. The question of the life cyle of Leydig cells is addressed and an increase in a certain cell after Leydig cell activity suggests that this cell type may be the precursor of Leydig cells.

ACKNOWLEDGEMENTS

My thanks are due to my supervisor, Dr. Ric Bernard (Rhodes University, Grahamstown), for his helpful advise and supervision throughout the study and to the electron microscopy unit for assistance in the electron microscopy and photography work. I am also grateful to Professor B. Millar (Medical School, Cape Town) for assaying the plasma testosterone samples. This study was funded by the CSIR and Rhodes University.

LIST OF TABLES

LIST OF FIGURES

1. GENERAL INTRODUCTION

Because of their world-wide distribution, adaptive diversity and unique physiological qualities, bats have been of interest to biologists for many years. Reproductive studies on bats, however, appear to be largely concerned with gross morphological reports on reproductive organs, while research on the physiology and endocrine events involved with reproduction are limited.

Bats are tropical in origin and primary distribution and annual periods of hibernation are observed in only the species which have become adapted to living in cooler temperate latitudes (Wimsatt, 1960). This move into temperate latitudes is significant in terms of its effect on the life-style and reproductive cycle of bats. Insectivorous bats, being small in size with large wings, have a large surface area to volume ratio. This fact, together with the seasonal insect abundance in temperate latitudes forced the bats to include a period of winter hibernation in their life-cycle. In other hibernating mammals, the periods of reproduction and hibernation do not overlap, whereas in hibernating bats, the period of hibernation falls astride the reproductive season and consequently profoundly influences the reproductive physiology of both sexes. Thus the reproductive cycles of hibernating members of the Vespertilionidae and Rhinolophidae have become modified to include the reproductive peculiarities for which bats are noted (Wimsatt, 1960; Racey, 1982). The majority of workers agree that in species from temperate latitudes, the period of hibernation or decreased activity necessitates the use of a reproductive delay such that the young are born when food is maximal (Mutere, 1967). These delay patterns are not typically observed in tropical species where the fluctuations in insect biomass are not so marked. The types of reproductive cycles found in the Vespertilionidae and Rhinolophidae are numerous and shall be discussed in greater detail in the introduction to the chapter on the seasonality of reproduction.

The aims of this study are threefold :

1. To determine the reproductive cycle of male Miniopterus schreibersii in the Eastern Cape and thus add to the reproductive data on this species.

2. To describe the cyclical changes in Leydig cell morphology in male Miniopterus schreibersii during the annual testicular cycle and to relate these changes to activity in the seminiferous tubules, accessory gland complex and to seasonal changes in plasma testosterone concentrations.

3. To determine a Leydig cell life cycle, since the seasonality of this bat clearly demands either a continuous renewal of these cells, or a cycle of activation and deactivation.

2. MATERIALS AND METHODS

2.1 SAMPLING

Specimens of male M. schreibersii were collected on a monthly basis for two years during 1986 and 1987. Specimens were obtained from deserted mine shafts of the Maitland Mines (33^{o} 58' S, 25^{o} 17' E) in the Eastern Cape and occasionally from the Storm water drains in Grahamstown. Specimens were killed by asphyxiation with carbon dioxide and the testes and accessory gland complex immediately removed and weighed on a balance to the nearest 0.0001 g.

2.2 LIGHT MICROSCOPY

Tissues were fixed in buffered neutral formalin and embedded in Paraplast. Sections of five um thick were cut and stained with Ehrlichs haemotoxylin and eosin, Periodic Acid Schiffs reagent (PAS) or Alcian blue.

The diameter of the seminiferous tubules of the testes were calculated by taking the mean of two measurements at right angles to each other using an ocular micrometer. Ten tubules from each of ten specimens were measured for each month.

2.3 ELECTRON MICROSCOPY

Small pieces of testes were fixed by immersion in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for approximately one day. The tissue was then washed with buffer, secondarily fixed in 1% osmium tetroxide for 90 minutes and washed with buffer once again. The tissue was then dehydrated through an alcohol sequence and embedded in Taab 812. Ultrathin sections were cut using a LKB ultramicrotome, stained with uranyl acetate for 30 minutes and lead citrate 1963) for five minutes and examined using a Jeol JEM 100CX11 transmission microscope.

The diameter of the smooth endoplasmic reticulum tubules (SER) and the size of Leydig cells were determined by calculating the mean of two measurements taken at right angles to each other. The number of SER tubules and Leydig cells measured varied from month to month depending on the number of micrographs (2-10) available for each month. This sample size is too small to allow for tests of statistical significance.

2.4 BLOOD SAMPLING

Blood was collected using a one millilitre syringe from the heart. The samples were then centrifuged at 1600g for three minutes and the plasma (typically between 0,15 ml and 0.2 ml) was pipetted out and stored at -20°C until assayed.

2.5 TESTOSTERONE ASSAY

Testosterone determinations were performed on 2.0 ml ether extracts of 0.2 ml plasma using TRK 402 tritiated testosterone (Radiochemical Centre, Amersham, U.K.) and a highly specific antiserum raised against testosterone-3 carboxymethyl-oximebovine serum albumin (Millar & Kewley, 1976). Cross reaction with all major naturally occuring steroids was 0.1% except for dihydrotestosterone for which it was 5.1%. The intra- and interassay coefficients of variation were 3.1 and 8.4% respectively. Sensitivity was 5 nmol/l.

2.6 GRAPHICAL DISPLAY OF DATA

Data are displayed both graphically and in a tabulated form such that the reader may refer to actual measurements. Throughout this report, the points plotted on the graphs are the mean values, vertical lines are one standard deviation and the numbers next to the points are the sample size.

2.7 STATISTICAL ANALYSES

Where appropriate, student's T-tests have been used to test for significant differences between means of different values.

3. SEASONALITY OF REPRODUCTION

3.1 INTRODUCTION

Most temperate-dwelling bats of the Suborder Microchiroptera either hibernate, undergo a period of torpor or show a pattern of decreased activity over the winter months. As a result of this characteristic, the reproductive cycle of these bats has become highly seasonal and further modified in different ways (Oxberry, 1979). Three reproductive strategies have been reported in the Microchiroptera. In the first strategy, delayed implantation, copulation, ovulation and fertilisation take place before the onset of winter and implantation is delayed for a period corresponding to the period of decreased activity/hibernation. In the second strategy, delayed embryonic development, development up to implantation is normal, while development of the embryo is retarded during winter. Delayed ovulation and sperm storage is the third strategy, where ovulation is delayed and sperm are stored in the female reproductive tract over winter. The effects of hibernation on the reproductive cycle, as mentioned above, have been reviewed by Wimsatt (1960, 1969), Carter (1970), Oxberry (1979) and Racey (1982).

Members of the genus Miniopterus (family Vespertilionidae : Subfamily Miniopterinae) occur widely, inhabiting the Old World, and employ the first strategy. Delaye d implantation, however is not restricted to this group of bats, but is also employed by the funnel-eared bat (Mitchell, 1965), by an equatorial fruit bat

(Mutere, 1967) and the Indian Rhinolophid bat (Ramakrishna & Rao 1977). This reproductive pattern differs from most temperate-dwelling Rhinolophidae and Vespertilionidae (excluding the Miniopterinae) which employ sperm storage (Oxberry, 1979).

Aspects of the reproduction of *Miniopterus* have been described for species from Australia, Japan, South Africa, Borneo, New Herbrides and France (Courrier, 1927 in Dwyer, 1963a,b; Baker & Bird, 1956; Dwyer, 1968; Medaway, 1971; Richardson, 1977; Bernard, 1980; Mori & Uchida, 1980; van der Merwe, 1980; McWilliam, 1988). At all latitudes for which data are available, spermatogenesis is highly seasonal and restricted to a few months prior to copulation. These authors are in agreement that the period of delayed implantation increases with increasing latitude (a period of up to three weeks at 4° S, three months at 28° S, Australia; four months at 30° S, South Africa; and five months at 45° N, France).

Changes in plasma testosterone have been recorded for a few bat species, where, as in most mammals, the peak plasma testosterone concentration has been found to occur during or shortly after spermiogenesis (Gustafson & Shemesh, 1976; Bernard, 1986; Krutzsch & Chrichton, 1987).

The role that Leydig cells play in the production and secretion of testosterone is now understood (Hall, 1988). The majority of workers agree that changes in either the configuration or volume of the SER as well as changes in the number of lipid droplets and mitochondria are an indication of the state of steroidogenic activity of the Leydig cells. For example, in the camel, the SER during the mating season is present in a highly condensed form, while this form is absent during the non-mating season (Friedlander et al., 1984). The study of changes in the ultrastructure of these cells is aided by the seasonality of reproduction in *Miniopterus schreibersii*.

3.2 RESULTS

3.2.i BAT MASS

Changes in the mean mass of the bats were recorded throughout the year (Fig. 1; Table 1). Bat mass is lowest in April and May, the first months of winter, and thereafter steadily increases to reach a mean peak in December of 12.27g. There is a significant difference between bat mass recorded at the height of male reproductive activity (April and May), compared to that recorded prior to the onset of spermatogenesis (November, December and January). ($p < 0.01$).

3.2.ii PATTERN OF WINTER ACTIVITY

During the colder winter months, April / May to September, *M. schreibersii* appeared to be less active, as indicated by an increase in the time taken to awaken once the bats were disturbed in the cave. The extent of this decreased activity varied, dependant upon prevalent ambient and cave temperatures. During particularly cold spells, for example during the month of June when the average cave temperature was 15.4^{o} C, the bats were apparently in deep torpor. Arousal of bats was more rapid when ambient and cave temperatures were raised as they were in March for example when the average cave temperature was recorded to be 19.5^{o} C.

Table 1 : Mean monthly mass of adult male <u>M. schreibersii</u>.

Collection	Mean mass (g) +/- SD (range)	n
March '87	10.3 +/- 1.89 (9.0 - 12.5)	3
April '87	8.0 +/- 0.71 (7.5 - 8.5)	2
May '87	7.95 +/- 0.35 (7.7 - 8.2)	2
June '87	10.25 +/- 0.35 (10.0 - 10.5)	2
July '87	9.33 +/- 1.04 (8.5 - 10.5)	3
August '87	10.34 +/- 0.96 (9.0 - 11.5)	5
September '87	10.5 +/- 0.5 (10.0 - 11.0)	3
October '87	10.79 +/- 1.19 (8.5 - 12.0)	7
November '87	11.17 +/- 2.52 (8.5 - 13.5)	3
December '87	12.27 +/- 0.4 (11.8 - 12.5)	3
January '88	11.66 +/- 0.58 (11.0 - 12.0)	3
February '88	11.0 +/- 1.58 (9.5 - 13.0)	4

3.2.iii SPERMATOGENESIS, MALE REPRODUCTIVE TRACT AND SEASONAL CHANGES IN THE ACCESSORY GLAND COMPLEX

Spermatogenesis does not occur in juvenile bats during their first autumn so that sexual maturity in the male is not achieved until the second year. Evidence for this comes from the structure of seminiferous tubules of juvenile bats during the periods of spermatogenic activity and winter inactivity in adults. There is no spermatogenic activity during March and April (when spermatogenesis is occuring in adults) and during winter inactivity, there are no lipid droplets in the Sertoli cells. Lipid droplets in Sertoli cells are a result of phagocytosis of residual bodies formed during spermiation (Pudney, 1986), and their absence indicates that the specimen had not been spermatogenically active during the preceding autumn. Furthermore, extremely low levels of plasma testosterone are measured in these immature bats during spermiogenesis, when elevated testosterone levels are measured in adult bats. In April of the first year, a testosterone concentration of 0.26 ng/ml was measured for an immature bat, while two concentrations of 4.76 ng/ml and 13.12 ng/ml were recorded for adult bats (Table 6).

During the colder winter months of April/May to September, when the bats are less active, the testes are spermatogenically inactive. The lumina of the seminiferous tubules are empty (Fig. 2) and no advanced spermatogenic stages can be seen. It is during this period that the lowest range of testis masses were recorded, 0.003 - 0.0064 g (Table 2; Fig. 3), as well as the smallest

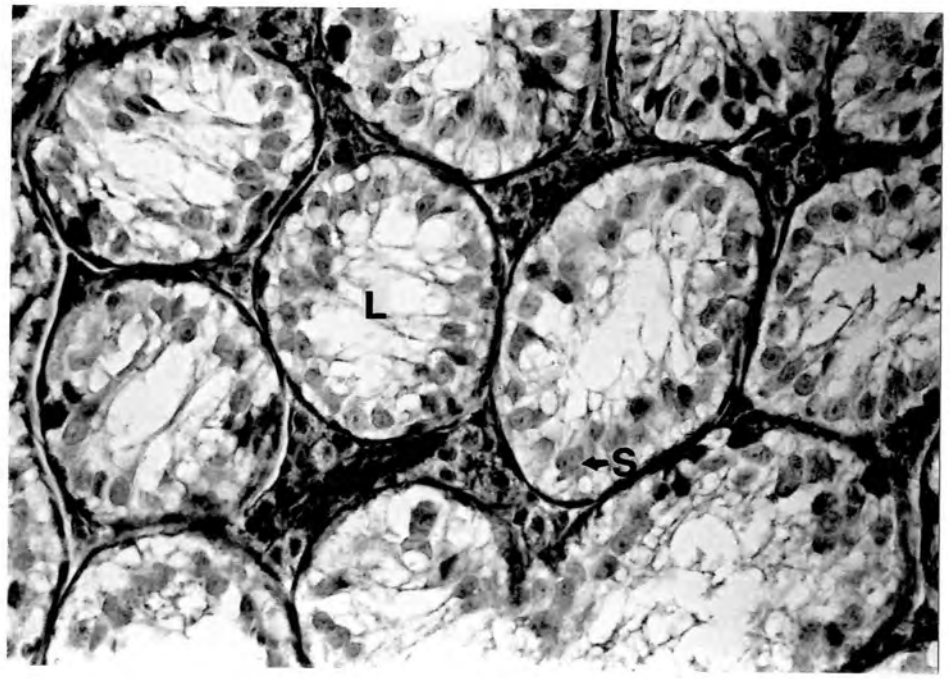


Figure 2 : Section through a spermatogenically inactive testis of _M. schreibersii_ as it appears during the months, April to December. The seminiferous tubules are reduced in diameter and the lumen (L) is filled with non-cellular material. The seminiferous epithelium contains Sertoli cells and occasional spermatogonia (S). X 458

Table 2 : Mean monthly testis weights of adult male *M. schreibersii*.

Collection	Mean testis weight +/- SD (g)	n
25 March '87	0.041 +/- 0.001	2
22 April '87	0.027 +/- 0.002	2
20 May '87	0.009 +/- 0.001	2
22 July '87	0.006 +/- 0.0002	2
24 August '87	0.004 +/- 0.001	6
23 September '87	0.003 +/- 0.0001	2
26 October '87	0.006 +/- 0.001	3
17 November '87	0.007 +/- 0.001	3
10 December '87	0.009 +/- 0.004	2
19 January '88	0.014 +/- 0.012	2
16 February '88	0.038 +/- 0.006	3

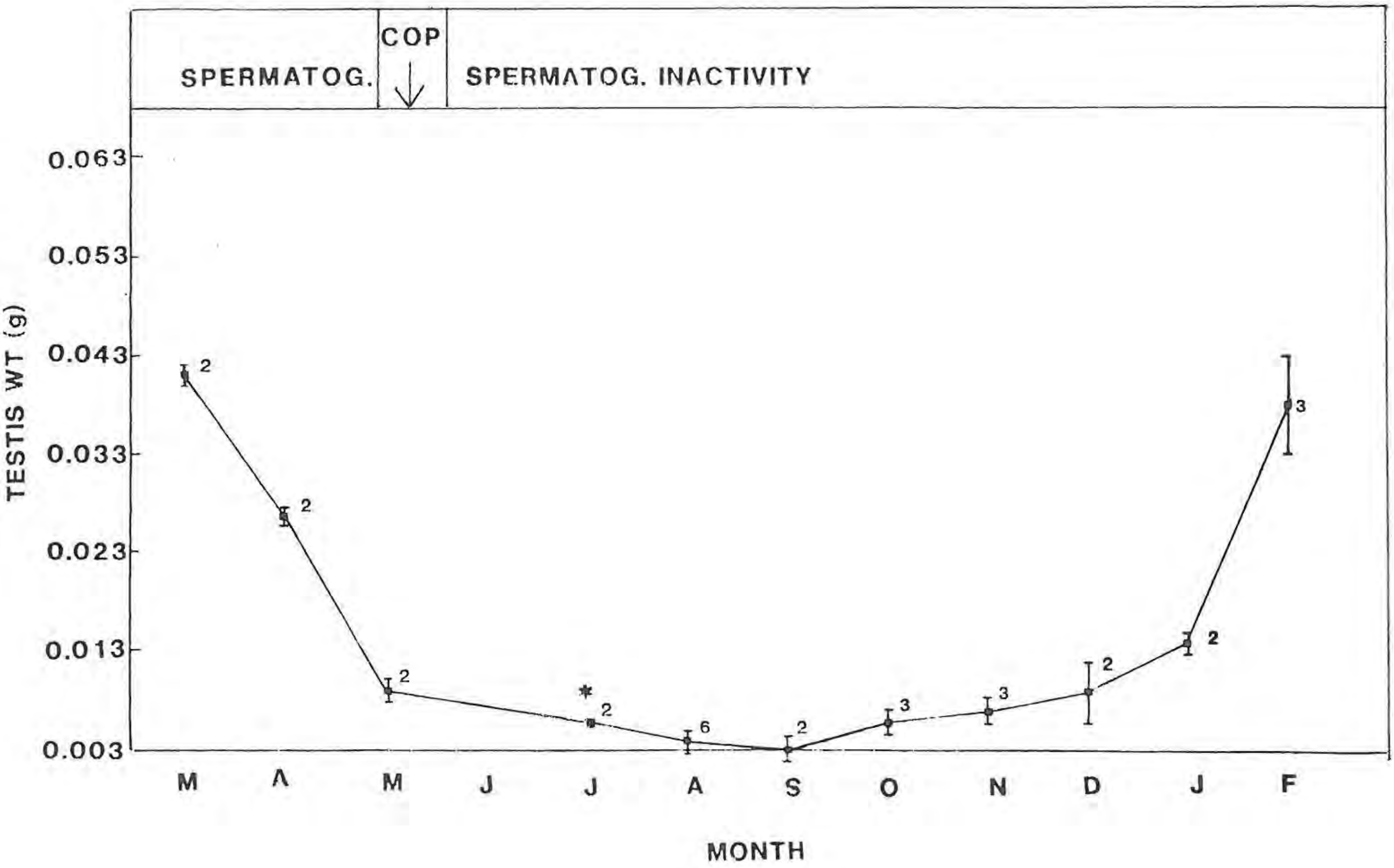


Figure 3 : Mean monthly testis weights of adult male M. schreibersii showing the order of magnitude increases in mass from spermatogenically inactive to active conditions.

seminiferous tubule diameter, 0.065 ± 0.010 mm (Table 3; Fig. 4). The testes remain in such an inactive form until the onset of spermatogenesis, the exact timing of which varies. Generally, initiation of this event is in February, when the germinal epithelium comprises two cell layers, indicating that the spermatogonia have begun to divide (Fig. 5).

Spermiogenesis takes place in March at which time the mean mass of the testes (0.041 g), is greatest (Table 2; Fig. 3). Spermiogenesis is confirmed by the presence of spermatids with condensing nuclei (Fig. 6). Mature sperm are present in the seminiferous tubules and cauda epididymis in late April and early May (Figs. 7,8) and copulation occurs in May. After copulation, the seminiferous epithelium reverts to the inactive form and is characterised by the presence of large lipid droplets in the Sertoli cell cytoplasm. The testes are then again at minimum dimensions (Table 2; Fig. 3). There is a statistically significant difference between the mean testis mass during spermatogenesis (February to April) compared to the mean mass of spermatogenically inactive testis (July to September). ($p < 0.01$). There is also a significant difference between the diameter of the seminiferous tubules of February to April when compared to that of July to September ($p<0.01$).

The gross organisation of the reproductive tract of male _M. schreibersii_ is similar to that described by Matthews (1941) of _M. dasythrix_. The accessory gland complex of _M. schreibersii_ (Fig. 9) is situated at the base of the bladder and comprises

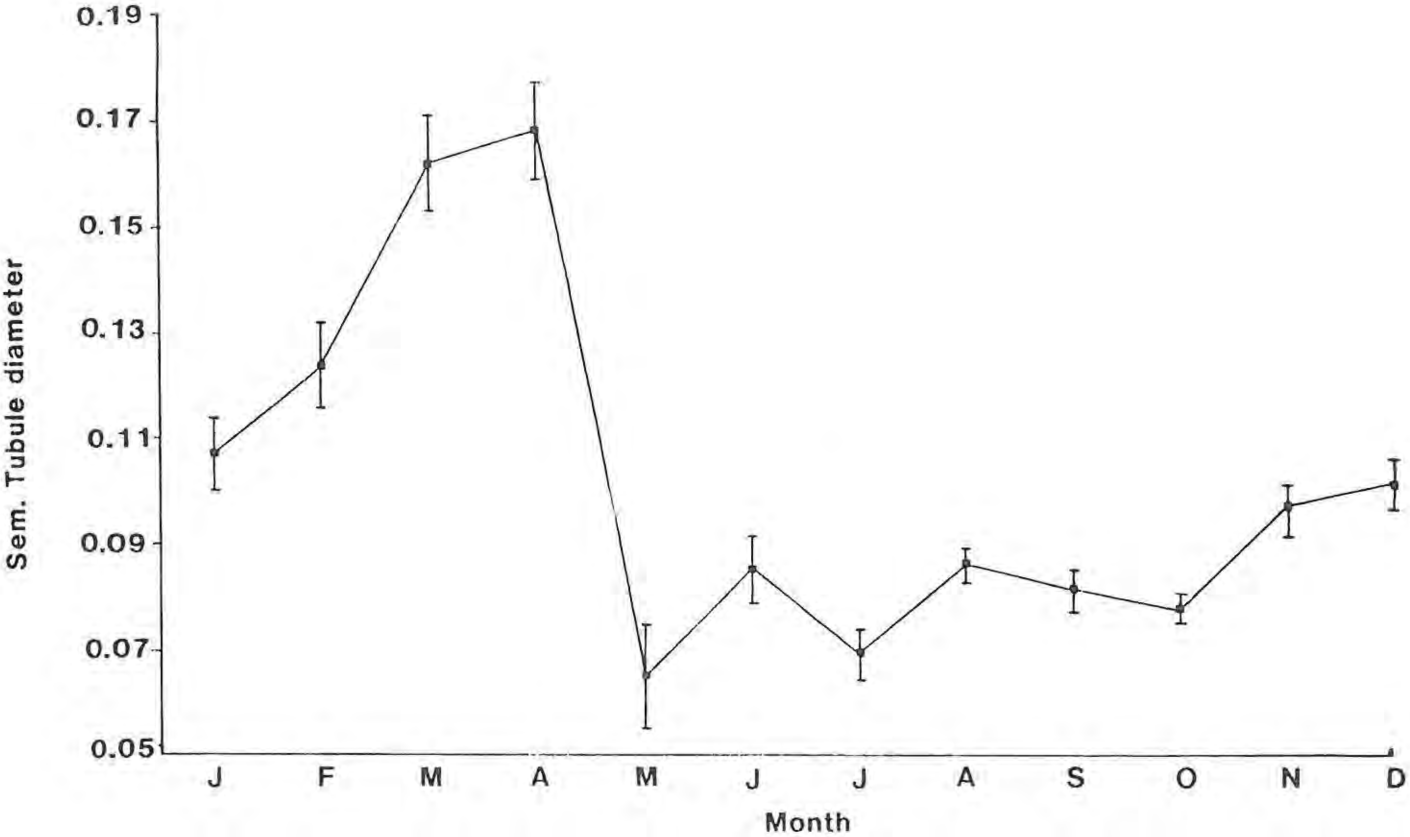
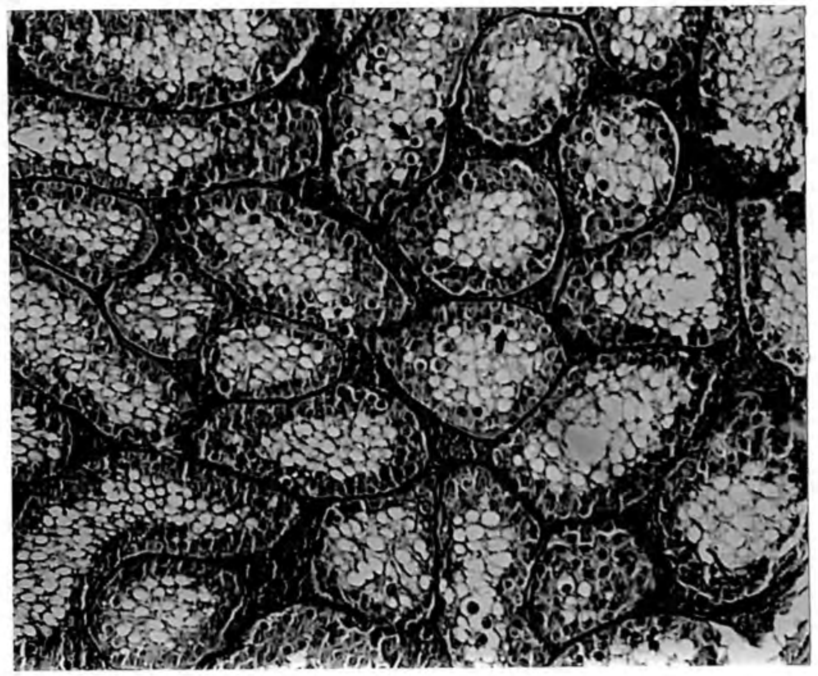


Figure 4 : Monthly changes in the mean diameter of the seminiferous tubules of M. schreibersii. Note the abrupt decrease in diameter following copulation.(mm)



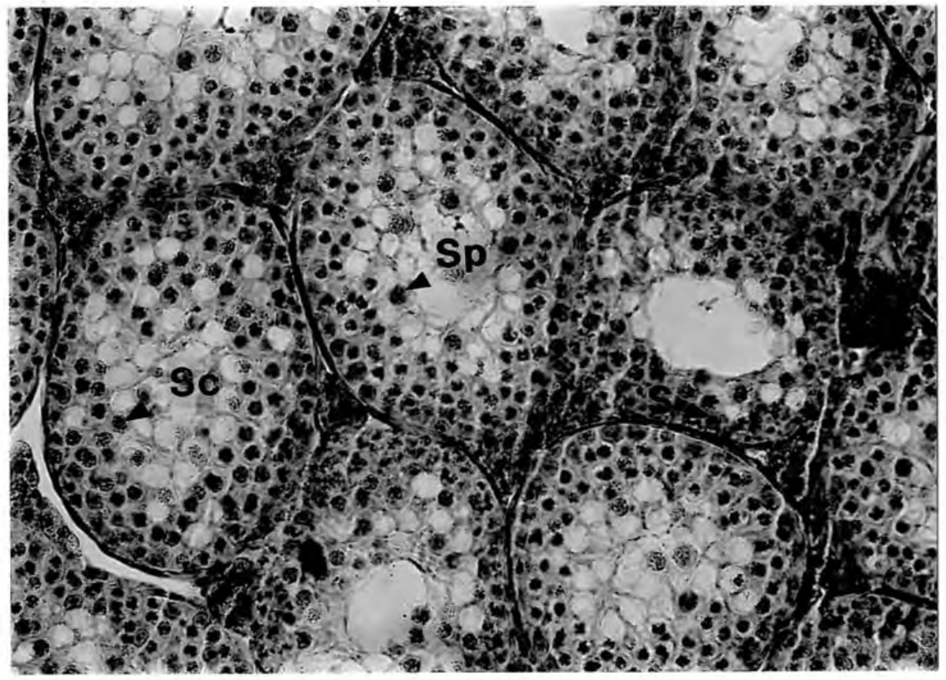
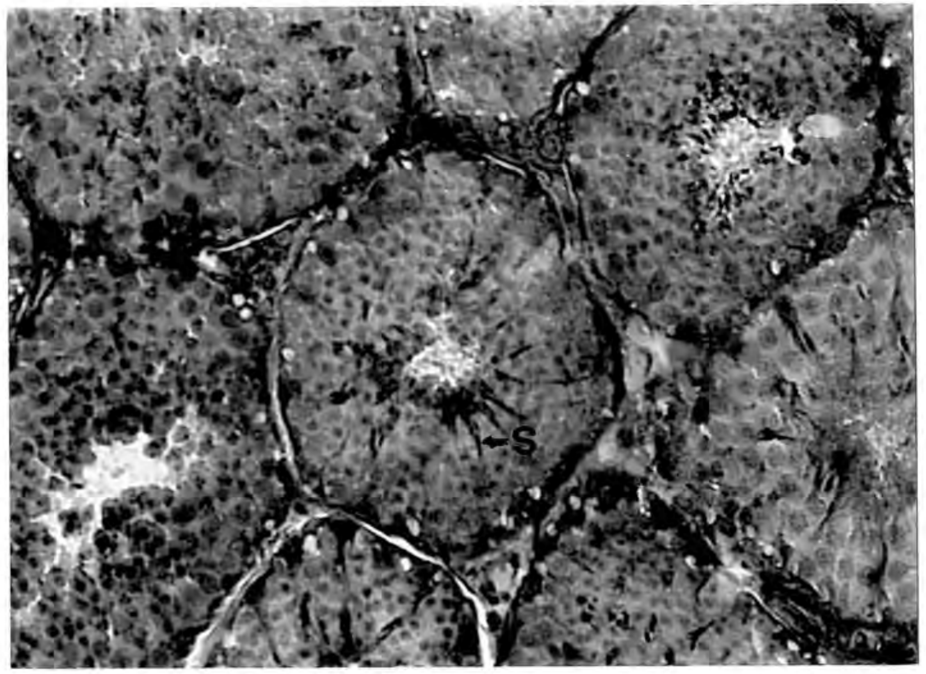

Sp
Sc

Figure 5 : Section through the testis of a specimen collected in February showing the onset of spermatogenic division (arrows). X 286

Figure 6 : Section through the testis of M. schreibersii from late March showing early spermiogenesis. The tubules are filled with spermatogonia (S) primary and secondary spermatocytes (Sc) and early spermatids (Sp). X 458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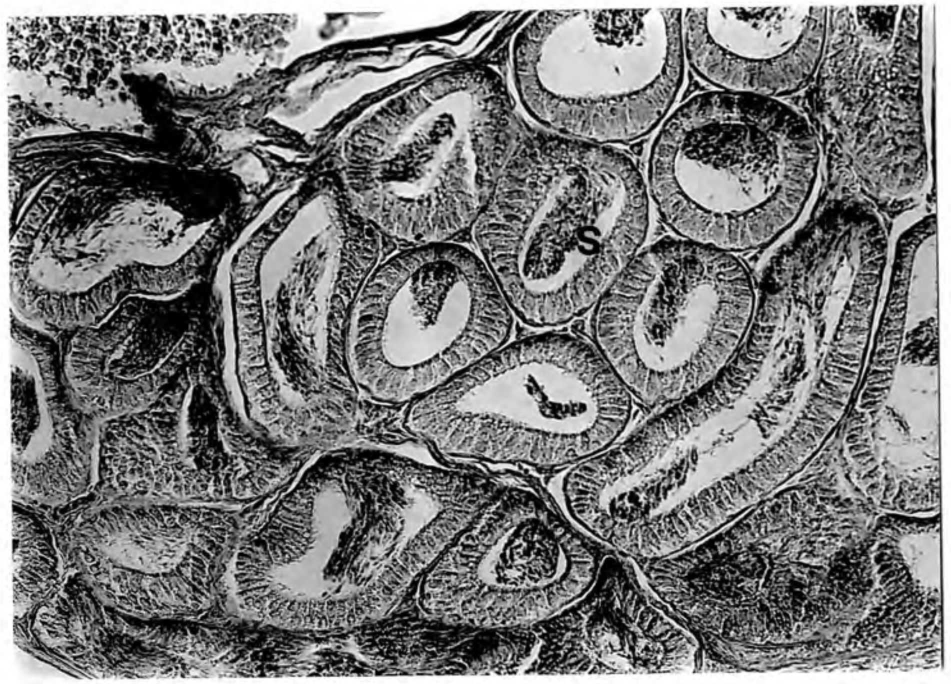
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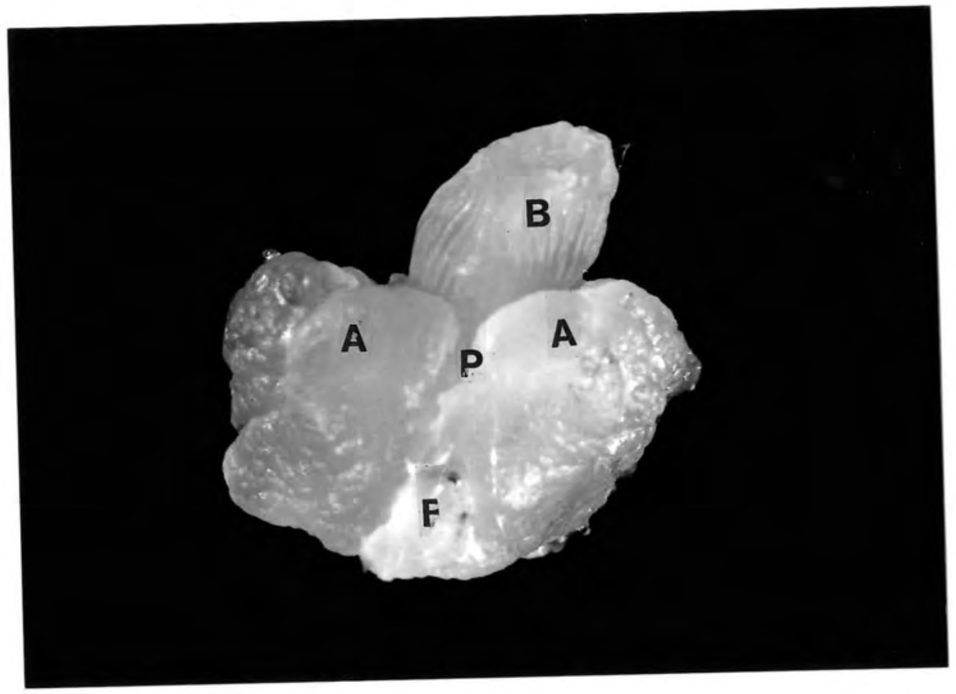
Figure 7 : Section through the testis of a specimen of M.schreibersii from late April, early May. Note the clump of late spermatids (S) close to the lumen of the seminiferous tubule. X 458

Figure 8 : Section through the cauda epididymis of M. schreibersii collected in May, showing the large number of stored spermatozoa. (S). X 286

three components, the urethra and urethral gland, the prostate and two ampullary lobes (Fig. 10). The urethra is a long canal which runs from the bladder, down through the accessory gland complex into the penis. The urethral gland comprises a set of tubular ducts that lie around and empty into the urethra. The ampullary gland is an alveolar gland with large alveoli and a thin cuboidal secretory epithelium. When active, this gland produces a uniform secretion in contrast to the prostate which produces two globular secretions. Larger droplets are secreted by a lobe of the prostate directly below the bladder, while smaller droplets are secreted from the prostate at the base of the ampullae. Both the prostate and the urethral glands are tubular glands with a columnar secretory epithelium.

The accessory gland complex of M. shreibersii varies seasonally in mass, size and secretory activity (Table 4; Fig. 11). Racey and Tam (1974) measured the changes in the height of the epithelium of the ampullary gland, but found that the variations were very small. Other histological criteria were thus sought as evidence of activity in the accessorry gland complex. During winter (June), when the accessory gland complex is secretorily inactive (as indicated by histological sections), the lowest mass of the complex (0.008g), was recorded (Table 4; Fig. 11). The mass of the complex remains relatively constant for the remainder of the year. In February, the mass of the complex begins to increase until the peak in April is reached (0.039 g). There is a statistically significant difference between the mean mass of the active accessory gland complex (March - May) when compared to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A
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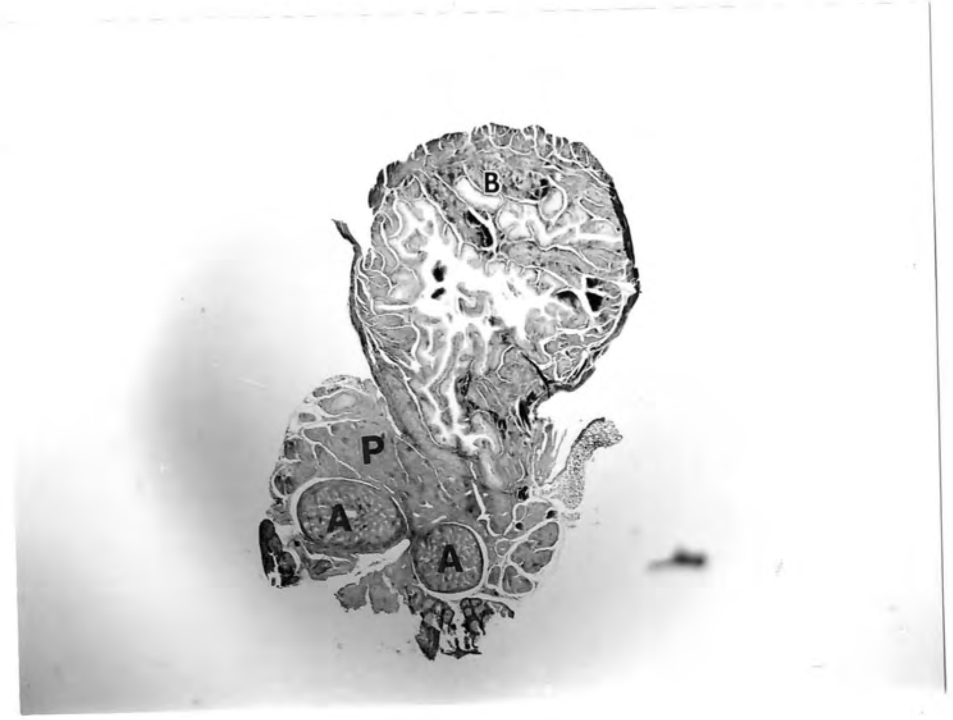
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Figure 9 : Dorsal view of the male accessory gland complex of *M. schreibersii*. B = bladder, P = prostate, A = ampulla, F = fat bodies. X 286

Figure 10 : Section through the inactive accessory gland complex of *M. schreibersii* illustrating the typical arrangement of the components. A = ampullary gland, B = bladder, P = prostate gland. X 286

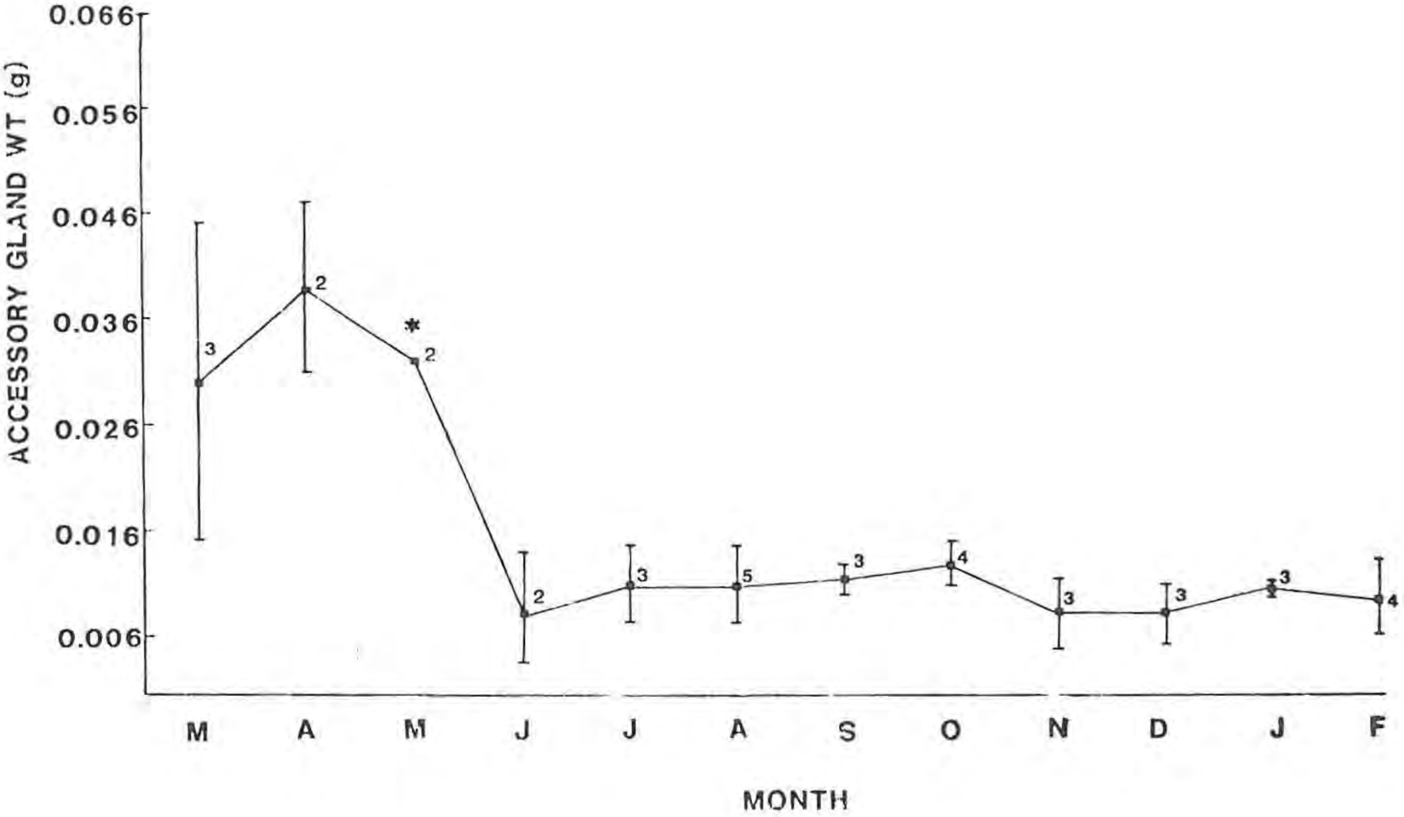


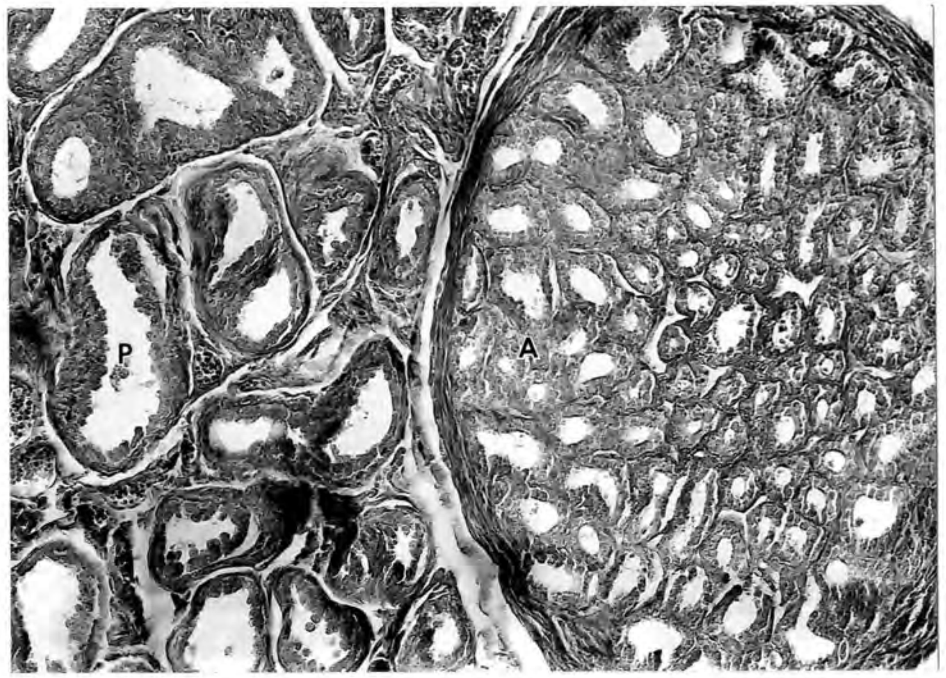
Figure 11 : Monthly changes in mean accessory gland weights of adult male M. schreibersii showng the statistically significant increase in mass at the same time as spermatogenesis is occuring. * = SD is too great to include on the axis.

that of the inactive complex (June - September) ($p<0.01$).

Histologically, the annual pattern of changes in the secretory activity of the ampullae and the prostate are similar. The urethral gland, however, appears to be active throughout the year as indicated by the presence of a secretion which stained positive with Alcian blue. Over the winter months, the prostate and ampullae are secretorily inactive and no secretions are present in either components (Fig. 12). Activity in these two components is initiated in March as indicated by the presence of secretory granules in the prostate and the presence of a uniform secretion in the ampullae, the latter staining positively with PAS (Fig. 13, 14,). The ampullae and prostate are active until early June, when secretions can no longer be observed in the complex. The bats then survive the colder winter months with an involuted accessory gland complex.

3.2.iv PLASMA TESTOSTERONE (T) CONCENTRATIONS

The changes in the levels of plasma testosterone during the annual reproductive cycle of M. schreibersii are shown numerically in Table 5 and graphically in Fig. 15. In May, when spermiogenesis is taking place and the accessory gland complex is beginning to involute, there is a surge in the levels of plasma testosterone to a maximum level of 15.83 ng/ml (n=1). The levels then decline over the winter months, coincident with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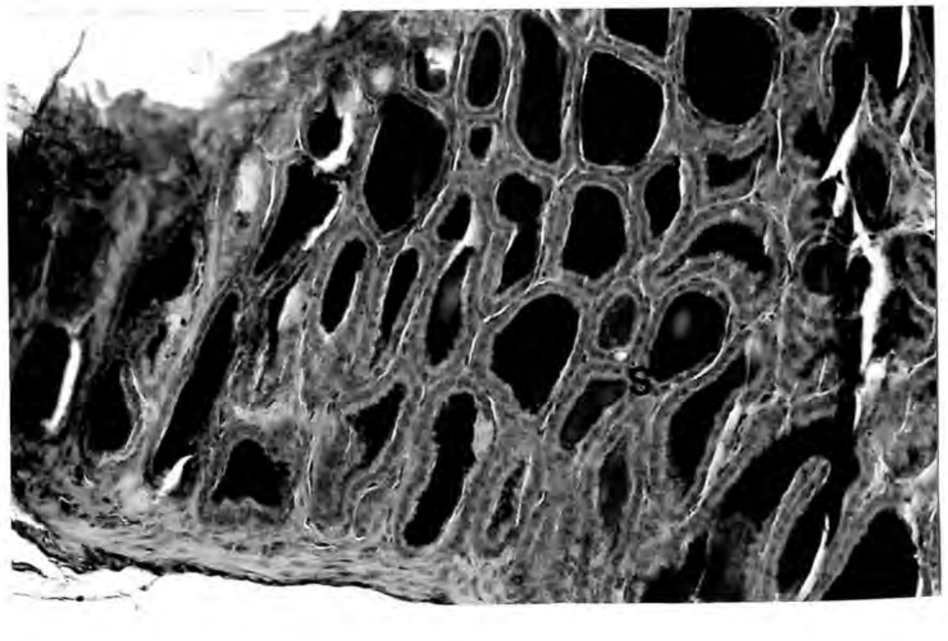

S

Figure 12 : Section through the accessory gland complex during the months June - March, depicting both an inactive prostate (P) and ampullary (A) gland. X 458

Figure 13 : Section through an actve prostate gland during the months March - early June, showing both types of granular secretions, small (S) and large (L) granules. X 458

Figure 14 : Section through an active ampullary gland during the months March - early June, showing the uniform secretion of the gland (S) stained with PAS. X 458

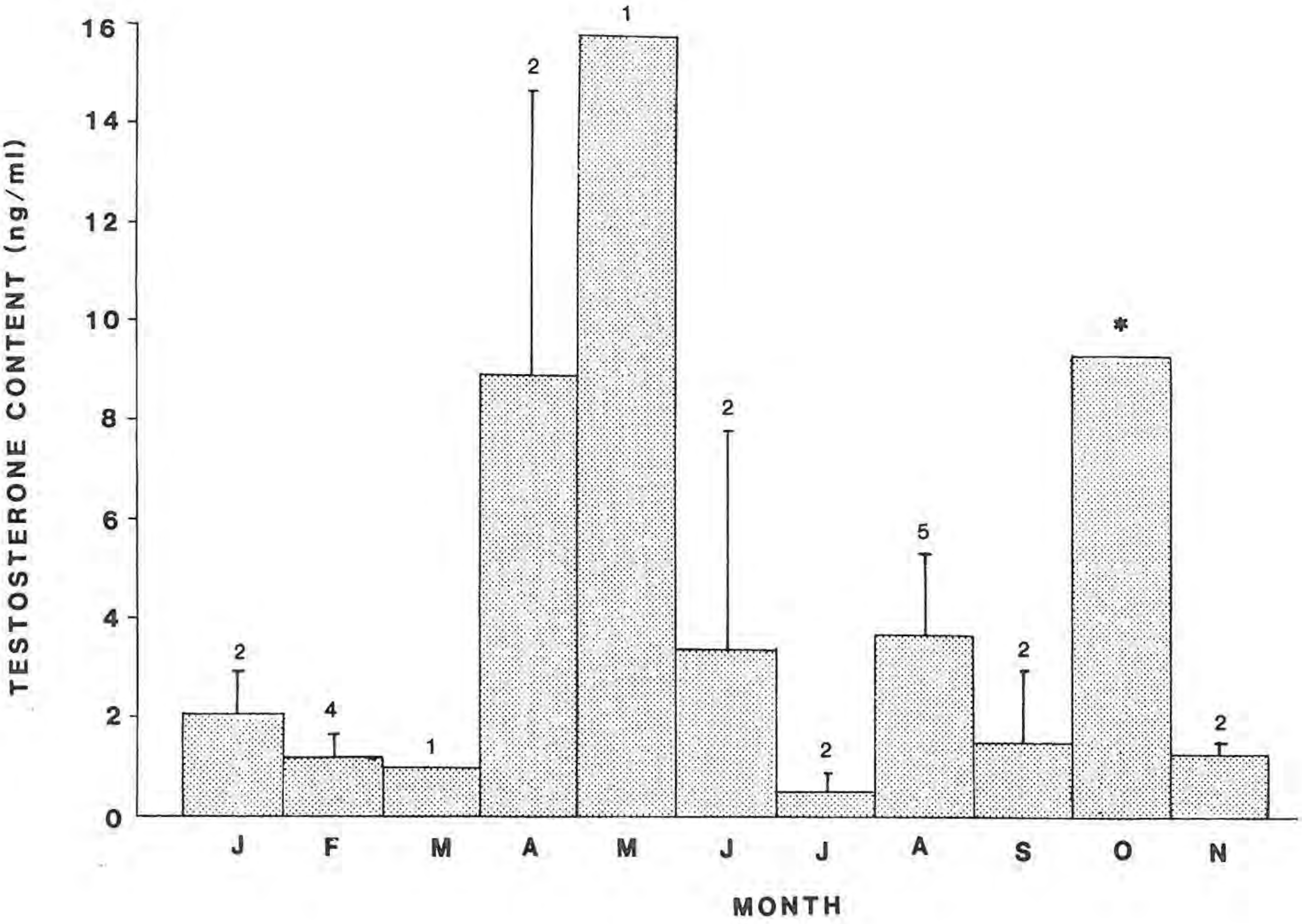


Figure 15 : Mean plasma testosterone concentrations in male adult M. schreibersii. Note the two peaks, one in April and May, coinciding wwith spermatogenic activity and the second in October. * = standard deviation too great to be included on the graph

quiescent seminiferous tubules, and the lowest recorded mean value for an adult occurs in July (0.59 ng/ml). At the end of winter, there is an increase in plasma testosterone levels, reaching a mean in October of 9.27 ng/ml. After October the levels steadily decrease until February, and this is followed by a steady increase in levels during spermatogenesis, reaching the previously mentioned peak in May.

Plasma testosterone levels of individual bats can vary greatly within a single month (Table 6; Fig. 16). In an attempt to explain this variation, the spermatogenic activity of these individuals was examined. In April of the first year, there was a large variation in plasma testosterone (T) levels between the two bats (4.76 ng / ml and 13.12 ng / ml). On examination, testes of both specimens were found to be spermiogenically active, the epididymes of both bats being filled with sperm. In early May, a concentration of 15.83 ng/ml was recorded for a single adult bat, while a sample taken at the end of the same month gave a concentration of plasma T of 6.4 ng/ml. The epididymes of both bats were filled with sperm. In October, an elevated individual plasma T concentration of 27.03 ng/ml was recorded, while a second bat had a plasma T concentration of 0.63 ng/ml. On examination of the testes, it was found that the bat with the lower plasma T concentration was an adult, due to the presence of lipid droplets in the Sertoli cells and that the testes were inactive. Testes from the specimen with high plasma T levels were not prepared for light microscopy. In March of the second year, an unusual variation was observed. The plasma T concentration of

16 February '87	10.0	adult*	1.01
16 February '87	10.5	adult*	0.75
25 March '87	12.5	adult	22.30
25 March '87	9.5	adult*	2.91
25 March ;87	9.0	adult	2.51
24 August '87	11.5	adult	4.59
24 August '87	10.2	adult	5.05
23 September '87	11.0	adult	0.52
23 September '87	10.5	adult	2.45
26 October '87	12.0	adult	0.78
26 October '87	10.5	adult	27.0

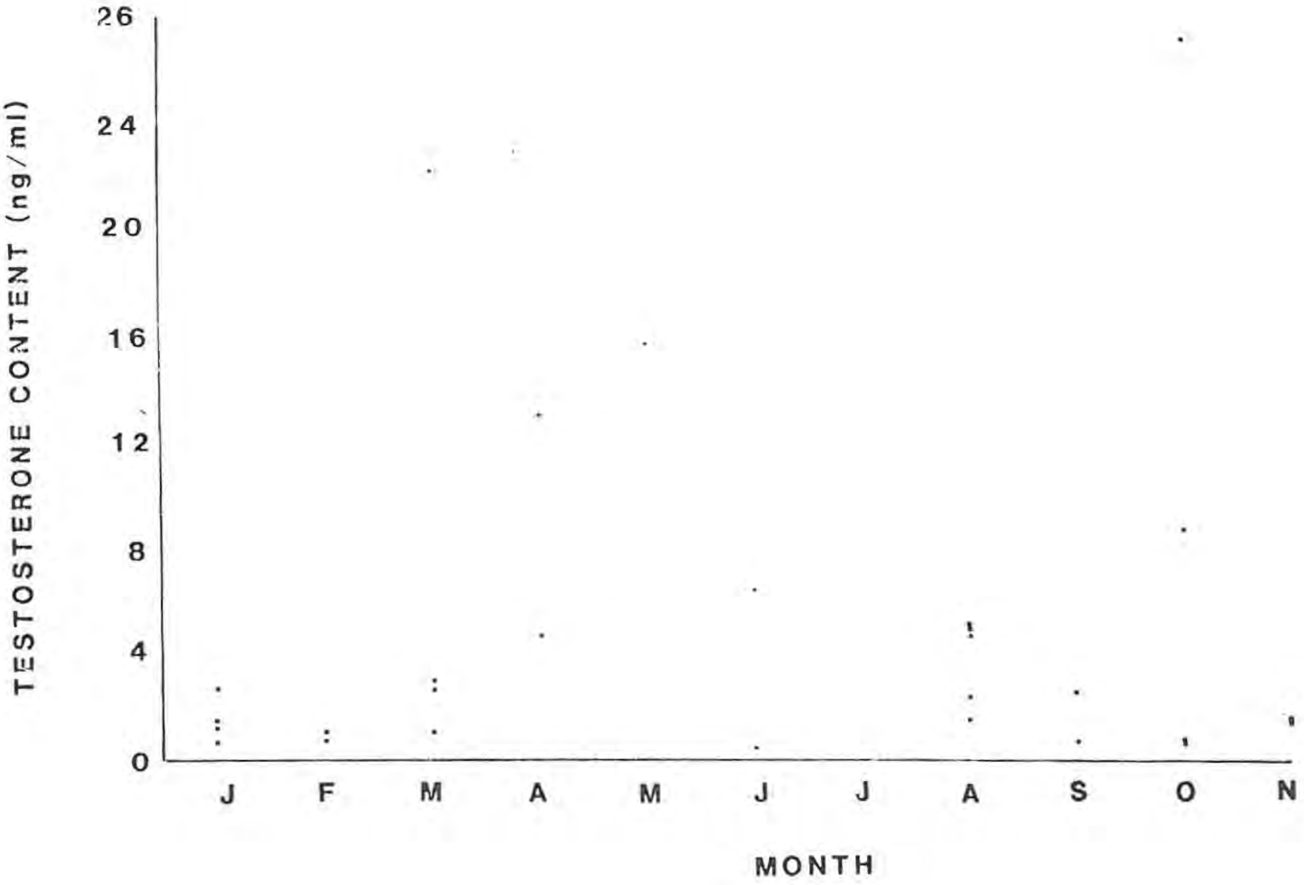


Figure 16 : Individual plasma testosterone levels

one bat was 22.3 ng/ml, while that of a second bat was 2.51 ng/ml. On examination of the seminiferous tubules, it was observed that the testes of both bats were undergoing spermiogenesis, but the testes of the bat with the higher plasma T level contained a greater number of seminiferous tubules containing spermatozoa than did the testes of the bat with a plasma T concentration of 2.51 ng/ml.

Plasma T concentrations from specimens in similar reproductive conditions are tabulated in Table 7. During winter, when the testes are spermatogenically inactive, higher plasma T levels are recorded in adult bats compared to juvenile bats. The difference in plasma T levels during spermatogenesis, prior to spermiogenesis and during spermiogenesis is unclear, although three of the four levels recorded prior to spermiogenesis are lower than the three levels recorded during spermiogenesis. An unusually high value of 22.3 ng / ml, the highest value recorded, was from a bat whose testes were undergoing spermatogenesis and not spermiogenesis, as might be expected. Due to the small sample size, there is no significant difference between plasma T levels recorded in April to June when compared to levels recorded from January to March.

Table 7 : Plasma testosterone (T) levels in male M. schreibersii recorded during winter, spermatogenesis (excluding spemiogenesis) and spermiogenesis.

Activity	Mean T +/- SD (range)	n
Adult / winter	0.99 +/- 1.57 (0.40 - 4.82)	7
Immature / winter	0.59 +/- 0.31 (0.37 - 0.81)	2
Spermatogenesis	7.21 +/- 10.1 (1.12 - 22.3)	4
Spemiogenesis	11.24 +/- 5.77 (4.76 - 15.83)	3

3.2.v LEYDIG CELL ACTIVITY

Seasonal changes in Leydig cell ultrastructure :

Light microscopy is not sufficient to determine changes in the organelles associated with steroidogenesis, and thus an electron microscopy study was undertaken. Leydig cell ultrastructure is variable, and based on general cell shape and the form and numbers of organelles known to be involved in the production and secretion of testosterone, three stages of Leydig cell have been recognised. Leydig cells from any one month are seldom found to be ultrastructurally similar. However, based upon the appearance of the majority of Leydig cells in one month, it is possible to describe seasonal changes in Leydig cell ultrastructure.

Stage 1 (Fig. 17):

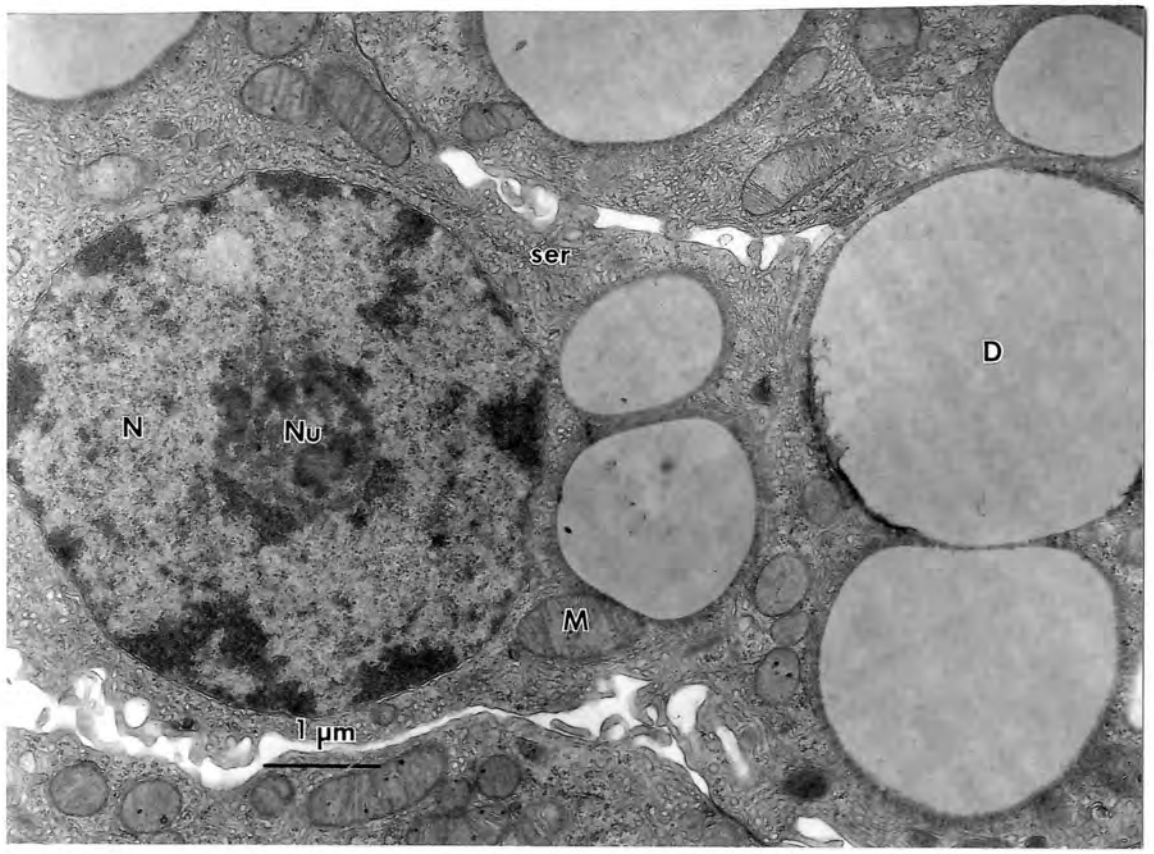
The Leydig cells in February and early March, coinciding with an initiation of spermatogenesis and increasing T levels, were typical of this condition. The smooth endoplasmic reticulum (SER), consisting of thin tubules, is the most abundant organelle. Mitochondria, with lamellar cristae and intermitochondrial granules, and lipid droplets are abundant in the cytoplasm. Leydig cells of this stage are generally polygonal in shape.

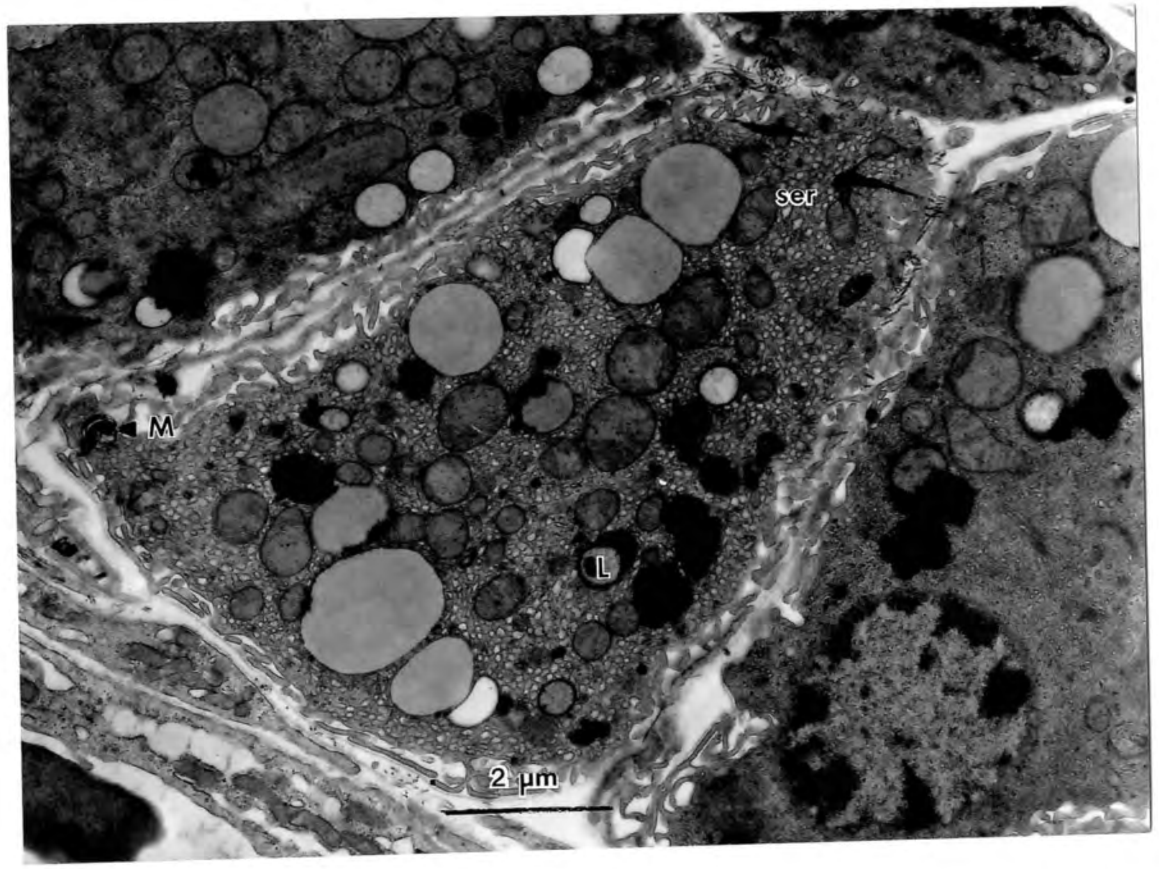
Stage 2 (Fig. 18):

Leydig cells in March during active spermiogenesis, are in this condition, although a number of these cells can still be observed

Figure 17 : Electron micrograph of a Stage 1 Leydig cell as it appeared in February. The majority of the cells are filled with SER. M = mitochondrion D = lipid droplet, N = nucleus, Nu = nucleolus.

Figure 18 : Electron micrograph of a Stage 2 Leydig cell (center) as it appeared in March. The SER tubules have a larger diameter and there is an increase in the numbers of lipofuscin granules (L) and myelin figures (M). Note the characteristic interdigitating cell membranes of the Leydig cells (arrows).


ser
D
N
Nu
M
1 µm


ser
M
L
2 µm

in the interstitial tissue until August. The cells take on a smaller, less polygonal form and generally appear less structured. The SER appears to be reduced in abundance and the SER tubules have a larger diameter (Fig. 18). In March and April, when the mean cell size is at its maximum value (9.6 um), the mean SER tubule diameter was 0.087 um and 0.057 um respectively. In June, a mean SER tubule diameter of 0.11 um was recorded. As a result of the decrease in cell size, the cytoplasm is reduced and the organelles appear more abundant. There is a steady decrease in the mean cell size from March (9.6 um) through to October (4.21 um). It was not possible to test for statistically significant differences because of the small sample size. There is a marked increase in the organelles involved in cellular degeneration, such as lipofuscin granules and myelin figures from March through to August.

Stage 3 (Fig. 19):

The appearance of these cells is one of degeneration and from May, when spermiogenesis is terminated and both the testes and accessory gland complex are inactive, examples of these cells could be seen in the interstitial tissue. These cells have an irregular shape, the sparse cytoplasm being filled with myelin figures, primary lysosomes, lipofuscin granules and peroxisomes. The ER is in the form of rough endoplasmic reticulum and appears disrupted. These cells , which occur in the interstitium from May until the following January, appear to be undergoing vacuolation due to the presence of a number of vesicles and vacuoles.

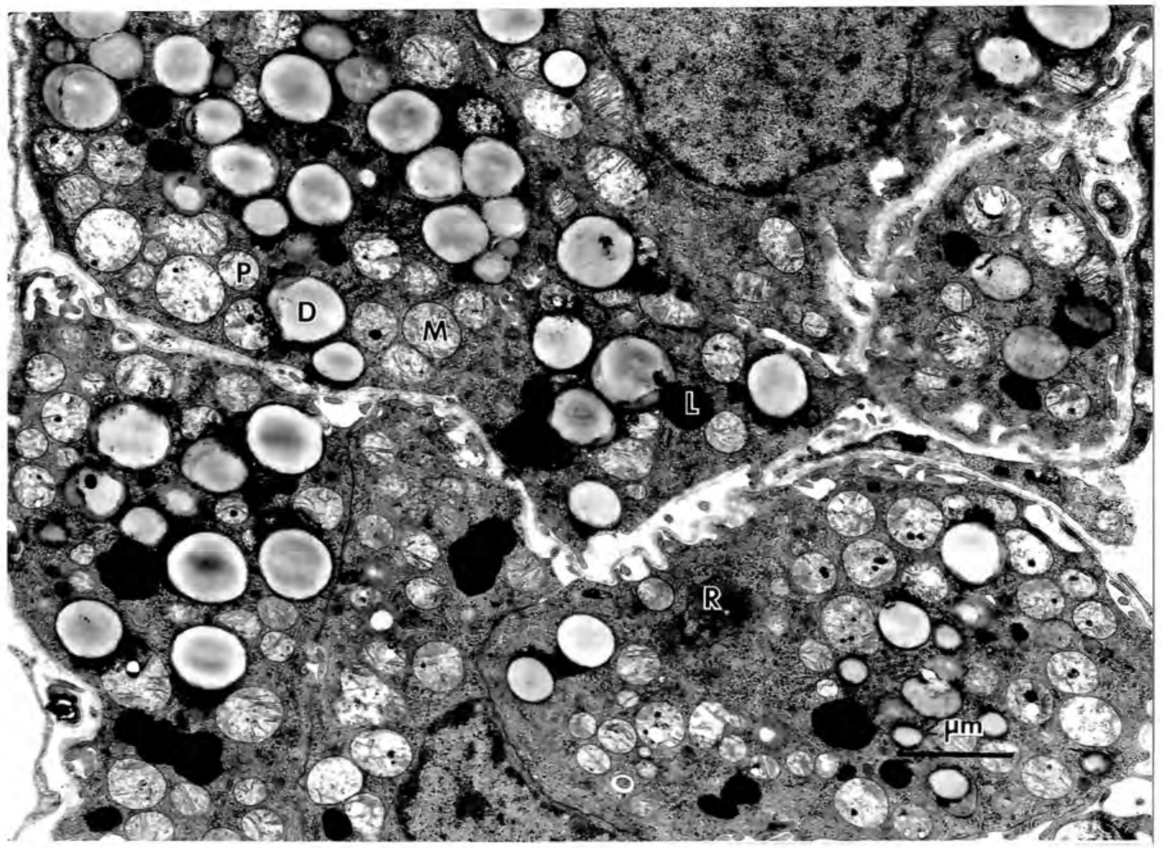
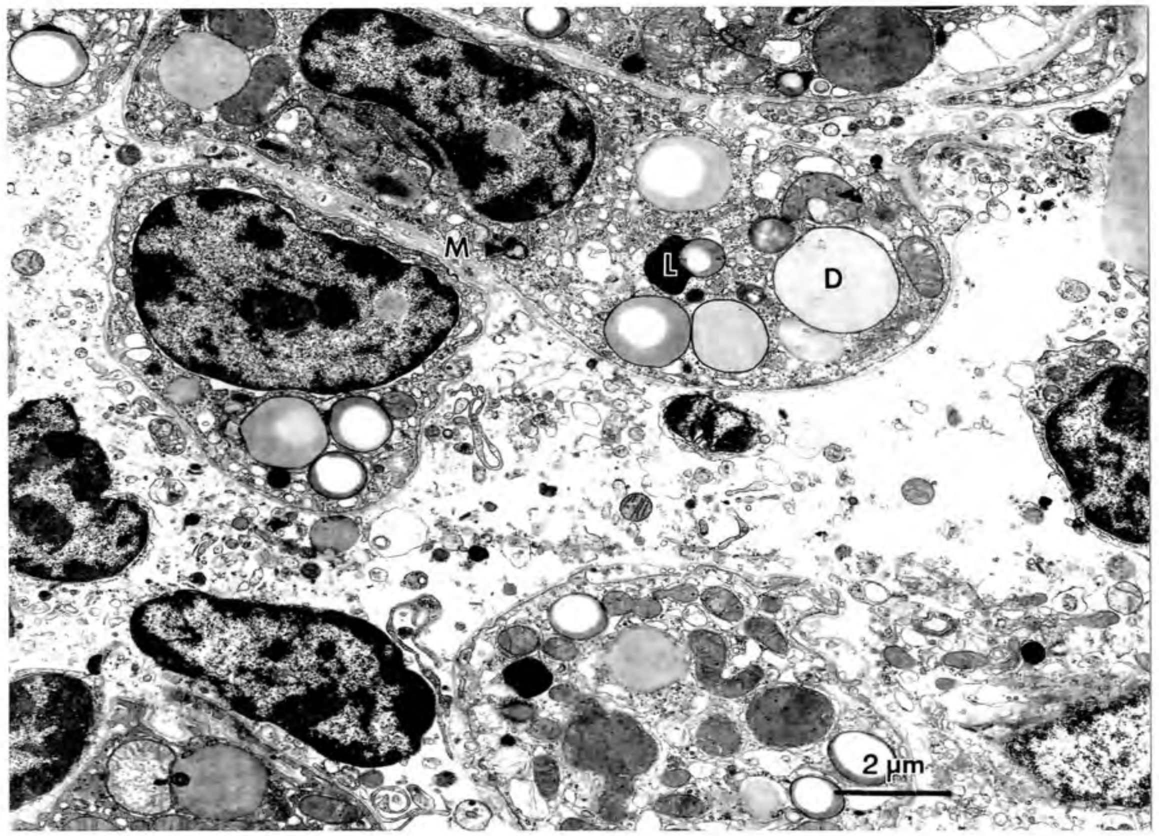


Figure 19 : Electron micrograph of a Stage 3 Leydig cell. The cells are less polygonal in shape and degeneration of the cell can be observed. A primary lysosome (P) fuses with a lipid droplet (D) and after breakdown of the cell, a lipofuscin granule (L) is formed. Ribosomes (R) and RER are more abundant in the cell

In addition to these degenerate cells, further signs of Leydig cell degeneration can be observed in the interstitial tissue from May until January. Following vacuolation of the cell (Fig. 20), the cellular membrane disintegrates and the cytoplasm and cellular organelles are released into the interstitial space (Fig. 21). Subsequently, naked Leydig cell nuclei, which are often highly folded, can be seen. Such nuclei are normally surrounded by cytoplasmic extensions of fibroblasts (Fig. 22). The fate of these nuclei is unknown.


M
L
D
2 µm

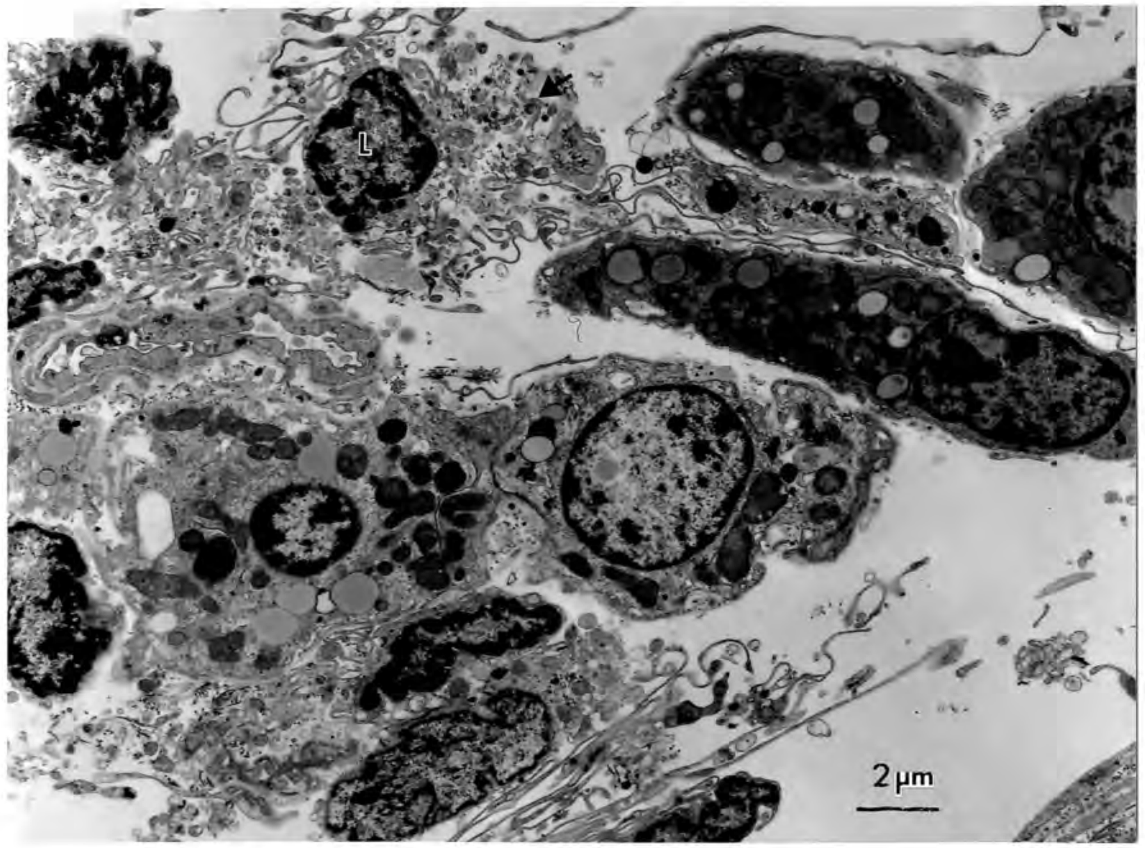

L
2 µm

Figure 20 : An electron micrograph showing vacuolation of the Leydig cell. The cell becomes filled with organelles involved in degeneration. Lipofuscin granules (L) break down the contents of lipid droplets (D) and an organelle found during breakdown of the cell, a myelin figure (M) is formed.

Figure 21 : An electron micrograph illustrating the cell membrane of the Leydig cell (L) breaking down (arrows) and the cytoplasm and organelles being released into the interstitium.

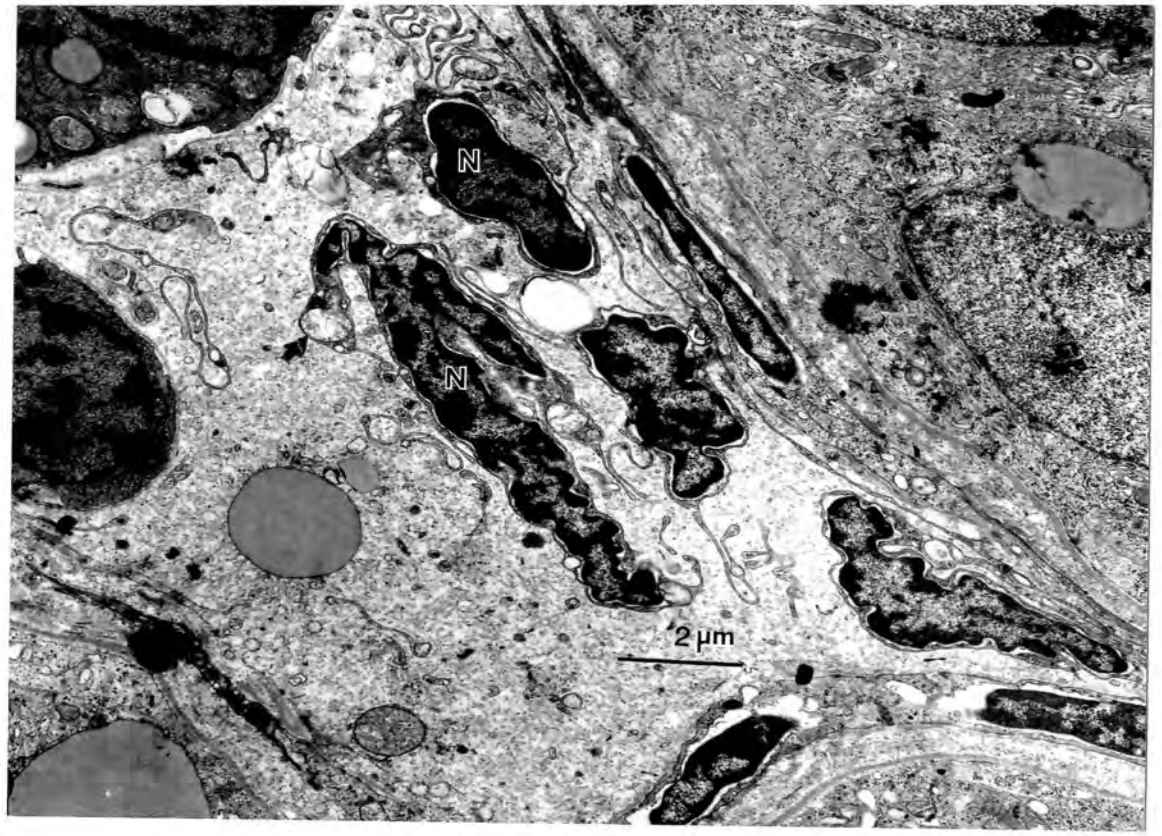


Figure 22 : The naked nucleus of the Leydig cell remains in the interstitium (N) surrounded by cytoplasmic extensions of fibroblasts (arrow)

3.3 DISCUSSION

3.3i BAT MASS

Mass loss is restricted to the early parts of winter and from mid-winter onwards, mass increases. M. schreibersii feeds throughout winter so that mass reduction at the onset of winter may be a result of a combination of testicular and accessory gland regression; the cost of mating behaviour and reduced food availability.

3.3ii MALE REPRODUCTIVE TRACT, SPERMATOGENESIS AND SEASONAL CHANGES IN THE ACCESSORY GLAND COMPLEX

The morphology of the male reproductive tract has been described for a range of vespertilionids (Matthews, 1941), and is highly variable (Krutzsch, 1979). The male reproductive tract has been described for M.dasythrix (=schreibersii) from South West Africa (Matthews, 1941), for M. schreibersii from Australia (Richardson, 1977) and for M. schreibersii fuliginosis (Pal, 1983). The descriptions correspond with that given in the present report.

Monthly changes in testicular and accessory gland mass and histological appearance, as well as changes in plasma testosterone concentration and Leydig cell ultrastructure indicate that male reproduction in M. schreibersii at 34° S is highly seasonal. Spermatogenic activity and associated changes in Leydig cell ultrastructure, elevated plasma T concentrations, and accessory gland activity are restricted to the end of summer, and copulation in autumn is followed by a lengthy period of reproductive inactivity.

Courrier (1927 in Dwyer, 1963a,b) was the first worker to describe the highly seasonal reproductive cycle of M. schreibersii (France). Further studies of the reproductive cycle of M. schreibersii from temperate latitudes by Baker and Bird (1936) in the New Herbrides (15^{0} 15'S), Dwyer (1963 a,b, 1968) in New South Wales (30^{0} S) and Medaway (1971), in Borneo (3^{0} 50'N) have confirmed the initial observation by Courrier that reproduction in this species is highly seasonal. In temperate latitudes, copulation, ovulation and fertilization all take place during late summer and autumn, and all male reproductive organs are quiescent on entry into hibernation or decreased activity during winter. The male reproductive cycle of Miniopterus minor at 4^{0} S has been described (McWilliam, 1988) where it was found that the highly seasonal rainfall pattern has necessitated a facultative short period of delayed implantation in the female over the more severe winters.

Unfortunately the above mentioned studies are based only upon gross morphological observations and recordings with no histological work to support the findings. Richardson (1977) undertook a histological study of reproduction in M. schreibersii and M. australis at 28.5^{0} S, in Eastern Australia and was able to accurately determine the duration and timing of spermatogenesis accessory gland activity, copulation and implantation. The results confirmed the previous observations of a highly seasonal male reproductive cycle.

This pattern of male reproductive activity in temperate latitudes is associated with a specialisation of the female reproductive cycle in which implantation is delayed for a period coinciding with winter. For M.schreibersii in Europe (45 ^{o}N), the duration of delayed implantation is five months, while in Australia (28^{o} 5' S), it is three months. In South Africa, at 29^{o} S - 30^{o} S, the period of delayed implantation was found to be four months, while for a hibernating population of Indian rhinolophid bats, Rhinolophus rouxi in India, the period was found to be one and a half months in duration (Planel et al., 1961, in Richardson, 1977; Peyre & Herlant, 1963, 1967, in Richardson, 1977; Richardson, 1977; Ramakrishna & Rao, 1977; Van der Merwe, 1977; Bernard, 1980). It has even been found that at a low latitude of 4^{o} S, female M. minor include a short period of delayed implantation during the long dry season. This suggests that the reproductive delay pattern may have originated in tropical latitudes (McWilliam, 1988).

Delayed implantation is one of three types of reproductive delay patterns which occurs in the Microchiroptera. Delayed implantation occurs not only in members of the genus Miniopterus, but also in the funnel - eared bat, Natalus stramineus (Mitchell, 1965), in the equatorial fruit bat, Eidolon helvum (Mutere, 1967) and in the Indian rhinolophid bat, Rhinolophus rouxi (Ramakrishna & Rao, 1977). Delayed fertilization is common in almost all of the other species of vespertilionid bats from temperate latitudes (Wilson, 1979). In delayed fertilization, copulation typically occurs in late summer and either the female or the male stores sperm over the cold winter period and ovulation and fertilization occurs in spring. The third pattern is that of delayed development.

The majority of authors agree that in temperate dwelling bat species, the period of hibernation necessitates the use of a reproductive delay so that the young are born at the most favourable time of the year. In the present study area, M. schreibersii inhabits an area in which the climate is highly seasonal, with warm to hot summers (September - April) and cool, dry winters (May - August) (Schulze, 1980). M. schreibersii gives birth in November and December, during which time the insect biomass is an order of magnitude higher than during the winter months (Earlé, 1981, 1985).

In M. schreibersii, hibernation is limited to the particularly cold spells, and specimens arouse during warmer spells (pers. obs./ Bernard, 1983; 1985a, unpublished data). As a result of the periods of decreased metabolic activity / hibernation associated with winter, the period suitable for all reproductive activity is the eight months from September to April. Spermatogenesis, which does not occur during hibernation (Racey, 1982), and is typically shorter than the period of follicular development takes between two to two and a half months. The genus specific minimum gestation length for Miniopterus is three and a half to four months (Wimsatt, 1969) based on a tropical - dwelling *M. australis* (Baker & Bird, 1936) where implantation follows immediately after spring ovulation. The period from parturition to weaning in Vespertilionids is one and a half to two months (Van der Merwe, 1978). And Shimoizumi (1959) suggests the time required after weaning to attain a fat store to survive the following winter is one month. The total length of the reproductive cycle thus varies from eight to nine and a half months. It would thus be difficult for a hibernating bat to reproduce without either gametogenesis occuring during winter or lactation and post weaning development in the following winter. Some form of reproductive adaptation is thus required in order to colonise a temperate latitude. In a recent study in coastal Kenya at 4° S, on a tropical-dwelling *M. minor*, it was found that even at this latitude, bats of this species undergo a period of torpor over the long, dry season. This is to ensure that parturition and lactation occur at a time of food abundance and for the benefits of mating with a fit male whose sexual cycle is also dependant upon periods of food surplus (McWilliam, 1988). A reproductive delay enables hibernating bats in South Africa to initiate the reproductive cycle in the middle of one summer and to complete the cycle in the middle of the following summer.

The ultimate factors controlling seasonal breeding in mammals are always climate, caloric availability and / or nutrient quality of an animals food (Baker, 1938 in Bronson, 1985). Reproductive periodicity in many bats from temperate latitudes reflects seasonal variations in food supply with birth during the summer months, when the insect food supply is most abundant (Oxberry, 1979).

Factors affecting specific reproductive events :

1. Spermatogenesis

Although the pineal gland is thought to be involved in mediating the effects of photoperiod on the hypothalamus, Pevet and Racey (1981) could find no evidence in P. pipistrellus that the pineal gland is involved in the inhibition of reproductive activity as it is in some other mammals. A second study on the possible influence of photoperiod on the reproductive cycle of the male pallid bat was undertaken by Beasley and Zucker (1984). The reproductive cycle of these bats was found to approximate to the reproductive sequence described in other temperate vespertilionid bats. These workers found that exposure of the bats to short days accelerated the attainment of an 'autumnal condition' (regressed testes and sperm storage), while bats kept in long days showed the 'summer reproductive condition' (spermatogenesis). These findings provide support for the theory that in pallid bats, reproduction may be timed by an endogenous circannual clock that can be phase advanced by short photoperiods.

Beasley and Zucker (1984) suggest that failure of the pipistrelle bats to respond to changes in photoperiod could be related to the time of the year during which experiments were carried out. Pipistrellus bats were held under experimental conditions during their normal period of hibernation, whereas pallid bats were kept under experimental conditions during periods of their normal activity. It is suggested that photoperiodic cues may be less important during times of hibernation.

Increased temperature and food availability during the second half of hibernation stimulates spermatogenesis in the little brown bat (Gustafson, 1979). Beasley and Zucker (1984) also suggest that temperature is more important than photoperiod in affecting the reproductive apparatus of pallid bats during their period of hibernation.

It thus appears that photoperiod may be a cue affecting reproductive processes, but that it may play a secondary role to temperature during hibernation. In *M. schreibersii* spermatogenesis is initiated in January / February. In the Eastern Cape at 29° S - 30° S the day : night length at this time is approximately 12 hours : 12 hours. It is suggested that the switch to shorter daylength may be the stimulus for the onset of spermatogenesis.

2. Delayed implantation

Mutere (1967) suggests that rainfall may stimulate the time of implantation in the fruit bat, *Eidolon helvum* as photoperiod varies little at the latitude at which he was working (0° 20'N). In *M. schreibersii* in Northern and Southern latitudes, implantation occurs when day length is 10 - 12 hours and increasing, suggesting that the winter solstice marks the onset of the stimulus for implantation (Racey, 1981).

Richardson (1977) suggests that because the period of delayed implantation coincides with the time spent in hibernation, one would expect the period of delayed implantation to be temperature dependant, conditioned by a pronounced lower metabolism. She notes that this form of control is unknown in any other mammal. Evidence for a temperature controlled period of embryonic development in M. screibersii fuliginosus has been provided by Uchida et al. (1984). These workers demonstrated that at elevated temperatures, the embryonic growth rate was accelerated, and consequently birth was advanced by a period equivalent to that of exclusion from hibernation as compared with that in the wild population. The results of the above study have been substantiated by work done by Kimura et al. (1987). Working on the same species, it was shown that elevated ambient temperatures and ample food supplies hastened embryonic development and resulted in an increase in the plasma progesterone levels. The workers suggest that the retarded embryonic development of this bat is a passive and direct response to the cold and depressed metabolism. Peyre and Herlant (1963) have produced evidence for M. schreibersii in France which implicates participation of the neurendocrine system, although the proximal factors underlying this system are as yet unknown (Kimura et al., 1987).

The question of whether variations in the duration of the period of delayed implantation are a facultative response or an adaptation incorporated into the genome is worthy of consideration. M. schreibersii gave birth in October in one study at Naracoorte in South Australia (Hamilton - Smith, 1972 in Racey, 1982) and in December in another year, but at the same sample site (Richardson, 1977). This suggests that a facultative variation in the timing of mating or gestation length exists.

3. PHYSIOLOGY OF REPRODUCTION

3.1 Spermatogenesis

The results of the present study indicate that the length of the spermatogenic cycle is approximately three months, from February until the end of April. The length of the spermatogenic cycle from the first division of the spermatogonia until the release of the sperm appears to be fairly similar throughout the Vespertilionidae. Typically in this family, spermatogenesis begins in late summer / autumn (July / August in the northern hemisphere) and sperm are released in Setember / October. This implies a brief spermatogenic cycle of between two and three months (Pearson et al., 1952; Krutzsch, 1956, 1961; Racey & Tam, 1974; Gustafson, 1977). The length of the spermatogenic cycle thus appears to be similar to that of M. schreibersii as indicated from the present study.

In bats belonging to families other than the Vespertilionidae, the length of the spermatogenic cycle is more variable. Racey (1974) reports for the male noctule bat, *Nyctalus noctula*, a cycle length of four to five months, beginning in summer (May) with sperm being released in August and September. Similar results were reported by Krutzsch et al. (1976) for the male leaf-nosed bat, *Macrotus waterhousii*. Krutzsch and Crichton (1987) on the little mastiff bat, *Mormopterus planiceps*, report a protracted period of spermatogenesis in spring / summer, followed by an autumn spermiogenic phase. Of interest are the studies on *Rhinolophus* in Southern Africa (Bernard, 1983, 1985a,b). Whereas the duration of the spermatogenic cycle of *R. clivosus* is approximately two to three months, as is usually found, that of the Cape horseshoe bat, *R. capensis*, is six months, beginning in mid summer with sperm being released in late Autumn (April / May in the Southern hemisphere). The adaptive significance of such variation in the length of the spermatogenic cycle remains obscure.

3.3ii Plasma testosterone (T) concentrations

The results of the present study on changes in plasma T concentration are in accordance with the results of other workers in this field, with peak plasma T concentrations being found during the period of spermatogenesis (Gustafson & Shemesh, 1976; Racey, 1974; Bernard, 1986). It is interesting to note that although there is an increase in mean plasma T levels from pre-spermiogenesis to during spermiogenesis, the highest value was found prior to spermiogenesis. Gustafson and Shemesh (1976), the first workers to analyse T concentrations in a wild population of seasonally - breeding, hibernating vespertilionid bat, Myotis lucifugus lucifugus, also reported the occurence of a peak in plasma T near the end of spermatogenesis. Certain observations on changes in the levels of this hormone during the year are more difficult to interpret. The first is the appearance of a second , although lower, peak in plasma T concentration in October. In October, M. schreibersii is out of hibernation or the period of decreased activity, and reproductive events have not begun. The occurence of a second peak, unrelated to the spermatogenic process, has been recorded in three other studies of plasma T concentrations in Chiroptera. They have been observed in the Cape horseshoe bat (Bernard, 1986), the little mastiff bat (Krutsch & Cricton, 1987) and in male noctule bats (Racey, 1974), although no explanation for the occurence is supplied. The second peak in these studies occured in August / September, when all spermatogenic activities were terminated, as in the present study.

More unusual are the large variations in plasma T concentrations in individuals from any single month and individuals in similar reproductive conditions. And such variations raise questions as to the plasma T levels required to trigger and control various reproductive events. If a plasma T concentration of 4.76 ng / ml is sufficient to trigger spermiogenesis, what would be the purpose of an elevated level of 15.83 ng / ml ? Furthermore, in spermatogenically active, but spermiogenically inactive testes, an elevated level of 22.3 ng / ml was recorded, the reason for which is not clear.

Although no study has yet been performed on plasma testosterone concentrations in M. schreibersii, several authors have compared plasma T concentrations of hibernating bats to plasma T concentrations of other mammals, and have found that the levels recorded in bats rank with the highest recorded levels of mammal species. Gustafson and Shemesh (1976) recorded a peak plasma T concentration of 68 ng / ml for M. lucifugus lucifugus and compared this peak concentration to those recorded for other mammals. Gustafson and Shemesh (1976) found the peak plasma T value of the bat to be 10 times greater than values found in man and some monkeys, 20 times those in stallions and 30 times those in shrews, dogs and some rabbits. Such high plasma T concentrations have also been reported in Pippistrellus pippistrellus (Racey & Tam, 1974) and Nyctalus noctula (Racey, 1974). Based on these findings, Gustafson and Shemesh (1976) suggest that the high levels may be a feature of seasonally - breeding mammals. When comparing plasma T concentrations of bats studied by other workers with those values recorded for M. shreibersii, it is evident that the mean maximum plasma T concentration (15.87 ng / ml) recorded for M. schreibersii is lower than the mean maximum plasma T concentrations of other bats, two exceptions being R. capensis (Bernard, 1986) and the little Mastiff bat (Krutzsch & Crichton, 1987). The mean minimum plasma T concentration of M. schreibersii (0.59 ng / ml) is the third lowest mean minimum plasma T concentration recorded for all bat species studied, both R. capensis and the Mastiff bat having the same lowest mean minimum plasma T concentration (0.1 ng / ml).

3.3iii Leydig cell activity

Leydig cells are responsible for the secretion of the hormone testosterone, which in turn triggers spermatogenesis and stimulates the accessory gland to produce secretions. As expected, notable differences in both the form of the Leydig cell as well as the organelles present in the cell, were observed between the mating and non - mating seasons.

The role that the various cellular organelles play in testosterone synthesis and secretion is now understood (Hall, 1984, 1988). Different steps of the process of androgen production in interstitial cells occur in different parts of the cell (Samuels & Eikness, 1968 in Setchell, 1978). Thus the fine structure of organelles can be related to androgen synthesis and secretion. The sequential involvement of various organelles are described briefly. Fatty acids probably constitute the main initial precursor for testosterone biosynthesis and enter through the plasma membrane. These are broken down into acetyl co-enzyme A in the mitochondria. Once formed, this leaves the mitochondria and after numerous reduction steps is synthesised into cholesterol which is carried out by enzymes that are tightly bound to the smooth endoplasmic reticulum (SER). After cholesterol has been synthesised, it is esterified and accumulates in lipid droplets. Cholesterol undergoes side-chain cleavage in the mitochondria and the resulting pregnenolone passes into the cytoplasm for further steps in the biosynthesis of testosterone. These enzymes are bound to the SER of Leydig cells (Christensen, 1975).

The most extensively studied Leydig cells are those of domestic or laboratory animals and man. In such species, the male typically produces sperm throughout the year, and these animals therefore show no seasonal variation in reproductive activities. The majority of workers on Leydig cells of different species have tried to correlate the ultrastructure of the Leydig cell with its steroidogenic activity. To determine the relationship between the structure and function of Leydig cells in these continuously reproducing animals it is necessary to examine the cells under experimental conditions.

In seasonal breeders, however, a cyclic sequence of different functional states of the Leydig cell is part of the normal pattern of reproductive activity. These animals thus provide a unique opportunity to study morphological manifestations of the variation in steroid production under physiological conditions.

Although it has been found that in seasonally - breeding mammals, the mating and non - mating seasons are related to markedly different plasma testosterone levels (Racey, 1978), corrosponding ultrastructural changes found in the Leydig cells vary from species to species. For example, Neaves (1973) reports an increase in the Leydig cell size during the breeding season, but no increase in Leydig cell number. Pearson et al. (1952) on the lump - nosed bat, in contrast, reports both an increase in Leydig cell size and number during the breeding season. Furthermore, Johnson et al. (1986) observed changes in Leydig cell number rather than cell size during non - breeding and breeding seasons. In the present study, a decrease in Leydig cell size was observed from March to October, although changes in the number of Leydig cells throughout the year was not recorded.

A second area of controversy concerns changes in the SER during breeding and non - breeding seasons. Friedlander et al. (1984) found that changes in the configuration of the SER could be correlated with changes in the reproductive activity of the camel. In the non-mating season, the SER tubules appeared to have a larger diameter when compared to those observed during the mating season. The highest testosterone concentration coincides with a gradual disappearance of the tubular form of SER and a proliferation of the highly condensed SER. In contrast, the volume of SER appeared to remain constant throughout both the mating and non-mating seasons (Friedlander et al., 1984).

Zirkin et al. (1981) on the rat and Neaves (1973) on the rock hyrax, in contrast, observed that changes in the volume of SER could be related to reproductive activities. Zirkin et. al (1980, 1981) compared the volume densities of SER, RER, mitochondria and lipids to testosterone secretion in the rat, rabbit, guinea-pig, hamster and dog testis and found that the only positive correlation was between the volume density of SER and testosterone secretion. Neaves (1973) reported an abundance of SER in Leydig cells from the hyrax whose testosterone levels are elevated. A two-fold increase in Leydig cell size was reported when comparing sizes of Leydig cells in the breeding and non-breeding hyrax. Distinct changes, similar to those found by Friedlander et al.(1984) in the configuration of the SER between mating and non-mating seasons in M. schreibersii were noted.

A series of studies have been done on the ultrastructure of Leydig cells of bats (Gustafson, 1975; Loh & Gemmel, 1980; Bernard, 1986). These workers are in agreement that the Leydig cells possess the machinery for androgen synthesis only during the summer spermatogenic period. During spermatogenesis, the Leydig cells are characterised by an increase in the diameter of lipid droplets, an increase in the volume of SER, and the SER consists of tubules with a small diameter which often form concentric rings around lipid droplets. In contrast, in the non - secretory condition, more RER is present, there is a decrease in the amount of SER and mitochondria with lamellar cristae are observed. These results are in accordance with those of the present study.

There appears to be discord in the results of studies on the changes in the ultrastructure of Leydig cells in other seasonally breeding mammals. Seasonal changes in the ultrastructure of these cells, coinciding with the results from the present study have been found in the woodchuck (Rasmussen, 1917), in the rock - hyrax (Neaves, 1973), the golden hamster (Wing & Lin, 1977) and in the ground squirrel (Pudney, 1986).

In contrast, numerous authors have reported little or no change in the ultrastructure of Leydig cells despite seasonality in reproduction. This has been found to be the case in the rabbit (Crabo, 1963), the sqirrel monkey (Belt and Cavazos, 1971) and in the European mole (Suzuki & Racey, 1978).

Changes in the ultrastructure of the Leydig cells of M. schreibersii as indicated by changes in the number and form of the cellular organelles provides evidence that these cells are producing testosterone during February and March. From the end of March, the cells begin to undergo a process of degeneration and the majority of Leydig cells remain in this form until summer.

4. LEYDIG CELL LIFE CYCLE

4.1 INTRODUCTION

Leydig cells are accompanied by a number of other interstitial cells in the testicular interstitium. These include myoid cells, macrophages, fibroblasts and a cell which appears to be the precursor cell of Leydig cells (Christensen, 1975).

The most contentious area of the studies on Leydig cells appears to be the origin and fate of Leydig cells. The reason for the controversy is perhaps the apparent confusion in the literature regarding the terminology of the 'precursor cell'. Unfortunately the majority of studies on this topic have been performed using continuously breeding mammals and thus the results are not very convincing. (Crabo, 1963; Hayashi & Harrison, 1971; Russo & de Rosas, 1971; Connell, 1972; Gondos & Renston, 1974; Connell & Christensen, 1975; Gondos et al., 1976, 1977). Continuously breeding mammals require a permanent supply of active Leydig cells and thus one would expect the interstitial tissue to contain active, inactive and precursor cells throughout the year. An attempt to determine the precursor cell of Leydig cells is thus, in studies on these mammals, more difficult. However, a number of workers have used various exogenous hormones and cytotoxic chemicals in an attempt to elucidate the precursor cell, providing more convincing results (Nistal & Paniagua, 1979; Christensen & Peacock, 1980; Chemes et al., 1985; Kerr & Sharpe, 1985a,b; Kerr et al., 1985; Molenaar et al., 1985; Jackson et al., 1986a,b; Morris et al., 1986;

Zaidi et al., 1988). The hormones and chemicals used in these studies either stimulate the formation of new Leydig cells or destroy the Leydig cells such that the repopulation of the interstitial tissue can be followed. Zaidi et al. (1988) observed that EDS destroys the fetal poulation of Leydig cells. After 25 days, a new population of Leydig cells were observed in the interstitium of the rat and they thus suggested that these cells constitute the precursor cells of Leydig cells. The consensus of the histologic literature has been that the adult Leydig cells arise from mesenchymal/fibroblastic cells, the exact morphology of which varies. Suggestions as to the fate of Leydig cells are no clearer, although the most accepted fate of these cells is one of degeneration (Rasmussen, 1917; Fawcett & Burgos, 1960; Mancini et al., 1963; Nishimura & Kondo, 1964; Friedlander et al., 1984; Neaves et al., 1985; Molenaar et al., 1985; Jackson et al., 1986b; Kuopio et al., 1986; Zaidi et al, 1988; Almahbobi et al., 1988).

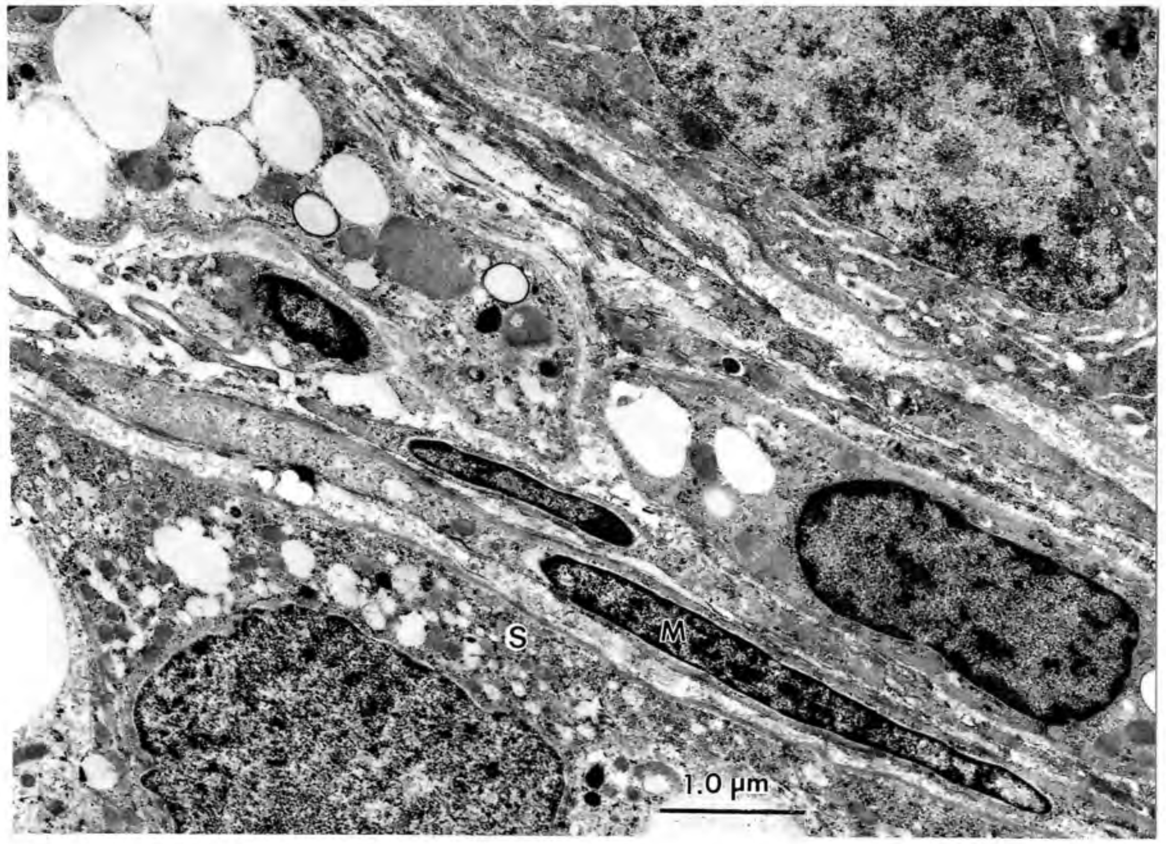
4.2 RESULTS

There are a number of other cells within the interstitial spaces of the testis of M. schreibersii besides Leydig cells.

A) Myoid cells (Fig. 23) are found lining the seminiferous tubules. They are generally elongated cells containing elongated nuclei. These cells are permanently in the interstitial tissue and comprise one or two layers of cells around the seminiferous tubules. Their organelle complement and shape is such that they can not be confused with Leydig cells.

B) Macrophages (Fig. 24) are highly varied in shape and contain a round nucleus. The cytoplasm of these cells is vacuolated and they have hence often been termed 'clear' cells. The cell membrane often has pseudopodia-like extensions. These cells are not frequently observed in the interstitium and once again, their form and organelle component clearly distinguishes them from Leydig cells.

C) Fibroblasts (Fig. 25, 26) are spindle-shaped cells , generally containing a small amount of cytoplasm, such that the nucleus occupies the majority of the volume of the cell. In certain of these cells collagen fibres can be observed. These cells are characterised by long cytoplasmic extensions which often surround Leydig cells.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M
1.0 µm

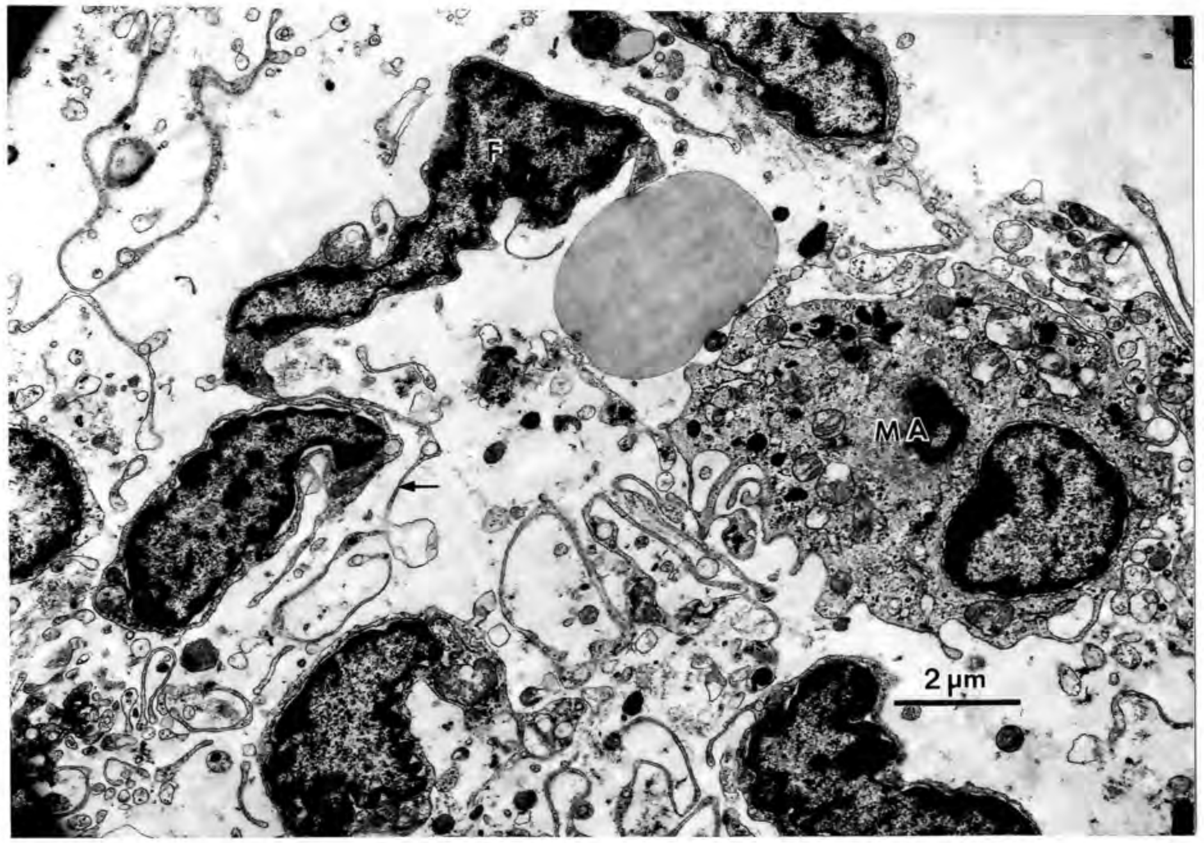
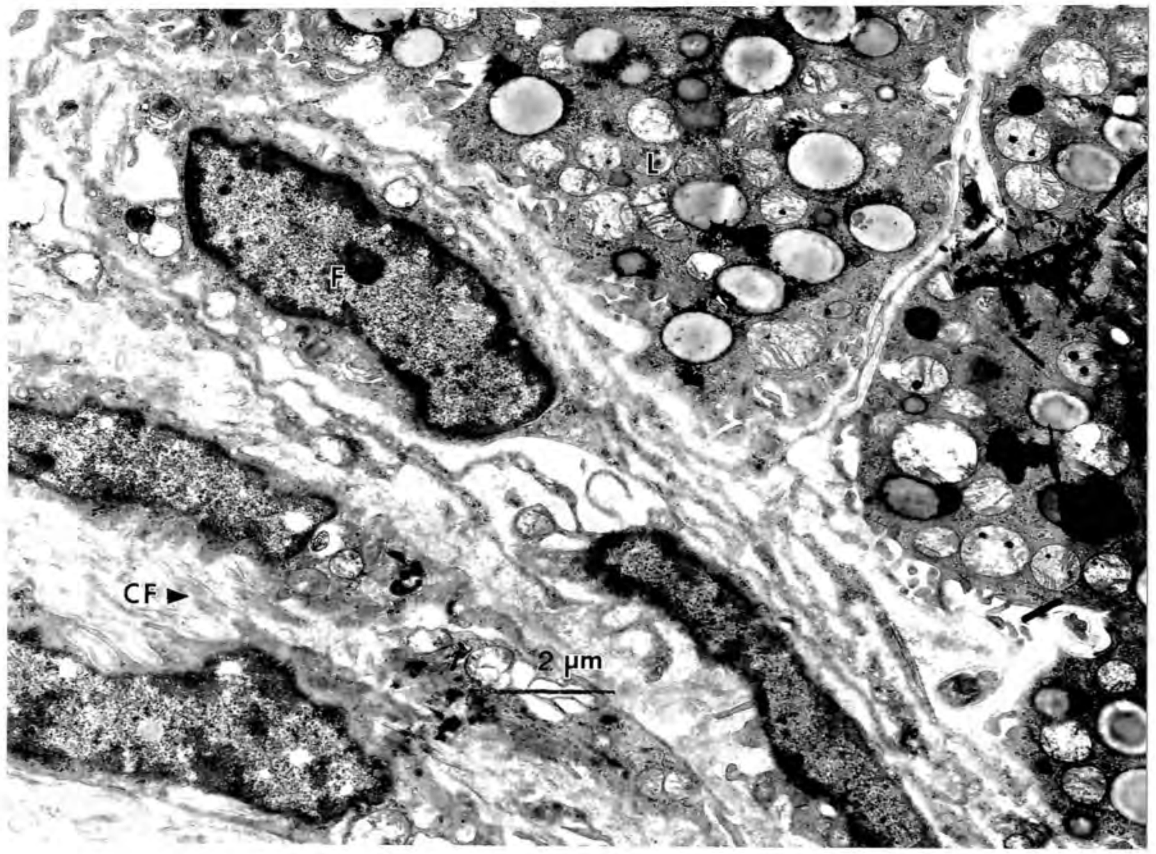

F
MA
2 µm

Figure 23 : Electron micrograph of elongated myoid cells (M) surrounding the seminiferous tubule (S).

Figure 24 : Electron micrograph of a macrophage (MA) seen in close proximity to a fibroblast (F) with long cytoplasmic extensions (arrow).


L
F
CF ►
2 µm


MA
F
CF
F
1.0 µm

Figure 25 : Electron micrograph of fibroblasts (F) seen in close proximity to a Stage 3 Leydig cell (L). Collagen fibres (CF) are often found in or in close proximity to fibroblasts.

Figure 26 : Electron micrograph of two fibroblasts (F) with collagen fibres in the cytoplasm (CF), in close proximity to a macrophage (MA).

D) A particular cell was observed in the interstitial tissue more frequently from May until January than the other months of the year (Fig. 27). This cell has a variable shape, small volume of cytoplasm and contains an irregularly-shaped nucleus. These cells appear to be similar to the 'immature' Leydig cells described by Kerr and Sharpe (1985b) and the 'precursor' cells of the Leydig cells described by Jackson et al. (1986a) and by Zaidi et al. (1988).

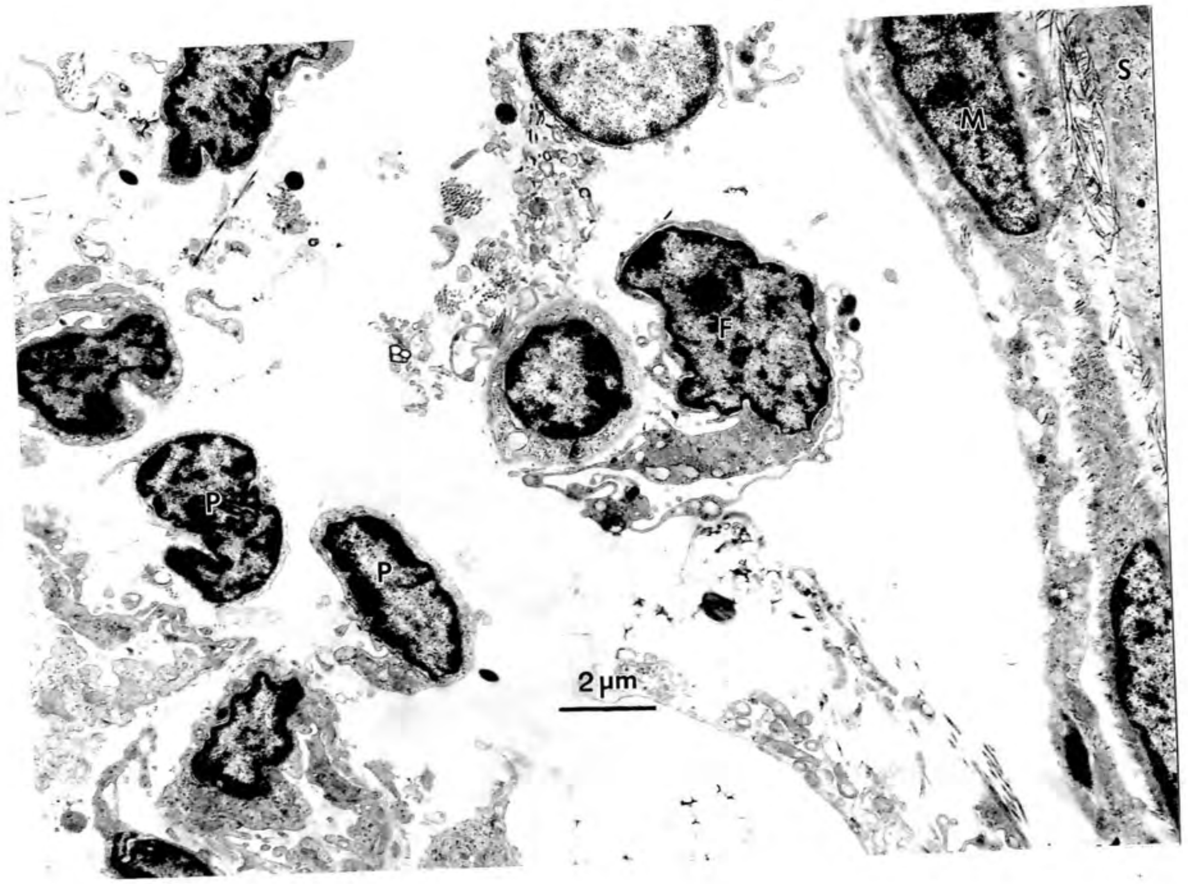


Figure 27 : Electron micrograph of presumptive precursor cells (P) in the interstitium together with a fibroblast (F) and a myoid cell (M) surrouding the seminiferous tubule (S).

4.3 DISCUSSION

Studies on the origin of Leydig cells may be divided into two sections. The first section consists primarily of early light microscopical and early electron microscopical studies on the Leydig cells of numerous species of mammals. The primary aim of these studies was to describe the appearance of Leydig cells. The question of the origin and fate of Leydig cells was, in these studies, generally a proposal based upon morphological similarities with other cells.

The consensus of the histologic literature has been that the adult Leydig cells arise from mesenchymal / fibroblastic cells. The exact nature of these cells remains unknown, however, primarily due to the fact that there appears to be confusion in the terminology that is applied to the primitive cell population.

Based merely upon histologic literature, the following cell types have been proposed as the 'presumptive precursor cell' of Leydig cells :

Undifferentiated, fusiform cells resembling fibroblasts (Hooker, 1948; Mancini et al.,1952; Sniffen, 1952; de la Balze et al., 1954; Fawcett & Burgos, 1960; Niemi & Kormano, 1964; de Kretser, 1967; Connell, 1972; Connell & Christensen, 1975, Kerr & Sharpe, 1985a,b; Kerr et al.; 1987).

Undifferentiated mesenchyme cells (Christensen & Fawcett, 1961; Black & Christensen, 1969; Gondos & Renston, 1974; Gondos et al., 1976; Kuopio et al., 1986).

Mature fibroblasts (Crabo, 1963; Mancini et al., 1963).

Young Leydig cells (Camantini & Franchi, 1981).

Cells in transition between fibrocytes and Leydig cells (Johnson & Neaves, 1981).

Primitive fibroblasts (Prince, 1984).

A second type of Leydig cell (Hadziselimovic, 1977).

Indeterminate connective tissue cells (Kerr et al., 1985).

Macrophages (Morris et al., 1986).

Elongated connective tissue cell (Jackson et al.; 1986a,b).

When studying published light or electron micrographs of these cells, it appears that the cell described, namely the presumptive precursor cell, has similar features throughout the studies, although the cell has been termed differently. Excluding the macrophages, this cell is typically fusiform or spindle - shaped, containing a small volume of cytoplasm, such that the nucleus, occasionally indented, almost fills the entire cell. The cytoplasm contains varying amounts of SER, RER, mitochondria and lipid inclusions. The cell often has short cytoplasmic processes, not to the extent of the fibroblastic extensions. It is important to note, however, that these are the general characteristics of the cell and many variations of these characteristics of the proposed precursor cell exist throughout these studies.

The second section on the Leydig cell life cycle are the studies concerned with the use of exogenous hormones and cytotoxic chemicals in an attempt to elucidate the precursor cell of the Leydig cell. Two types of cytotoxic chemicals and one hormone have been used in these studies.

A number of authors have made use of HCG (human chorionic gonadotropin) in order to elucidate the precursor cells of Leydig cells. HCG has been administered to prepubertal boys in previous studies where it was found to induce a rapid increase in serum testosterone levels. Results from studies using HCG generally observed cells in the interstitium resembling fibroblasts (Nistal & Paniagua, 1979; Christensen & Peacock, 1980; Chemes et al., 1985, Jackson et al., 1986a,b). It has also been suggested that these fibroblastic cells arise from the wall of the seminiferous tubule, acquire the characteristics of Leydig cells and migrate into the interstitium (Chemes et al., 1985), although other authors using the same chemical do not report this.

A single dose of EDS (ethylene-1,2-dimethanesulphonate) disrupts spermatogenesis and causes Leydig cell degeneration. Workers administering this cytotoxic chemical were thus able to note the re-development of the Leydig cell population after degeneration and hence propose a Leydig cell precursor. There appear to be discrepencies in the results obtained from studies using EDS. Several authors suggest that the Leydig cells arise from differentiation of fusiform fibroblast-like cells or cells intermediate between Leydig cells and connective tissue cells

(Kerr et al., 1985; Jackson et al., 1986a,b; Zaidi et al., 1988). Morris et al. (1986), however, suggested that macrophages may be the precursor cell based on similarities between this cell and Leydig cells, although no intermediate cells were identified.

FSH (follicle stimulating hormone) has a stimulatory effect on Leydig cell function by increasing the luteinizing hormone (LH) receptor numbers. Since differentiation of Leydig cells is principally dependant upon LH, it should be possible to determine the precursor cells of Leydig cells. Kerr and Sharpe (1985a) studied the effects of follicle stimulating hormone (FSH) on the Leydig cell population and suggested that Leydig cells arise from spindle-shaped mesenchymal cells at the periphery of the seminiferous tubule.

Several authors have suggested that Leydig cells arise from mitotic division of former Leydig cells, although based upon the number of occasions that mitotic figures have been observed, this seems an unlikely possibility. The presence of mitotic figures has been reported by Chemes et al., (1976), Christensen and Peacock (1980), Kerr and Sharpe (1985a,b) and Kerr et al. (1985) on rare occasions, although Roosen-Runge and Anderson (1959) report frequent mitosis in the Leydig cells of fetal rats.

A third proposal as to the origin of Leydig cells is that after Leydig cell activity, these cells revert to an 'undifferentiated state' after which they redifferentiate back into normal Leydig cells (Sniffen, 1950; Christensen, 1975; Gondos et al., 1977; Payer et al, 1979; Schulze, 1984; Neaves et al, 1985). This suggestion, however, has never been strongly supported, based on the fact that the majority of Leydig cells have been observed undergoing degeneration after the period of activity (Rasmussen, 1917; Fawcett & Burgos, 1960; Mancini et al., 1963; Nishimura & Kondo, 1964; Friedlander et al., 1984; Neaves et al., 1985; Molenaar et al., 1985; Jackson et al., 1986a,b; Kuopio et al., 1986; Almahbobi et al., 1988; Zaidi et al., 1988).

It appears then, that generally both histological studies and studies performed using exogenous hormones and cytotoxic agents are in consensus that Leydig cells arise from a fusiform cell resembling a fibroblastic cell. This is in accord with results from the present study where cells morphologically similar to those described in the above studies were observed. The increase in numbers of these cells between May and January, the period of Leydig cell inactivity, suggests that these cells may be the precursor cells which differentiate into Leydig cells.

The ultimate fate of Leydig cells is a point perhaps of as little consensus in observations and deductions as any other single consideration with respect to these cells.

The majority of workers dealing with this aspect suggest degeneration as the fate of Leydig cells. This process involves vacuolation of the cell, at this stage usually packed with myelin figures (Rasmussen, 1917; Fawcett & Burgos, 1960; Mancini et al., 1963; Nishimura & Kondo, 1964; Friedlander et al., 1984;

Neaves et al., 1985; Molenaar et al., 1985; Jackson et al., 1986a,b; Kuopio et al., 1986; Almahbobi et al., 1988; Zaidi et al., 1988). This is in accord with results from the present study (see results, chapter 3) where it was found that the majority of Leydig cells undergo vacuolation and cytoplasmic dissolution. The cellular membrane eventually disintegrates and the cytoplasmic organelles are released into the interstitial spaces. A final observation is that of a naked nucleus often surrounded by the filamentous extensions of fibroblasts.

A second suggestion as to the fate of Leydig cells is dedifferentiation or reversion to fibroblasts or cells resembling fibroblasts. Other authors term this an 'undifferentiated state' (Rasmussen, 1917; Sniffen, 1950; Christensen, 1975; Gondos et al., 1977; Payer et al., 1979; Schulze , 1984; Neaves et al, 1985). Results from the present study suggest that such dedifferentiation of Leydig cells is unlikely based on the fact that degeneration of these cells was observed.

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