The precipitated aglycone was separated by filtration. The aqueous solution was neutralised ($BaCO_3$), filtered and evaporated under reduced pressure to a syrup which was found to contain rhamnose by paper cochromatography with an authentic sample and osazone formation, m.p. 191°. Quantitative analysis[6] revealed the presence of 1 mole of sugar.

The aglycone was purified by paper chromatography and crystallised from EtOAc: petroleum ether mixture as yellow needles, m.p. 110° (d). The homogeneity was checked by TLC and PC in different organic solvent systems. λ_{max}^{EtOH} 290, 330 (sh) nm; $\lambda_{max}^{EtOH+AlCl_3}$ 288 nm; $\lambda_{max}^{EtOH+NaOAc}$ 323 nm.; ν_{max}^{KBr} 1020, 1120, 1170, 1205, 1265, 1340, 1360, 1420, 1450, 1500, 1550, 1600, 1680, 2870, 2920 and 3400 cm^{-1} (Found: C, 63·55; H, 4·62; OCH_3, 10·25; $C_{16}H_{14}O_6$ requires; C, 63·57; H, 4·63. $-OCH_3$; 10·26%). Acetyl derivative prepared by Ac_2O/Py method and crystallised from acetone: methanol as pale yellow needles melted at 130–35° (d) and did not give any colour with $FeCl_3$ (Found; C, 61·67; H, 4·66; acetyl, 30·14; $C_{22}H_{20}O_9$ requires; C, 61·68; H, 4·67; 3 × acetyl, 30·14%).

Demethylation of the Aglycone

The aglycone (150 mg) in glacial acetic acid (10 ml) was refluxed with 50% HBr (10 ml) for 10 h and worked up as usual. The resultant product was crystallised from EtOAc: Petroleum ether 1 : 1 v/v) as light yellow needles, m.p. 267–68°; λ_{max}^{EtOH} 290 nm; $\lambda_{max}^{EtOH+AlCl_3}$ 315 nm; $\lambda_{max}^{EtOH+NaOAc}$ 325 nm; R_f 0·85 in butanol: 27% gl. ACOH; (1 : 1 v/v). It was identified as eriodictyol by spectral means, colour reactions and degradation studies.

NMR Spectrum of the Aglycone

It was recorded at 100 MHz; $(CD_3)_2CO$; −60° C; signals at τ, 7·21 (d, Jgem 17Hz), 3H (eq); 6·70 (q Jgem 2Hz), 3H (ax); 6·20 (s), OMe; 2·91 (s) 6 and 8–H; 3·20 (5′–H); 2·2 (2′ and 6′–H).

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ADENOSINE TRIPHOSPHATASE ACTIVITY IN THE SPERMATOGENIC AND ANDROGENIC COMPONENTS OF THE TESTIS OF *TAPHOZOUS LONGIMANUS* HARDWICKE (MICROCHIROPTERA : MAMMALIA)

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ABSTRACT

Histochemical site and distribution of adenosine triphosphatase (ATPase) activity in the spermatogenic and androgenic cells of the testis of sexually mature males of an insectivorous Microchiroptera, *Taphozous longimanus* Hardwicke exhibited differences of intensity. It is suggested that varying levels of activity of this enzyme represent the metabolic levels of testicular cell populations and energy requirements in relation to the transport of chemicals, secretion, meiosis and process of differentiation and maturation during spermatogenesis. ATPase may also serve as one of the "link" enzymes which regulate the catalysis of spermatozoal fructolysis and glycolysis.

HISTOCHEMICAL and biochemical evidence indicates that cellular specialisation during spermatogenesis is accompanied by molecular individualisation. Enzyme studies have offered new insight into the biochemical changes associated with meiosis, differentiation and maturation in the mammalian testes[1–9]. Once the profile of various testicular enzymes is known, it would be possible to use them as "finger prints" for elucidating control and regulatory mechanism(s) of spermatogenesis. However, despite their worldwide distribution, unique aerial life and curious reproductive mechanisms, there is no study on testicular

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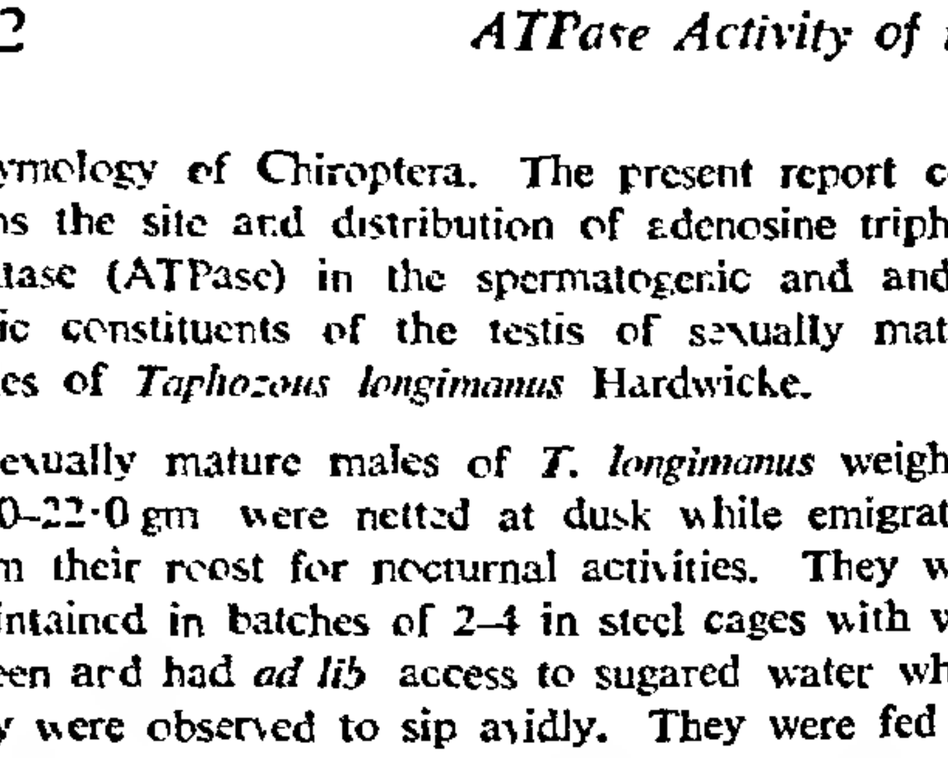
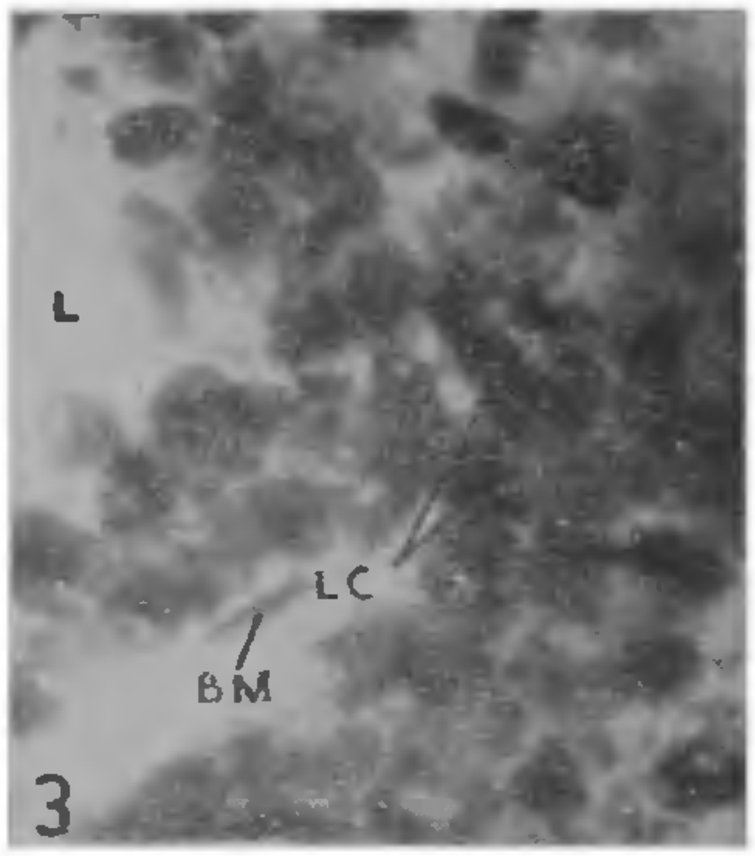
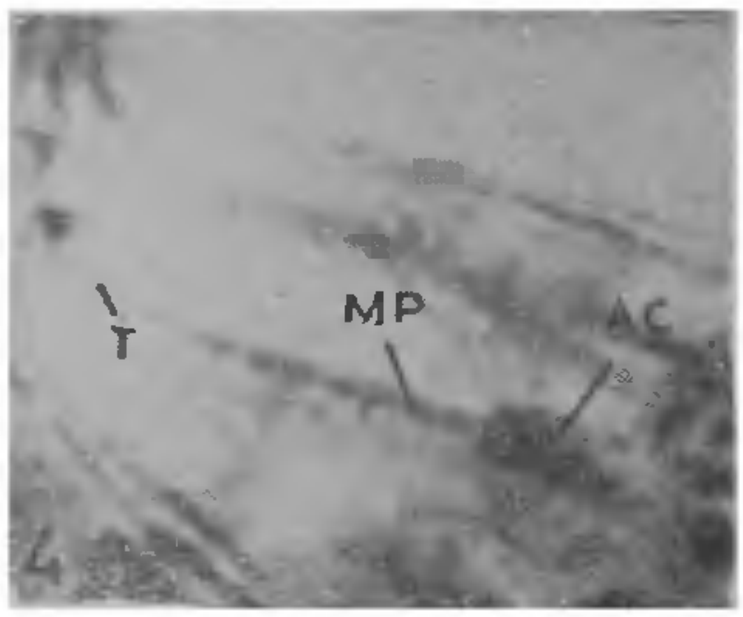
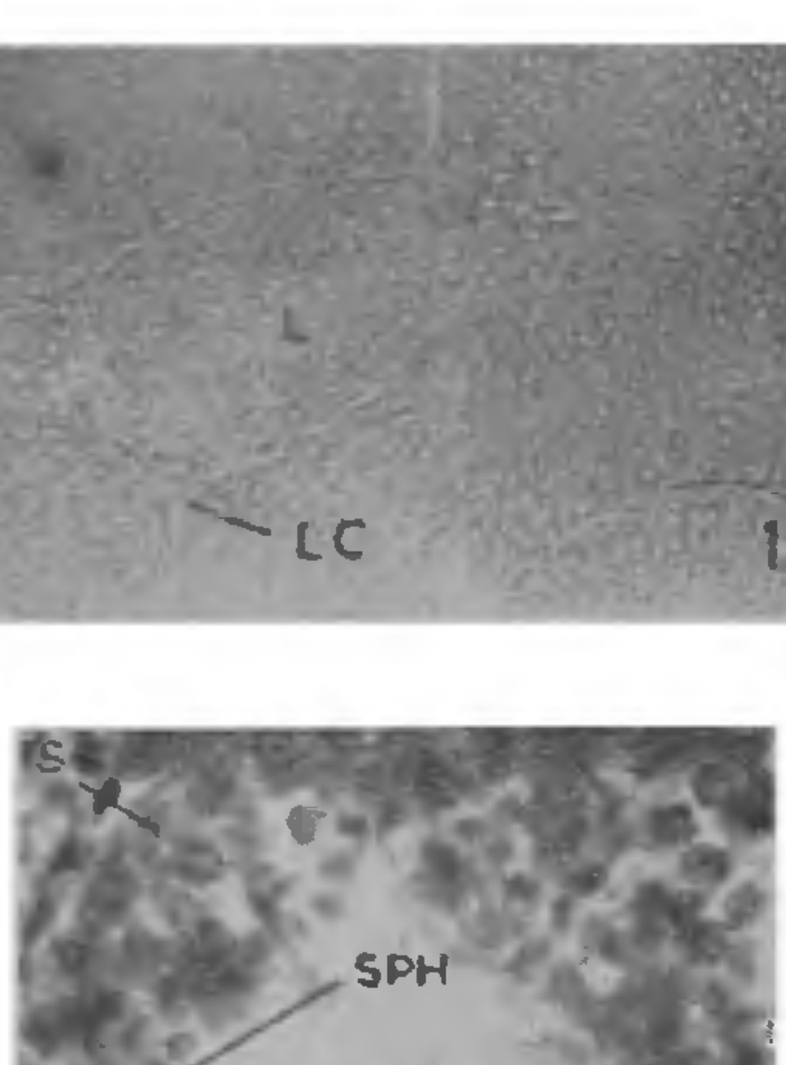
enzymology of Chiroptera. The present report concerns the site and distribution of adenosine triphosphatase (ATPase) in the spermatogenic and androgenic constituents of the testis of sexually mature males of *Taphozous longimanus* Hardwicke.

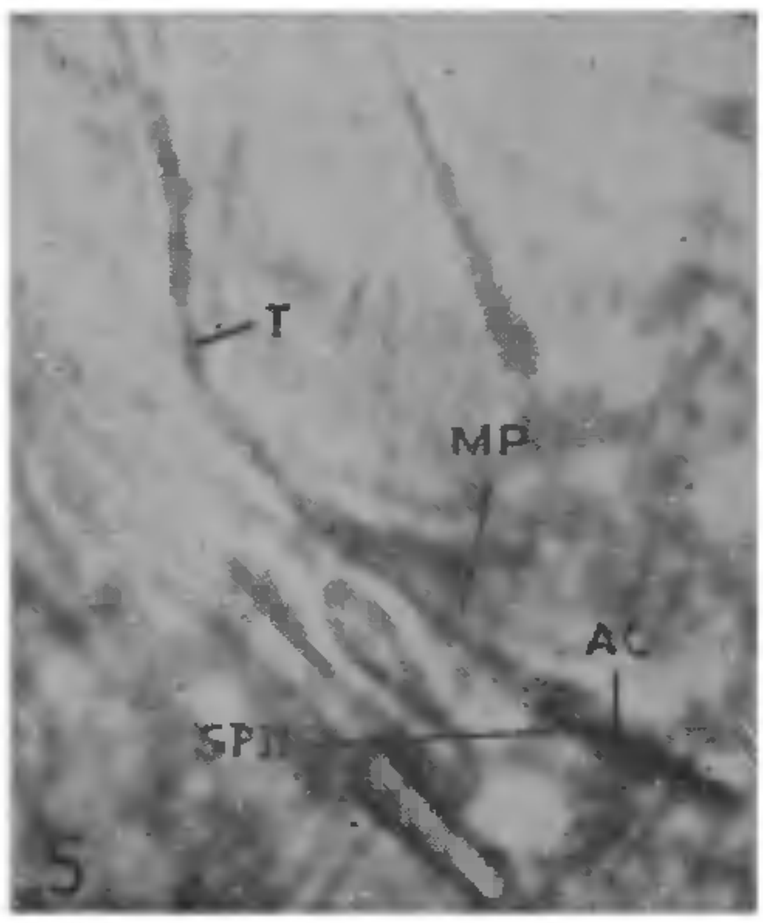
Sexually mature males of *T. longimanus* weighing 20·0–22·0 gm were netted at dusk while emigrating from their roost for nocturnal activities. They were maintained in batches of 2–4 in steel cages with wire screen and had *ad lib* access to sugared water which they were observed to sip avidly. They were fed on crushed roaches.











FIGS. 1–5. Fig. 1. ATPase activity in the testis of *Taphozous longimanus*. Control section (10 μM) of testis showing negative enzyme reaction (100 × enlarged). Figs. 2–3. ATPase activity in the spermatogenic and androgenic elements of the testes 400 × enlarged) (Note the differential ATPase activities). Figs. 4–5. Dimorphic spermatozoa showing enzyme reactions in the head and mitochondrial segments (1000 ×).

(SG = spermatogonia; SPH = sperm head; SPT =sperm tail; PRS = spermatocytes; LC = Leydig cells; AC = Acrosome; T = Tail; MP = Mid-piece; BM = Basal membrane; L = Lumen.)

The animals were sacrificed by cervical dislocation and the testes were quickly removed surgically under aseptic conditions. They were fixed in chilled neutral formalin (10% at 4° C). Frozen sections were cut at 10 μM. ATPase was locallsed using the disodium salt of ATPase substrate according to the method of Padykula and Herman[10]. Control sections were incubated in substrate free media, but otherwise processed identically.

The testes of *T. longimanus* showed the presence of spermatogonia, spermatocytes, spermatids and mature sperms (Fig. 2). The dimorphic spermatozoa are aligned in a characteristic manner with the tail oriented towards the lumen of the seminiferous tubule and the head towards the periphery (Figs. 4–5). The interstitial space between the seminiferous tubules is prominently occupied by Leydig cells (Fig. 2). The cellular constituents of seminiferous tubules indicated variable intensities of ATPase activity (Figs. 2–3). The basement membrane of the testes displayed intense ATPase reaction. Similar pattern of activity was discerned in the nuclear membrane of the spermatogonia and the spermatocytes (Figs. 2 and 3). The enzyme activity in the spermatids was much more strong as compared to spermatogonia and spermatocytes. The sertoli cells showed moderate ATPase reaction. The mitochondrial elements and the sperm head gave strongly positive ATPase activity (Figs. 4 and 5). Leydig cells were also ATPase positive.

Control sections were ATPase negative (Fig. 1).

The present study highlights the histochemical site and distribution of ATPase in the spermatogenic and androgenic cells of the testis of *T. longimanus*. The differential ATPase activity in these cells may be related to variable energy requirements of these cells. Thus, intense ATPase activity in spermatids (Figs. 3 and 4) is probably a sign of high energy requirements for spermatogenesis. Varying patterns of ATPase activity in spermatogonial cells population may be involved with differential rates of secretion, growth and differentiation. The activity of this enzyme in the head and mitochondrial segment of the spermatozoa may be of considerable importance in metabolic functions of the male gamete conducive to survival and maintaining functional abilities. ATPase may thus play an important 'link' in the chain of regulatory enzymes which catalyze spermatozoal fructolysis and glycolysis. This is in consonance with the earlier reports on mammalian sperm metabolism[10–12]. In view of the total lack of information on the testicular enzymology of Chiroptera, we compare our results with other mammals. Our results are at considerable variance with the reports on *Hemiechinus* and house shrew in which nearly uniform ATPase activity has been observed[7–8]. It seems that species specific differences exist in mammals in relation to enzyme profiles in the testis. Their bearing on duration and kinetics of spermatogenesis may be an interesting speculation.

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