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A COMPARISON OF ACID PHOSPHATASE ACTIVITY IN THE SPERMATOGENIC AND ANDROGENIC CELLS OF THE TESTES OF IMPUBERAL AND SEXUALLY COMPETENT *PTEROPUS GIGANTEUS GIGANTEUS* BRÜNNICH (MEGACHIROPTERA: MAMMALIA)

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ABSTRACT

A comparison of the acid phosphatase (AcPase) activity in the spermatogenic and androgenic cells of the impuberal and spermatogenically active testes of *Pteropus giganteus giganteus* displayed characteristic differences. In the impuberal testis, intense AcPase reaction was discerned in the Leydig cells but relatively less enzyme reaction was manifested by cells of the seminiferous epithelium. Spermatogonia, spermatocytes, spermatids, sertoli and Leydig cells of spermatogenically active testis displayed positive but varying AcPase staining. The acrosome of the dimorphic spermatozoa presented positive enzyme reaction. The role of AcPase in relation to growth, cell division and differentiation is discussed. It is suggested that AcPase may be involved in potentiating the male gamete to overcome egg membrane barriers and facilitate at least some of the early steps in the intricate process of fertilisation.

IN mammals, spermatogenesis becomes established sometimes after birth. The factors which initiate, sustain and facilitate completion of spermatogenic and androgenic activities in the testes have been well investigated. Metabolic enzymes have been implicated in the growth, cell division and differentiation of testicular cells and their activities[1–4]. However, very little is known about the comparative enzymatic pattern of spermatogenic and androgenic cells of impuberal and sexually competent males[5–6]. The literature provides very little information with respect to Chiroptera[7–9].

The present study was aimed to determine the histochemical site and pattern of distribution of acid phosphatase (AcPase)—a lysosomal 'marker' enzyme in the seminiferous epithelial cells and Leydig cells of the testis of impuberal and sexually competent *Pteropus giganteus giganteus*.

MATERIAL AND METHODS

Males of *P. g. giganteus* were trapped/shot throughout the year from their roosting sites on mango, guava and date palm trees. Animals with undescended (inguinal), small sized and aspermatogenic testis were considered to denote impuberal state. This was confirmed by histological examination. Males with scrotal, deeply pigmented, large-sized and spermatogenically active testes were accepted as mature and sexually competent individuals.

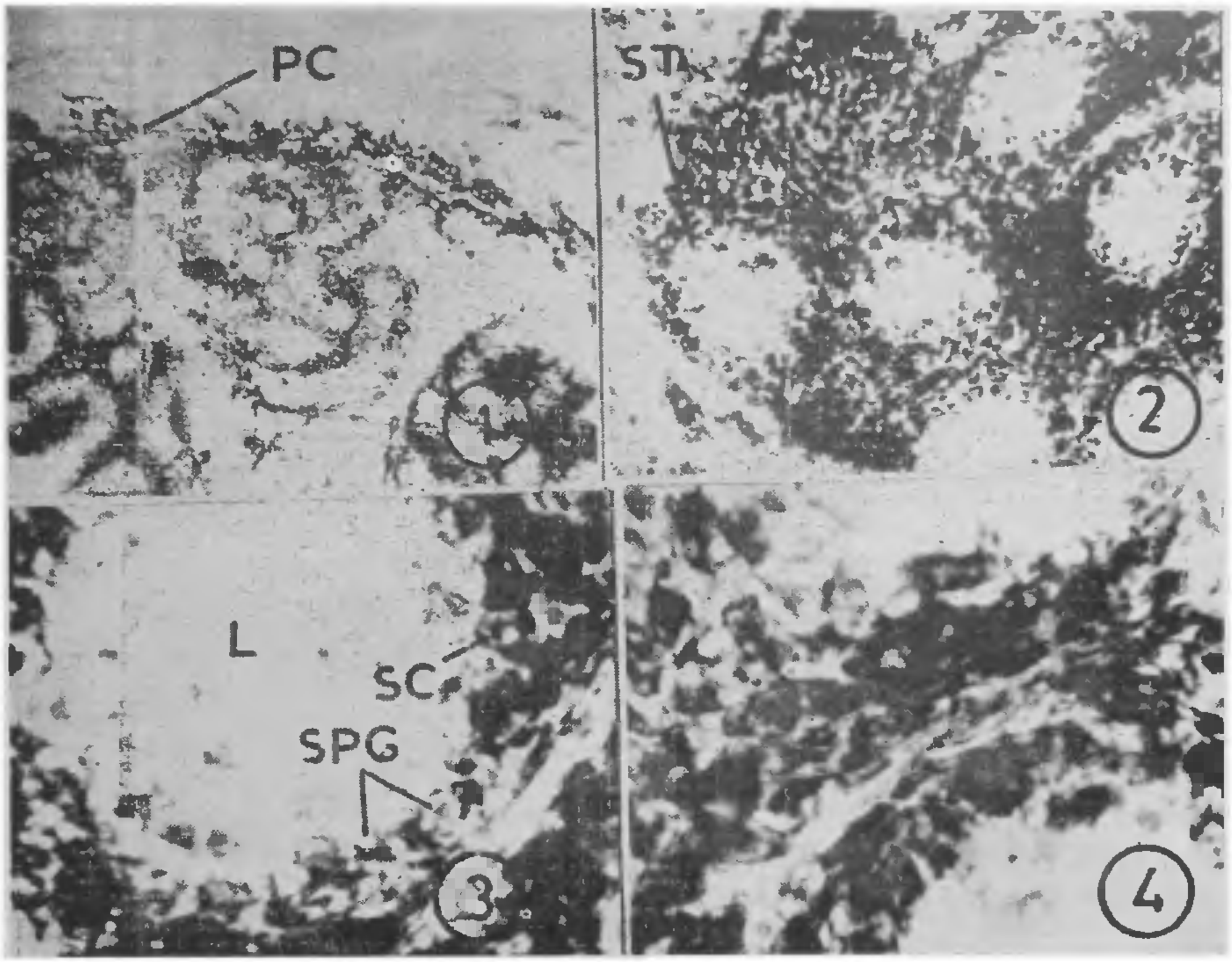
The animals were sacrificed by cervical dislocation. Recovery of tissues and subsequent fixation in chilled neutral formalin (10% at 4° C) was as described earlier[7].

Frozen sections (10 μM) of impuberal and spermatogenically active testis were processed for determining the histochemical site and pattern of distribution in the seminiferous epithelium and Leydig cells, according to Gomori's method as described by Pearse[10]. Suitable controls were run simultaneously.

Enzyme activity in testicular cells was visually appraised and graded as described earlier[7].

RESULTS AND DISCUSSION

In the impuberal testis of *P. g. giganteus* all the seminiferous tubules appeared to be similar. Spe-



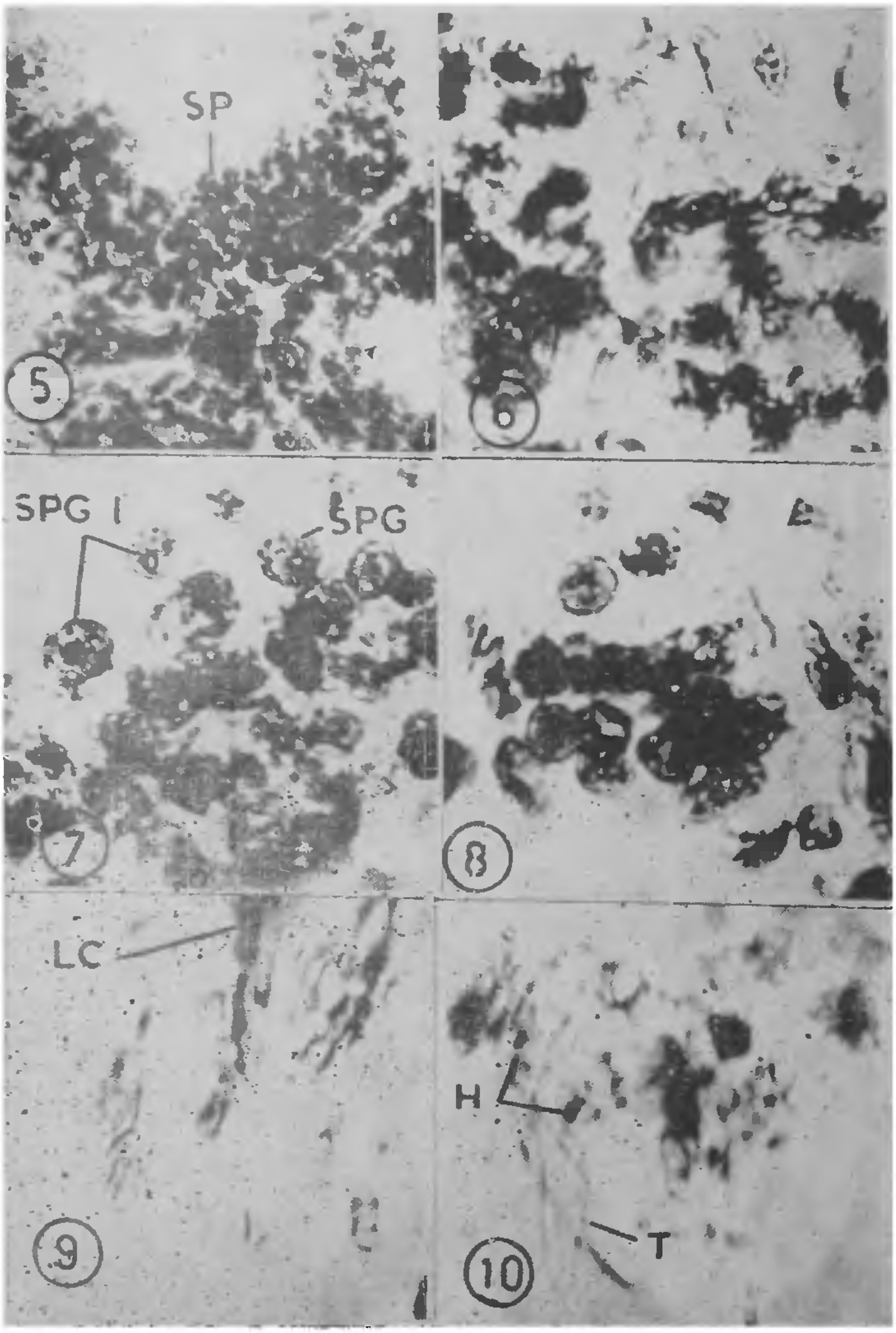
FIGS. 1–4. Acid phosphatase (AcPase) activity in the spermatogenic and androgenic cells of the tests of impuberal and sexually active *Pteropus giganteus giganteus*. Impuberal testis : Note the differential AcPase reaction in the spermatogonia sertoli and Leydig cells and in the small population of spermatocytes. Pigmenting into the interstitium. (SPG = spermatogonia; SC = sertoli cells; LC = Leydig cells; PC = pigmentation.)

matogonia, sertoli cells and spermatocytes could be clearly discerned in the seminiferous epithelium. The interstitium was occupied by regressed Leydig cells.

Variable AcPase reactions were discerned in the seminiferous cells, with spermatogonia exhibiting the most intense, and the sertoli cells the mildest enzyme reaction. High AcPase activity was discerned in the Leydig cells; but the tunica albuginea cluttered with pigmentation was AcPase negative (Figs. 1–4).

The spermatogenically active testes were characterised by dissimilar seminiferous tubules exhibiting various cell types in different patterns of cell associations. Stages in spermiogenesis could also be seen. The Leydig cells were hypertrophied. Intense AcPase activity was observed in the spermatogonia, leptotene and pachytene spermatocytes and Leydig cells; while moderate to mild reactions were observed in the spermatids and sertoli cells. The nucleus, chromatin material and the nucleolus showed positive AcPase activity in the spermatogenic cells and also in the Leydig cells. The dimorphic spermatozoa aligned in a characteristic manner displayed positive enzyme activity in the head, confined to the acrosome. The testicular fluid also exhibited mild AcPase activity (Figs. 5–10).

One of the common features of the impuberal and spermatogenically active testis was the occurrence of intense AcPase activity in the spermatogonia. In the mature testis alone, the pachytene and leptotene spermatocytes exhibited intense enzyme reaction and their chromatin figures were very distinctly AcPase positive. AcPase, by its hydrolytic action, may be responsible for generating metabolites of trophic importance for maintaining the cellular integrity, anabolic mechanisms of cell division and differentiation. The positive enzyme reaction in the tubular luminal fluid is a histo-



FIGS. 5–10. Acid phosphatase (AcPase) activity in the spermatogenic and androgenic cells of the testis of impuberal and sexually active *Pteropus gigantius gigantius*. Spermatogenically active testis: Note the intense AcPase reaction in the spermatogonia, leptotene and pachytene spermatocytes, and Leydig cells. Sperm acrosome can be seen to exhibit positive enzyme reaction. (SPGI = leptotene spermatocytes; SPG = pachytene spermatocytes; H–T = head and tail of the spermatozoa aligned in a characteristic manner.)

chemical evidence of enzyme elaboration. This may affect the chemical composition and amount of testicular fluid. It is interesting to record here that the testicular fluid has been considered to be the logical pathway for the feedback of information to the hypophysis about the kinetics of spermatogenesis[11]. Such factors are obviously not present in the aspermatogenic testis of impuberal males.

Positive AcPase reaction in the sperm acrosome may be of functional importance in facilitating the male gamete to overcome the egg membrane barrier(s) and thus could promote the initiation of some of the early steps in the process of fertilization. Allison and Hartree[12] suggested that sperm acrosome is a transformed lysosome(s). In bull and rabbit this has been confirmed electron microscopically.

A comparison of AcPase profile in the testicular cells of bat with other mammals indicates several differences. Thus, Singh and Mathur did not observe any AcPase activity in the testes of Hemiechinus[13]. In the testicular cells of rat, hamster and guinea pig, uniform AcPase activity has been described[14–16]. Barbacka and Surowiak[17] have observed circadian rhythm in AcPase activity patterns. They recorded that weaker AcPase activity in the testis is linked with low yield of spermatozoa and androgens.

Thus, the differential AcPase reaction in the cellular constituents of impuberal and adult testis of *Pteropus giganteus giganteus* may also be related to the functional differences, *e.g.*, active spermatogenesis and androgenesis *versus* lack of sperm production. AcPase activity may also be related to androgenesis and the concomitant testicular activity, *i.e.*, spermatogenesis.

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