# The prolonged storage and survival of spermatozoa in Chiroptera

## P. A. Racey

*Department of Zoology, University of Aberdeen, Aberdeen AB9 2TN, U.K.*

The prolonged storage of spermatozoa by bats was first reported by Pagenstecher in 1859, and subsequently attracted the attention of several early comparative anatomists and histologists whose work has been thoroughly reviewed by Hartman (1933). Hartman was initially sceptical about the fertilizing capacity of the stored spermatozoa (Hartman & Cuyler, 1927) and it was left to Wimsatt (1942, 1944) to undertake the first critical experiments of isolating inseminated females from males and observing the developing embryo in the reproductive tract after arousal from hibernation in spring. Further work, reviewed by Wimsatt (1969), has shown that sperm storage is one facet of the complex reproductive adaptations of bats living in temperate latitudes where a period of hibernation occurs during prolonged oestrus.

This review aims to provide a concise account of the major contributions to the study of prolonged storage and survival of bat spermatozoa, and where possible to compare the properties of bat spermatozoa with those of other mammals.

### Occurrence of sperm storage amongst Chiroptera

Sperm storage has so far been reported in members of the family Rhinolophidae and the subfamily Vespertilioninae of the family Vespertilionidae, all of which are insectivorous. The phenomenon was long thought to be confined to those species living in temperate latitudes where decreasing ambient temperatures result in a decrease in the insect food supply shortly after copulation. As a result, the bats' daily periods of adaptive hypothermia become extended into prolonged hibernation. Prolonged sperm storage has been reported in members of the following genera living in temperate latitudes (see Hartman, 1933, for references):

Family Vespertilionidae
    subfamily Vespertilioninae
        *Myotis, Pipistrellus, Nyctalus, Eptesicus, Vespertilio, Chalinolobus* (Kitchener, 1975),
        *Plecotus*
Family Rhinolophidae
    *Rhinolophus*

This list has recently been extended to include some tropical vespertilionids. In Central India (at about 21°N) Gopalakrishna & Madhavan (1971) observed that *Pipistrellus ceylonicus* copulates early in June but does not ovulate until the following month. Sperm storage has also been established in *Scotophilus heathi* by Gopalakrishna & Madhavan (1978) in Central India (at about 21°N) and by Krishna & Dominic (1978) in North-East India (at about 25°N). In Malaysia (at about 3°N) Medway (1972) observed that *Tylonycteris pachypus* and *T. robustula* were inseminated in November and December but did not ovulate until early January. Sperm storage has also been established in the neotropical species *Lasiurus ega, Eptesicus furinalis* and *Myotis albescens* studied by Myers (1977) between 22 and 25°S in Paraguay. It seems likely, in view of these reports, that sperm storage may yet be established in members of other tropical genera.

## Duration of sperm storage

In rhinolophids and vespertilionids living in temperate latitudes, spermatogenesis takes place during summer, spermatozoa are released from the testis during August and September and copulation then commences. The earliest insemination recorded in England was on 23 September for a pipistrelle taken in a mist net while feeding (Racey, 1972). Pipistrelles do not appear in their hibernacula until the end of November (Racey, 1973c) so that sperm storage coincides with periods of activity as well as with periods of torpor. Since ovulation occurs in April and May in this species (Potts & Racey, 1971), spermatozoa may be stored in the female reproductive tract for up to 7 months.

After the completion of spermatogenesis, the testes regress but spermatozoa are stored in the cauda epididymidis of males until the following summer. Individual pipistrelles (*Pipistrellus pipistrellus*), with epididymides distended with spermatozoa released from the testes the previous autumn, have been observed during June and July, before the current summer's production of gametes has reached the stage of spermiogenesis (Racey, 1972). Spermatozoa may thus remain in the cauda epididymidis for up to 10 months. However, the duration for which spermatozoa are stored does not necessarily coincide with the duration of their viability or their fertility. Spermatozoa may die or lose their fertilizing capacity and remain in storage organs as a result of the torpor of their hosts and a reduction in the rate of those processes which might otherwise eliminate defective or redundant gametes. In practice it is impossible to distinguish between live and fertile spermatozoa, although if vital dyes such as eosin stain their plasma membranes, then they are assumed to be dead.

Experiments to determine the fertilizing life of spermatozoa stored in the female reproductive tract have been undertaken in only a few species (Table 1), and involve isolating females from males as soon as possible after insemination and recording the subsequent presence of developing embryos or the occurrence of live births.

**Table 1.** The fertilizing life of spermatozoa stored by female bats

| Species | Fertilizing life (days) | Reference |
|---|---|---|
| *Pipistrellus ceylonicus* | 16 | Gopalakrishna & Madhavan (1971) |
| *Tylonycteris pachypus* | 21 | Marshall, A. G.; cited in Medway (1972) |
| *Myotis sodalis* | 68 | Gates (1936) |
| *M. lucifugus* | 138 | Wimsatt (1944) |
| *P. pipistrellus* | 151 | Racey (1973a) |
| *Eptesicus fuscus* | 156 | Wimsatt (1944) |
| *P. abramus* | 175 | Hiraiwa & Uchida (1956) |
| *Nyctalus noctula* | 198 | Racey (1973a) |

The only experiments aimed at determining the fertilizing life of spermatozoa stored by male bats are those of Racey (1973a). Mating was delayed in a captive breeding colony of noctule bats (*Nyctalus noctula*) and females were admitted to males at intervals during winter. Males which had stored spermatozoa for up to 5 months were found to have fathered young born the following summer. To extend the period over which the fertilizing capacity of the stored spermatozoa could be demonstrated, spermatozoa were taken from the cauda epididymidis of male noctules after 7 months storage and inseminated into the uterus of females. The birth of young during the summer following this artificial insemination demonstrated that the spermatozoa had maintained their fertilizing capacity for the period of storage.

Experimental work has thus demonstrated that both male and female bats are able to store fertile spermatozoa for extended periods. Which sex actually stores the spermatozoa destined to

fertilize the eggs varies with the species. Since all female noctules (*Nyctalus noctula*) and pipistrelles (*Pipistrellus pipistrellus*) taken from hibernation are inseminated, it would appear that in these species the burden of sperm storage falls on the female. In other species, the storage of spermatozoa destined to fertilize eggs is shared by both sexes. There have been frequent reports of bats copulating during winter (Wimsatt, 1945; Gilbert & Stebbings, 1958; Stebbings, 1965) and Strelkov (1962) found that an increasing proportion of females of 4 species (*Myotis daubentoni, M. dasycneme, M. mystacinus* and *Plecotus auritus*) were inseminated as hibernation progressed.

Tropical bats store spermatozoa for much shorter periods than those which hibernate. In *Pipistrellus ceylonicus* (Gopalakrishna & Madhavan, 1971) and *Scotophilus heathi* (Gopalakrishna & Madhavan, 1978; Krishna & Dominic, 1978), up to 5 weeks elapse between copulation and ovulation. In *Tylonycteris* this interval may be as long as 2 months (Medway, 1972), and in the neotropical vespertilionids studied by Myers (1977) it varies between 1 and 3 months.

## Sites of storage

The cauda epididymidis and ductus deferens act as sperm stores in bats as in all male mammals, and mammalian epididymal function has been reviewed recently by Hamilton (1977). The possibility also exists that spermatozoa are stored within the testis of *Pipistrellus hesperus*. Cross-sections of seminiferous tubules from overwintering individuals containing few premeiotic germ cells but many spermatozoa are illustrated by Krutzsch (1975: Plate 5, Fig. 24).

**Table 2.** The sites of sperm storage in female bats

| Site | Species | Reference |
|---|---|---|
| Oviduct | *Tylonycteris pachypus, T. robustula* | Racey, Suzuki & Medway (1975) |
| | *Pipistrellus ceylonicus* | P. A. Racey, unpublished |
| | *Chalinolobus gouldii* | Kitchener (1975) |
| | *Rhinolophus hipposideros* | Racey (1975) |
| | *R. ferrumequinum* | Racey (1975) |
| Utero-tubal junction | *Myotis lucifugus* | P. H. Krutzsch, personal communication |
| | *M. daubentoni* | Racey (1975) |
| | *Miniopterus schreibersii fuliginosus* | T. Mori & T. A. Uchida, pers. comm. |
| | *Scotophilus heathi* | Krishna & Dominic (1978); Gopalakrishna & Madhavan (1978) |
| Uterus | *M. nattereri* | Racey (1975) |
| | *P. pipistrellus* | Racey (1975) |
| | *P. abramus* | Hiraiwa & Uchida (1955) |
| | *Nyctalus noctula* | Racey (1975) |

The sites of sperm storage in female bats varies with species (Table 2), although spermatozoa have been observed throughout the reproductive tract in different species, from the periovarian space (Uchida, 1953) to the vagina (Racey, 1975). However, the presence of spermatozoa in any part of the female reproductive tract, such as the vagina, is no indication that they are stored there and it is not always easy to decide which part of the tract fulfils that function. Useful criteria are the identification of special relationships, such as attachment between spermatozoa and their storage organs (see below), or the presence of healthy spermatozoa in the uterine or oviductal glands.

In noctules and pipistrelles the uterus is massively distended with semen. No spermatozoa are found in the oviduct during hibernation and neither the utero-tubal junction nor the cervix appears patent until the time of ovulation. The cervix of overwintering noctules is occluded by connective tissue hypertrophy (Grosser, 1903) and the vagina of pipistrelles also becomes occluded by concentric layers of cornified epithelial cells, the result of continued oestrogen secretion by the theca cells of developing follicles (Pl. 1, Fig. 2). Occasional spermatozoa are also found in the centre of these cornified vaginae. Kitchener (1975) has also described distension of the vagina as a result of extensive cornification in *Chalinolobus gouldii*. He observed spermatozoa in the core of the vaginal plug and refers to it, as well as the uterus and oviduct, as a site of storage. Similarly, workers on horseshoe bats, notably Harrison Matthews (1937) and Gaisler (1966), refer to the vaginal plug as a site of sperm storage. In these bats the plug is formed from the coagulated secretions of the male urethral gland and Racey (1975), as a result of finding spermatozoa in the hollow chamber in the centre of a plug from a greater horseshoe bat to be decapitate and eosinophilic, assumed that their presence there was fortuitous. In histological sections spermatozoa in the homogeneous plugs from lesser horseshoe bats also appear decaptitate (Racey, 1975). The available evidence thus suggests that the vagina is unlikely to be a site of sperm storage. However, the presence of vaginal plugs suggests that monogamous mating systems may obtain in these species and both field (van Heerdt & Sluiter, 1965; Panyutin, 1963) and laboratory observations (Racey & Kleiman, 1969) support this hypothesis for the noctule. In contrast, the vagina of *Myotis lucifugus* is not occluded by a plug, and in this species mating is random and promiscuous (D. W. Thomas, M. B. Fenton & R. M. Barclay, personal communication).

### The morphological relationships between spermatozoa and their storage organs

Whenever appropriate investigations have been carried out, close spatial relationships have been established between the stored spermatozoa and the epithelium lining the storage organs. Racey & Potts (1970) showed that spermatozoa at the periphery of the mass of uterine semen are orientated with their heads towards the uterine epithelium and in contact with the epithelial microvilli (Pl. 1, Fig. 1). Although this orientation may be explained by spermatozoa moving

---

PLATE 1

**Fig. 1.** Spermatozoa orientated towards the uterine epithelium of an overwintering pipistrelle (*P. pipistrellus*). Contact is established between the epithelial microvilli and the plasma membrane of the sperm head. To obtain such micrographs, uteri distended with spermatozoa are immersed in fixative, embedded in celloidin and sectioned at 80 μm. Thick sections are re-embedded serially in araldite and areas are selected for thin sectioning (Racey & Potts, 1970). ×8500.

**Fig. 2.** A longitudinal section of the vagina of a pipistrelle (*P. pipistrellus*) with the cervix at the top. Once formed, the plug of cornified epithelial cells probably constitutes a barrier to further insemination. ×70.

**Fig. 3.** A longitudinal section of the ductus deferens of a pipistrelle (*P. pipistrellus*) at the point where it passes through the ampullary gland. Orientation of spermatozoa towards the epithelium was particularly evident in this region of the ductus deferens. ×400.

**Fig. 4.** Contact between stored spermatozoa and the epididymal epithelium, as illustrated here, was observed most frequently when the tubules were not distended with spermatozoa and the epithelium had become pseudostratified. ×350.

PLATE 1

PLATE 2
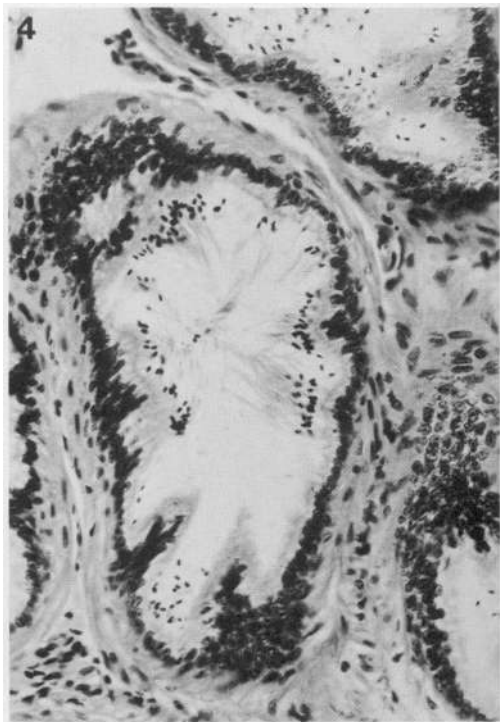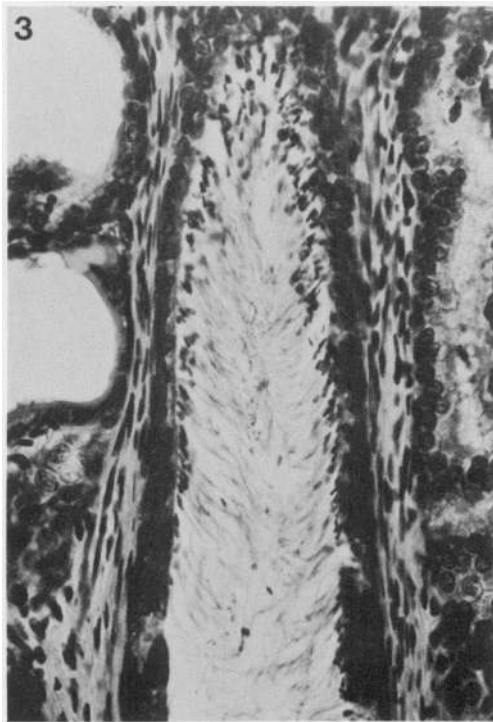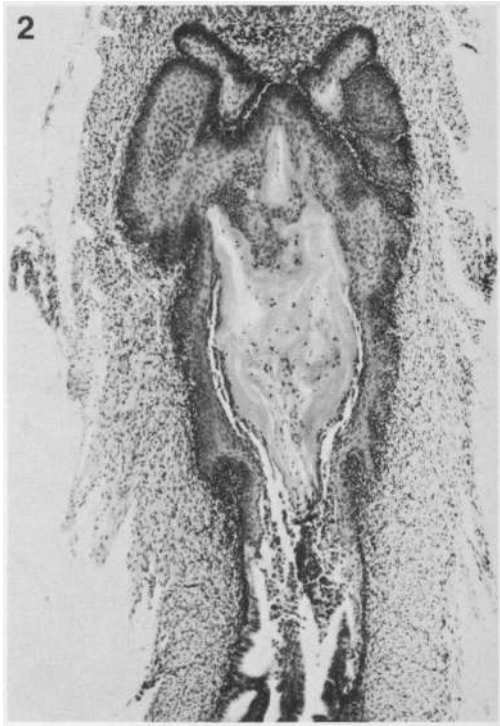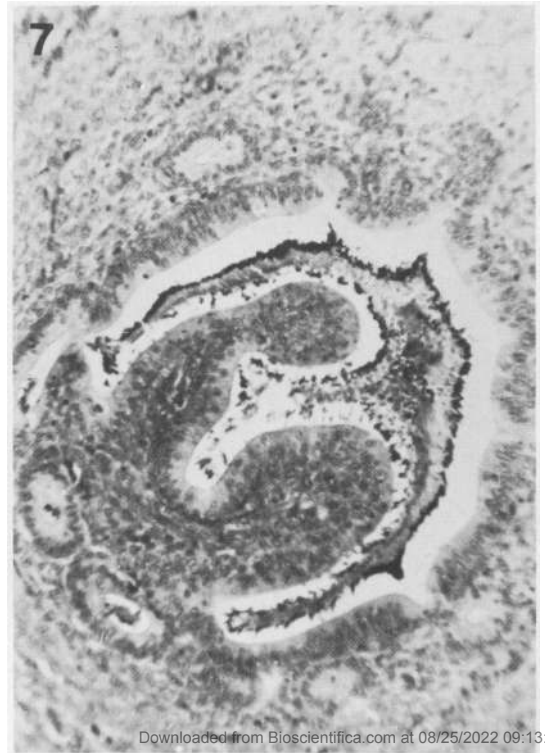
away from an area of high density to one of low density, the effect is to bring the sperm cells into contact with the microvilli of the uterine epithelium, an area where secretory products of the epithelium must be in highest concentration, and where the $Po_2$ must also be highest. A trophic effect on the spermatozoa need not be postulated since the system may be mimicked by enclosing a drop of uterine semen in paraffin oil on a cavity slide: spermatozoa soon become orientated at the paraffin oil–semen interface (Racey, 1975: Plate 2).

Orientation of spermatozoa towards the uterine epithelium has also been observed for *Pipistrellus abramus* with the light microscope (Hiraiwa & Uchida, 1955). Spermatozoa are similarly orientated towards the oviductal epithelium in *Pipistrellus ceylonicus* (A. Gopalakrishna, personal communication) and towards the epithelium of the uterus and uterotubal junction in *Scotophilus heathi* (Pl. 2, Figs 6 and 7; Krishna & Dominic, 1978).

Similar relationships between stored spermatozoa and the oviductal epithelium are apparent in *Tylonycteris pachypus* and *T. robustula* (Racey, Suzuki & Medway, 1975), in which the heads of most spermatozoa are situated in indentations in the luminal surface of the epithelial cells (Pl. 2, Fig. 5). These indentations vary in depth from those which enclose only the acrosome to those which enclose the entire head.

In the male reproductive tract orientation of spermatozoa towards the epithelium of the ductus deferens was found in over half (29/55) the pipistrelles (*P. pipistrellus*) examined (Pl. 1, Fig. 3). Orientation between spermatozoa and the epithelium of the cauda epididymidis was much less common and involved fewer spermatozoa (Racey, 1975). It was found in tubules which were not distended and in which the epithelium had therefore become pseudostratified (Pl. 1, Fig. 4).

The close relationships described above are a consistent and repeatable feature of sperm storage in the bat species mentioned. Similar relationships also occur in other mammalian species, such as the shrew, *Blarina brevicauda* (Pearson, 1944), golden hamster, *Mesocricetus auratus* (Yanagimachi & Chang, 1963), and the bitch, *Canis familiaris* (Marshall & Jolly, 1905; Doak, Hall & Dale, 1967). Sperm heads are also intimately associated with epithelial microvilli in the rabbit vagina (Phillips & Mahler, 1977), although this may be a prelude to phagocytosis since the epithelial cells are frequently filled with vacuoles containing the remains of spermatozoa. However, clear evidence that bat spermatozoa which have established a close relationship with epithelial microvilli may be degenerating has been obtained in the ductus deferens for only one individual pipistrelle (Racey, 1972).

## Sperm metabolism

That bat spermatozoa can survive prolonged storage raises questions regarding their metabolism during this period. If the spermatozoa are metabolically active then the necessary substrates

---

PLATE 2

**Fig. 5.** Sperm heads embedded in indentations in the oviductal epithelium of *Tylonycteris pachypus*. Close apposition occurs between the plasma membranes of the sperm head and the epithelium (arrow). Such attachment anchors the spermatozoa and brings them into close juxtaposition to a source of exogenous nutrients and high $Po_2$. ×40 000.

**Fig. 6.** Stored spermatozoa in the uterus of *Scotophilus heathi*. The orientation of spermatozoa towards the epithelium is apparent, although separation has occurred between them as a result of the preparative techniques.

**Fig. 7.** Stored spermatozoa in the utero-tubal junction of *Scotophilus heathi*. Orientation of spermatozoa towards the epithelium is also apparent.
(Figs 6 and 7 were kindly provided by Mr A. Krishna.)

must be continuously available to them. In his review of sperm metabolism, Harrison (1977) has differentiated between metabolism to provide energy for motility and metabolism for maintaining the integrity of the cells. In all species, energy for sperm motility is provided in the form of adenosine triphosphate (ATP) which is produced as a result of glycolysis and oxidative phosphorylation. In general, all mammalian spermatozoa are able to glycolyse such sugars as glucose, mannose and fructose, and oxidize such substrates as lactate, pyruvate, fatty acids and citric acid cycle intermediates. Since all these substrates are constitutents of body fluids, Harrison (1977) has argued that spermatozoa are always able to acquire sufficient substrates for metabolism from the secretions with which they are in contact. Since there is no reason to suppose that bat spermatozoa differ substantially from those of other mammals, there would not seem to be any obvious problem in the supply of substrates for metabolism.

Glycogen is particularly abundant in the uterine epithelium of pipistrelles during the period of sperm storage (Nakano, 1928, for *Pipistrellus abramus*; Racey, 1975, for *P. pipistrellus*). Although it is much less abundant than in the uterus, the occurrence of glycogen in the epididymal epithelium of pipistrelles is similar in amount and distribution to that recorded in other mammalian species by Nicander (1957). However, mammalian spermatozoa are incapable of glycogenolysis (Mann, 1964) and the polymer would have to be degraded to glycolysable sugars before it would be of use to the spermatozoon. Fructose is present in the uterine plasma of *P. pipistrellus* (Racey, 1975) and may either have originated from epithelial glycogen or have been transferred to the uterus in the seminal plasma. Its presence has been demonstrated in the seminal vesicles of the closely related noctule bat (*Nyctalus noctula*) (Racey, 1974) and also in the ampullary glands of *Scotophilus heathi* (Mokkapati & Dominic, 1976). The presence of sorbitol dehydrogenase in the midpiece of spermatozoa of *Myotis lucifugus* (Wimsatt, 1969) and *P. pipistrellus* (Racey, 1975) also indicates activity of the sorbitol pathway by which glucose is converted to fructose via sorbitol.

Racey (1975) attempted to localize some of the enzymes associated with carbohydrate metabolism in the spermatozoa and epithelia lining the storage organs in *P. pipistrellus*. Phosphorylase could not be found, but this enzyme is also absent from all other mammalian spermatozoa so far examined (Harrison, 1977). Glycogen and alkaline phosphate are often closely associated within cells (Maneely, 1955; Pearce, 1968) and this had led to the suggestion of a metabolic relationship between the two. Although alkaline phosphatase was found in the apical and basal regions of the epididymal epithelial cells in the pipistrelle (as in other mammals; see for example, Malone & Bower, 1962) its occurrence in only occasional cells of the uterine epithelium suggests cellular specialization of that tissue.

Glucose 6-phosphate is a key metabolite, linking the glycolytic pathway and the pentose cycle, and the occurrence of an enzyme hydrolysing this substrate in the acrosome of pipistrelle spermatozoa (Racey, 1975) may be significant, particularly since orientation of spermatozoa towards the endometrium brings the acrosome close to the source of exogenous nutrients.

Much attention has been paid in the past to endogenous substrates for sperm metabolism. Phospholipids in particular have been proposed as an endogenous energy source (Scott, 1973) and these are found primarily in the midpiece mitochondria of mammalian spermatozoa. The numbers of mitochondria recorded by Fawcett & Ito (1965) in the midpiece of *Myotis lucifugus* spermatozoa (Table 3) are, with the exception of some rodents, at the top of the range listed by Fawcett (1970) for mammalian spermatozoa in general. In a comparative study, Uchida & Mori (1972) noted that *Miniopterus schreibersii*, which does not store spermatozoa for a prolonged period, has fewer smaller mitochondria than those bat species which do (Table 3) so that a large volume of mitochondrial phospholipid may be one of the factors contributing to prolonged survival.

Harrison (1977) has criticized the concept that phospholipids act as an energy source and offers an alternative suggestion. Carnitine, which is accumulated by spermatozoa as they pass through the epididymis (Casillas, 1973), functions as a carrier of acetyl and acyl groups in the

**Table 3.** The numbers and sizes of mitochondria
in bat spermatozoa

| Species | No. of mitochondria | Size |
|---|---|---|
| *Myotis lucifugus** | 114–120 | —– |
| *M. nattereri*† | 135 | Large |
| *M. macrodactylus*† | 117 | Large |
| *Pipistrellus abramus*† | 138 | Large |
| *Rhinolophus cornutus*† | 130 | Small |
| *R. ferrumequinum*† | 160 | Small |
| *Miniopterus schreibersii*† | 78 | Small |

* Fawcett & Ito (1965).
† Uchida & Mori (1972).

mitochondria. In the presence of substrates which yield acetyl moieties (such as fructose, lactate and acetate) a large proportion of the available carnitine becomes acetylated (Casillas & Erickson, 1975), and the acetyl groups can subsequently become oxidized via the citric acid cycle. Spermatozoa of several mammalian species can store large quantities of acetyl moieties in this way, and Harrison (1977) suggests that this store may be utilized in the absence of exogenous substrates. Whatever the case, bat spermatozoa do not appear to have evolved any specialized substrates, and neither do they show any substantial structural differences from other spermatozoa. Fawcett & Ito (1965) described the fine structure of epididymal spermatozoa in *Myotis lucifugus* and *Eptesicus fuscus* and these observations were extended by Wimsatt, Krutzsch & Napolitano (1966) to include the uterine spermatozoa of *M. lucifugus*. Although no specific morphological specializations were described which could account for the prolonged survival of spermatozoa, the arrangement of the mitochondria in the midpiece of *Myotis* spermatozoa appears to be unique (Fawcett & Ito, 1965; Fawcett, 1970), in that the end-to-end junctions of the pairs of mitochondria in successive turns are aligned in register along the dorsal and ventral aspects of the midpiece, instead of being randomly aligned as in most mammals. However, the functional significance of this arrangement remains to be established.

A problem very relevant to sperm storage is the maintenance of cellular integrity, particularly the repair of cell membranes and the maintenance of intracellular ion levels. ATP may be required for these processes, but at much lower levels than for motility. More importantly, extra phospholipids may be required to maintain and repair sperm membranes (Harrison, 1977). In this respect it may be relevant that the spermatozoa from those bat species in which prolonged storage occurs contain more mitochondria and hence more phospholipid than do those of other species. Phospholipids have also been identified in the apical region of the epithelial cells of the uterus and epididymis of *Pipistrellus pipistrellus* (Racey, 1975). They may be secreted as such or as free fatty acids, which would then be available for the maintenance and repair of sperm membranes.

Having considered the possible sources of metabolic substrates available to the stored bat spermatozoa, the question still remains: are these cells metabolically active? Within 1 h of a systemic injection of tritiated glucose into hibernating pipistrelles (*P. pipistrellus*), radioactivity was detected in the uterine spermatozoa (Racey, 1975), suggesting that such spermatozoa are metabolically active, although the extent of such activity has yet to be determined.

In those species such as the noctule (*Nyctalus noctula*) and pipistrelles (*P. abramus*, Hiraiwa & Uchida, 1955; *P. pipistrellus*, Racey, 1975) in which the uterus is distended with semen, spermatozoa are at their highest recorded density (Mann, 1964). Thus 5 to 8 × 10⁶ spermatozoa/μl were recorded in the noctule uterus by Schwab (1952) and Hiraiwa & Uchida (1956) record a range from 0·6 to 10·0 × 10⁶ spermatozoa/μl. It is difficult to establish the

extent to which such spermatozoa are motile. Hartman (1967) examined stored oviductal spermatozoa *in vivo* by transillumination and noted that they were motionless, and Hiraiwa & Uchida (1956) reported that the concentration of uterine spermatozoa in *P. abramus* increased during winter and was negatively correlated with their index of motility *in vitro*. Racey (1972) found that spermatozoa stored in the uterus of *P. pipistrellus* are intensely motile (4 on the 0–4 scale of Emmens, 1947) when released from the uterus and exposed to the atmosphere.

The prediction of Redenz (1929) that the motility of pipistrelle spermatozoa was inhibited *in vivo* by their density as well as by the accumulation of carbon dioxide is now well supported by experimental work *in vitro* on the inhibitory effect of high $P_{CO_2}$ on the motility of mammalian spermatozoa (Mann, 1964). Although Redenz (1929) suggested that high $P_{CO_2}$ might obtain in uteri distended with spermatozoa, in those species which store relatively few spermatozoa, measurements in other mammals suggest that the $P_{O_2}$ may be sufficient to support aerobic respiration (Bishop, 1956; Mastroianni & Jones, 1965).

It is interesting to speculate whether the metabolism of bat spermatozoa may be depressed or inhibited to slow down those processes which might otherwise result in ageing and cell deterioration. An inhibitory effect of rat uterine fluid on bovine spermatozoa has been observed under certain conditions (Smith & Klebanoff, 1970; Klebanoff & Smith, 1970), and specific inhibitors such as zinc, which inhibits the metabolism and motility of human spermatozoa, are present in human seminal plasma (Eliasson, 1971; Eliasson, Johnson & Lindholmer, 1971). In the hamster, Morton, Harrigan-Lum, Albagli & Jooss (1974) have shown that spermatozoa removed from the epididymis are immotile until exposed to calcium ions when immediate activation occurs, accompanied by a sudden increase in intracellular cyclic AMP and a simultaneous fall in the high level of intracellular ATP. Similar activation may occur in bat spermatozoa exposed to the changing pattern of secretions that characterize mammalian oviducts and uteri at ovulation (Hamner, 1973; Hutchinson & Holdsworth, 1975).

## Antigenicity of stored spermatozoa

The antigenicity of the reproductive organs of male *Myotis lucifugus* has been evaluated by Hunter, Barker, Johnson, Fahning & Schultz (1971). The antigenic spectrum and cross-reactivity of male reproductive organs did not differ substantially from that reported for species in which the spermatozoa have a limited life-span, although 1 of the 9 proteins detected electrophoretically in the seminal vesicles relaxed smooth muscle, increased the proportion of circulating heterophils and reduced the proportion of circulating lymphocytes. Hunter, Johnson, Barker, Fahning & Schultz (1971) hypothesized that this protein is transferred to the female tract at insemination where its action on smooth muscle may inhibit transport of spermatozoa to the site of fertilization. They also suggest that the protein inhibits leucocytic phagocytosis of stored spermatozoa.

When compared with the massive invasion of leucocytes into the reproductive tract which occurs after insemination in some other mammals (Phillips & Mahler, 1977), the reproductive tracts of sperm-storing bats are remarkably free of leucocytes (Racey, 1975). Guthrie (1933) is alone in reporting large accumulations of such cells. This is in marked contrast to bat species which do not store spermatozoa for long periods such as *Miniopterus schreibersii*, and where the uterus is invaded by leucocytes following copulation (Uchida & Mori, 1974; T. Mori & T. A. Uchida, personal communication).

Mucosubstances have been found on the luminal surfaces of the epithelial cells lining sperm storage organs in pipistrelles (Racey, 1975). Such mucosubstances, which are widely distributed in mammalian reproductive tracts, may have a protective and immunosuppressive function.

### Fertilization and removal of spermatozoa

Uchida (1953) and Uchida & Mori (1975) consider that fertilization in *Pipistrellus abramus* takes place in the periovarian sac and if this is verified it would be exceptional among mammals. Uchida (1953) was impressed by the paucity of spermatozoa in the oviduct and periovarian sac, and Uchida & Mori (1975) recorded 10 spermatozoa in the ampulla of the oviduct. In a study of implantation in *P. pipistrellus*, Potts & Racey (1971) found a fertilized tubal egg, together with several adjacent spermatozoa, but few spermatozoa elsewhere in the oviduct. After ovulation, the removal of the mass of semen from the uterus is remarkably rapid in *P. pipistrellus* and although small numbers of morphologically normal spermatozoa were found in the uterine glands at the time when the developing morula entered the uterus, no leucocytes were observed (Potts & Racey, 1971).

It seems likely that in those species in which the uterus is distended with semen, this is lost by drainage through the cervix (as has been described in the hamster by Yanagimachi & Chang, 1963) once the plug of vaginal cornification has been lost. While catching bats in the field during spring, tiny translucent plugs have occasionally appeared on our hands, and the vulval areas of some bats have appeared damp and matted with liquid with the appearance and viscosity of semen.

Phagocytosis of spermatozoa by the epithelia of storage organs may also be important in removing spermatozoa. Spermatozoa have thus been found in the uterine epithelial cells of *P. pipistrellus* (Racey, 1975) and *Myotis lucifugus* (Wimsatt *et al.*, 1966), and in the oviductal epithelium of *Rhinolophus ferrumequinum*, *P. pipistrellus* (Austin, 1960) and *P. abramus* (Mori & Uchida, 1974).

Gopalakrishna, Phansalkar & Sahasrabudhe (1970) noted that spermatozoa are present in the oviduct and uterus of *P. ceylonicus* for about 4–5 days after fertilization, after which they quickly degenerate, presumably as the environment within the genital tract changes under the influence of luteal hormones. This is supported by their observations on a megachiropteran bat *Pteropus giganteus* in which only one side of the reproductive tract is functional during each pregnancy cycle. All the spermatozoa in the horn ipsilateral to the corpus luteum degenerate soon after its formation, whereas spermatozoa in the contralateral horn remain unaffected until the embryo has reached the neural groove stage. It has been suggested that a portal circulation in *P. giganteus* carries progesterone directly from the corpus luteum to the ipsilateral horn (Marshall, 1953), and this may bring about the changes which are obviously inimical to the stored spermatozoa.

### Origin of sperm storage

For a century mammalian sperm storage was thought to be a reproductive adaptation confined to heterothermic vespertilionids and rhinolophids living in temperate latitudes, where the shortage of insect food during winter necessitated hibernation, and where the reproductive cycles of both male and female bats had become interrupted by this event.

However, in recent years, sperm storage has been established in vespertilionids in India (Gopalakrishna & Madhavan, 1971, 1978; Krishna & Dominic, 1978), Malaysia (Medway, 1972), and the neotropics (Myers, 1977). Medway (1973) has also demonstrated experimentally that *Tylonycteris* can become hypothermic, a condition from which it can spontaneously rewarm, and A. Krishna & C. J. Dominic (personal communication) have observed that *Scotophilus heathi* is naturally torpid during December. It seems, therefore, that sperm storage and heterothermy are closely associated.

The first bats were found in Eocene fossil beds in subtropical latitudes and were insectivorous (Jepsen, 1970). The thermoregulatory ability of contemporary tropical microchiropterans is

highly variable but is generally imprecise (Lyman, 1970). If this is an ancient characteristic, as seems likely, it may have led to the development of adaptive hypothermia and allowed bats to follow their insect food into more temperate latitudes. Whenever falling temperature caused a shortage of insects, the bats could become torpid with the result that reproductive processes slowed or halted. Prolonged food shortage and consequent torpor occurring after insemination may thus have led to a long delay in ovulation and fertilization. Racey (1973b) has demonstrated that adaptive hypothermia can occur even during gestation with no ill effects on the fetus, whose development is slowed or halted.

In all cases for which information is available, insemination among the Chiroptera precedes ovulation. When ovulation in vespertilionids living in temperate latitudes occurs in response to experimental increases in food supply and environmental temperature, the result is an impressive synchrony of births (Racey, 1973b). If such a response also occurs in tropical sperm-storing vespertilionids, then birth synchrony may have been the ultimate cause of sperm storage.

Since it has long been thought to be confined to bats, the discovery of the prolonged fertilizing life of spermatozoa in hares (Martinet & Raynaud, 1975) and mice (Ullman, 1976) is particularly significant because it emphasizes that mammalian spermatozoa in general may not have such a short life-span as has formerly been believed and that changes in their environment are more important than chronological age in the loss of fertilizing capacity. Rather than look for specific physiological adaptations unique to bat spermatozoa to explain their prolonged survival, a better understanding may come from investigating the existence of those mechanisms (Restall, 1967; Mann, 1968; see also review by Hutchinson & Holdsworth, 1975) which assure the survival of spermatoza in the genital tracts of mammals in general.

## References

Austin, C.R. (1960) Fate of spermatozoa in the female genital tract. *J. Reprod. Fert.* **1**, 151–156.

Bishop, D.W. (1956) Oxygen concentration in the rabbit genital tract. *Proc. 3rd Int. Congr. Anim. Reprod., Cambridge* Section 1, p. 53.

Casillas, E.R. (1973) Accumulation of carnitine by bovine spermatozoa during maturation in the epididymis. *J. biol. Chem.* **248**, 8227–8232.

Casillas, E.R. & Erickson, B.J. (1975) The role of carnitine in spermatozoa metabolism: substrate induced elevations in the acetylation state of carnitine and coenzyme A in bovine and monkey spermatozoa. *Biol. Reprod.* **12**, 275–283.

Doak R.L., Hall, A. & Dale, H.E. (1967) Longevity of spermatozoa in the reproductive tract of the bitch. *J. Reprod. Fert.* **13**, 51–58.

Eliasson, R. (1971) Inhibitory effects of human seminal plasma on the oxygen consumption of mitochondria. *Life Sci.* **10**, 407–409.

Eliasson, R., Johnson, O. & Lindholmer, C. (1971) Effects of zinc on human sperm respiration. *Life Sci.* **10**, 1317–1320.

Emmens, C.W. (1947) The motility and viability of rabbit spermatozoa at different hydrogen ion concentrations. *J. Physiol., Lond.* **106**, 471–481.

Fawcett, D.W. (1970) A comparative view of sperm ultrastructure. *Biol. Reprod.* **2**, 90–127.

Fawcett, D.W. & Ito, S. (1965) The fine structure of bat spermatozoa. *Am. J. Anat.* **116**, 567–610.

Gaisler, J. (1966) Reproduction in the lesser horseshoe bat (*Rhinolophus hipposideros hipposideros* Bechstein, 1800). *Bijdr. Dierk.* **36**, 45–64.

Gates, W.H. (1936) Keeping bats in captivity. *J. Mammal.* **17**, 268–273.

Gilbert, O. & Stebbings, R. (1958) Winter roosts of bats in West Suffolk. *Proc. zool. Soc. Lond.* **131**, 329–333.

Gopalakrishna, A. & Madhavan, A. (1971) Survival of spermatozoa in the female genital tract of the Indian vespertilionid bat, *Pipistrellus ceylonicus chrysothrix* (Wroughton). *Proc. Indian Acad. Sci. B* **73**, 43–49.

Gopalakrishna, A. & Madhavan A. (1978) Viability of inseminated spermatozoa in the Indian vespertilionid bat *Scotophilus heathi* (Horsefield). *Indian J. exp. Biol.* **16**, 852–854.

Gopalakrishna, A., Phansalkar, R.B. & Sahasrabudhe, J.D. (1970) Degeneration of the inseminated spermatozoa after ovulation in two species of Indian bats. *Curr. Sci.* **39**, 489–490.

Grosser, O. (1903) Die physiologische bindegewebige Atresie des Genitalkanales von *Vesperugo noctula* nach erfolgter Kohabitation. *Verh. anat. Ges. Jena* **17**, 129–132.

Guthrie, M.J. (1933) Notes on the seasonal movements and habits of some cave bats. *J. Mammal.* **14**, 1–19.

Hamner, C.E. (1973) Oviducal fluid—composition and physiology. In *Handbook of Physiology*, Section 7, Volume 2, Female Reproductive System, pp. 141–151. Ed. R. O. Greep, American Physiological Society, Washington D.C.

Hamilton, D.W. (1977) The epididymis. In *Frontiers in Reproduction and Fertility Control*, Part 2, pp. 411–426. Eds R. O. Greep & M. A. Koblinsky. MIT, Cambridge, Massachusetts.

**Harrison, R.A.P.** (1977) The metabolism of mammalian spermatozoa. In *Frontiers in Reproduction and Fertility Control*, part 2, pp. 379–401. Eds R. O. Greep & M. A. Koblinsky. MIT, Cambridge, Massachusetts.

**Harrison Matthews, L.** (1937) The female sexual cycle in the British bats *Rhinolophus ferrumequinum insulanus* Barrett-Hamilton and *R. hipposideros minutus* Montagu. *Trans. Zool. Soc. Lond. B* **23**, 224–255.

**Hartman, C.G.** (1933) On the survival of spermatozoa in the female genital tract of the bat. *Q. Rev. Biol.* **8**, 185–193.

**Hartman, C.G.** (1967) Research should spell FUN. *J. Reprod. Fert., Suppl.* **2**, 1–10.

**Hartman, C.G. & Cuyler, W.K.** (1927) Is the supposed long life of the bat spermatozoa fact or fable? *Anat. Rec.* **35**, 39.

**Hiraiwa, Y.K. & Uchida, T.** (1955) Fertilization in the bat *Pipistrellus abramus abramus* (Temminck). II. On the properties of semen stored in the uterus. *Sci. Bull. Fac. Agric. Kyushu Univ.* **30**, 255–266.

**Hiraiwa, Y.K. & Uchida, T.** (1956) Fertilization capacity of spermatozoa stored in the uterus after the copulation in the fall. *Sci. Bull. Fac. Agric. Kyushu Univ.* **31**, 565–574.

**Hunter, A.G., Barker, L.D.S., Johnson, W.L., Fahning, M.L. & Schultz, R.H.** (1971) Antigenicity, toxicity, and cross reactions of male bat (*Myotis lucifugus*) reproductive organs. *J. Reprod. Fert.* **24**, 171–177.

**Hunter, A.G., Johnson, W.L., Barker, L.D.S., Fahning, M.L. & Schultz, R.H.** (1971) Bat seminal vesicle protein: its characterization and physiological properties. *J. Reprod. Fert.* **24**, 179–186.

**Hutchinson, J.S.M. & Holdsworth, R.J.** (1975) Bibliography (with review) on the uterine environment. *Biblphy Reprod.* **26**, 117 *et seq.*

**Jepsen, G. L.** (1970) Bat origins and evolution. In *The Biology of Bats*, Vol. 1, pp. 1–64. Ed. W. A. Wimsatt. Academic Press, New York.

**Kitchener, D.J.** (1975) Reproduction in female Gould's Wattled Bat *Chalinolobus gouldii* (Gray) (Vespertilionidae) in Western Australia. *Aust. J. Zool.* **23**, 29–42.

**Klebanoff, S.J., & Smith, D.C.** (1970) The source of $H_2O_2$ for the uterine fluid-mediated sperm-inhibitory system. *Biol. Reprod.* **3**, 236–242.

**Krishna, A. & Dominic, C.J.** (1978) Storage of spermatozoa in the female genital tract of the Indian vespertilionid bat, *Scotophilus heathi* Horsefield. *J. Reprod. Fert.* **54**, 319–321.

**Krutzsch, P.H.** (1975) Reproduction of the Canyon bat, *Pipistrellus hesperus*, in south-western United States. *Am. J. Anat.* **143**, 163–200.

**Lyman, C.P.** (1970) Thermoregulation and metabolism in bats. In *The Biology of Bats*, Vol. 1, pp. 301–330. Ed. W. A. Wimsatt. Academic Press, New York.

**Malone, T.E. & Bower, M.C.** (1962) Regional localization of polysaccharide complexes and alkaline phosphate in the hamster epididymis. *Amer. Zool.* **2**, 427–428, Abstr.

**Maneely, R.B.** (1955) The distribution of polysaccharide complexes and of alkaline glycerophosphatase in the epididymis of the rat. *Acta anat.* **24**, 314–329.

**Mann, T.** (1964) *The Biochemistry of Semen and of the Male Reproductive Tract.* Methuen, London.

**Mann, T.** (1968) Biochemical aspects of gamete survival in the male and female genital tract. *Proc. 6th Int. Congr. Anim. Reprod. & A. I., Paris* **1**, 3–30.

**Marshall, A.J.** (1953) The unilateral endometrial reaction in the giant fruit-bat (*Pteropus giganteus* Brunnich). *J. Endocr.* **9**, 42–44.

**Marshall, F.H.A. & Jolly, W.A.** (1905) Contributions to the physiology of mammalian reproduction 1. The oestrous cycle in the dog. *Phil. Trans. R. Soc. Ser. B* **198**, 99–122.

**Martinet, L. & Raynaud, F.** (1975) Prolonged spermatozoa survival in the female hare uterus: explanation of superfetation. In *The Biology of Spermatozoa: Transport, Survival and Fertilizing Capacity*, pp. 134–144. Eds E. S. E. Hafez & C. G. Thibault. Karger, Basel.

**Mastroianni, L. & Jones, R.** (1965) Oxygen tension within the rabbit fallopian tube. *J. Reprod. Fert.* **9**, 99–102.

**Medway, Lord** (1972) Reproductive cycles of the flat-headed bats *Tylonycteris pachypus* and *T. robustula* (Chiroptera: Vespertilionidae) in a humid equatorial environment. *Zool. J. Linn. Soc.* **51**, 33–61.

**Medway, Lord** (1973) Hypothermia in flat-headed bats (Chiroptera, Vespertilionidae: genus *Tylonycteris*). *Mammalia* **37**, 646–653.

**Mokkapati, S. & Dominic, C.J.** (1976) Sites of production of fructose and citric acid in the accessory reproductive glands of three species of male chiropterans. *Biol. Reprod.* **14**, 627–629.

**Mori, T. & Uchida, T.A.** (1974) Electron microscopic analysis of the mechanism of fertilization in Chiroptera. II. Engulfment of spermatozoa by epithelial cells of the Fallopian tube in the Japanese house bat *Pipistrellus abramus. Zool. Mag.*, Tokyo **83**, 163–170.

**Morton, B., Harrigen-Lum, J., Albagli, L. & Jooss, T.** (1974) The activation of motility in quiescent hamster sperm from the epididymis by calcium and cyclic nucleotides. *Biochem. biophys. Res. Commun.* **56**, 372–379.

**Myers, P.** (1977) Patterns of reproduction of four species of vespertilionid bats in Paraguay. *Univ. Calif. Publ. Zool.* **107**, 1–41.

**Nakano, O.** (1928) Uber die Verteilung des Glykogens bei den zyklischen Veranderungen in den Geschlechts-organen der Fledermaus. Und uber die Nahrungsaufnahme der Spermien in den weiblichen Geschlechtswege. *Folia Anat. Jap.* **6**, 777–828.

**Nicander, L.** (1957) On the regional histology and cytochemistry of the ductus deferens in rabbits. *Acta morph. neerl.-scand.* **1**, 99–118.

**Pagenstecher, H.A.** (1859) Uber die Begattung von *Vesperugo pipistrellus. Verh. naturh.-med. Ver. Heidelb.* **1**, 194–195.

**Panyutin, K.K.** (1963) Reproduction in the common noctule. *Uchen. Zap. mosk. Obl. Pedagog. Inst.* **126**, 63–66. (In Russian: from Biol. Abstr. (1965) **46**, 21, No. 96256).

**Pearse, A.G.E.** (1968) *Histochemistry, Theoretical and Applied*, Vol. 1. Churchill, London.

**Pearson, O.P.** (1944) Reproduction in the shrew (*Blarina brevicauda* Say). *Am. J. Anat.* **75**, 39–93.

Phillips, D.M. & Mahler, S. (1977) Phagocytosis of spermatozoa by the rabbit vagina. *Anat. Rec.* **189**, 61–72.

Potts, D.M. & Racey, P.A. (1971) A light and electron microscope study of early development in the bat *Pipistrellus pipistrellus. Micron* **2**, 322–348.

Racey, P.A. (1972) *Aspects of reproduction in some heterothermic bats.* Ph.D. thesis, University of London.

Racey, P.A. (1973a) The viability of spermatozoa after prolonged storage by male and female European bats. *Period. Biol.* **75**, 201–205.

Racey, P.A. (1973b) Environmental factors affecting the length of gestation in heterothermic bats. *J. Reprod. Fert., Suppl.* **19**, 175–189.

Racey, P.A. (1973c) The time of onset of hibernation in pipistrelle bats (*Pipistrellus pipistrellus*). *J. Zool., Lond.* **171**, 465–467.

Racey, P.A. (1974) The reproductive cycle in male noctule bats, *Nyctalus noctula. J. Reprod. Fert.* **41**, 169–182.

Racey, P.A. (1975) The prolonged survival of spermatozoa in bats. In *The Biology of the Male Gamete*, pp. 385–416. Eds J. G. Duckett & P. A. Racey. Academic Press, London.

Racey, P.A. & Kleiman, D.G. (1969) Observations on noctule bats breeding in captivity. *Lynx* **10**, 65–77.

Racey, P.A. & Potts, D.M. (1970) Relationship between stored spermatozoa and the uterine epithelium in the pipistrelle bat (*Pipistrellus pipistrellus*). *J. Reprod. Fert.* **22**, 57–63.

Racey, P.A., Suzuki, F. & Medway, Lord (1975) The relationship between stored spermatozoa and the oviducal eptithelium in bats of the genus *Tylonycteris*. In *The Biology of Spermatozoa: Transport, Survival and Fertilising Capacity*, pp. 123–133. Eds E. S. E. Hafez & C. G. Thibault. Karger, Basel.

Redenz, E. (1929) Das Verhalten der Saugetierspermatozoen zwischen Begattung und Befruchtung. *Z. Zellforsch. mikrosk. Anat.* **9**, 734–749.

Restall, B.J. (1967) The biochemical and physiological relationships between the gametes and the female reproductive tract. *Adv. Rep. Physiol.* **2**, 181–212.

Schwab, H. (1952) Beobachtungen uber die Begattung und die Spermakonservierung in den Geschlechtsorganen bei weiblichen Fledermausen. *Z. mikrosk.-anat. Forsch.* **58**, 326–357.

Scott, T.W. (1973) Lipid metabolism of spermatozoa. *J. Reprod. Fert., Suppl.* **18**, 65–76.

Smith, D.C. & Klebanoff, S.J. (1970) A uterine fluid-mediated sperm-inhibitory system. *Biol. Reprod.* **3**, 229–235.

Stebbings, R.E. (1965) Observations during sixteen years on winter roosts of bats in West Suffolk. *Proc. Zool. Soc. Lond.* **144**, 137–143.

Strelkov, P. (1962) The peculiarities of reproduction in bats (Vespertilionidae) near the northern border of their distribution. *Int. Symp. Meth. Mammal. Invest. Brno*, pp. 306–311. Praha.

Uchida, T. (1953) Studies on the embryology of the Japanese house bat, *Pipistrellus tralatitius abramus* (Temminck). II. From the maturation of the ova to the fertilization, especially on the behaviour of the follicle cells at the period of fertilization. *Sci. Bull. Fac. Agric. Kyushu Univ.* **14**, 153–168.

Uchida, T.A. & Mori, T. (1972) Electron microscope studies on the fine structure of germ cells in Chiroptera. 1. Spermiogenesis in some bats and notes on its phylogenetic significance. *Sci. Bull. Fac. Agric. Kyushu Univ.* **26**, 399–418.

Uchida, T.A. & Mori, T. (1974) Electron microscopic analysis of the mechanism of fertilization in Chiroptera. 1. Acrosomal reaction and consequences to death of the sperm in the Japanese long-fingered bat, *Miniopterus schreibersii fuliginosus. Sci. Bull. Fac. Agric. Kyushu Univ.* **28**, 177–184.

Uchida, T.A. & Mori, T. (1975) Cell movement in male gametes. *Iden* (Genetics) **29**, 6–16 (in Japanese).

Ullman, S.L. (1976) Anomalous litters in hybrid mice and the retention of spermatozoa in the female tract. *J. Reprod. Fert.* **47**, 13–18.

van Heerdt, P.F. & Sluiter, J.W. (1965) Notes on the distribution and behaviour of the noctule bat (*Nyctalus noctula*) in the Netherlands. *Mammalia* **29**, 463–477.

Wimsatt, W.A. (1942) Survival of spermatozoa in the female reproductive tract of the bat. *Anat. Rec.* **83**, 299–305.

Wimsatt, W.A. (1944) Further studies on the survival of spermatozoa in the female reproductive tract of the bat. *Anat. Rec.* **88**, 193–204.

Wimsatt, W.A. (1945) Notes on breeding behaviour, pregnancy and parturition in some vespertilionid bats of the Eastern United States. *J. Mammal.* **26**, 23–33.

Wimsatt, W.A. (1969) Some interrelations of reproduction and hibernation in mammals. *Symp. Soc. exp. Biol.* **23**, 511–549.

Wimsatt, W.A., Krutzsch, P.H. & Napolitano, L. (1966) Studies on sperm survival mechanisms in the female reproductive tract of hibernating bats. 1. Cytology and ultra-structure of intra-uterine spermatozoa in *Myotis lucifugus. Am. J. Anat.* **119**, 25–60.

Yanagimachi, R. & Chang, M.C. (1963) Infiltration of leucocytes into the uterine lumen of the golden hamster during the oestrous cycle and following mating. *J. Reprod. Fert.* **5**, 389–396.