THE INTRINSIC INNERVATION OF THE MALE REPRODUCTIVE SYSTEM OF A FRESHWATER TURTLE *TRIONYX GANGETICUS* (CUVIER). A BREEDING CYCLE STUDY

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SUMMARY

The intrinsic nerve supply of the male reproductive system of *Trionyx gangeticus* has been studied by histological and histochemical methods. Thin nerve fibres penetrate the tunica propria and enter the cellular portion of the seminiferous tubule. Some nerve fibres are also associated with blood vessels and interstitial cells. Nerve endings are observed in close association with interstitial cells. The functional relationship of the nerve supply to interstitial cells is discussed. The nerve supply of the testis differs along with the changes that occur in the testis. During the breeding season, no clear-cut asssociation of nerve fibres, either with vessels or with Leydig cells, could be established. Acetylcholinesterase-positive nerve fibres are present in the testes surrounding each seminiferous tubule. Cholinesterase activity is mainly restricted to the intertubular space. The epididymis and vas deferens contain few nerve fibres and the density of innervation differs in different regions of the spermatic duct is discussed.

INTRODUCTION

Autonomic influence may be far more widely involved in the regulations of reproductive process and in their dysfunction than has been generally recognised. There can be little doubt of the prime importance of the central nervous system in sexual activity, even though the details regarding which are still far from clear. Many workers who studied the innervation of the testes and their respective ducts in mammals had divergent views with respect to the distribution of nerve fibres (Kuntz, 1919; Mitchell, 1938; Okkels and Sand, 1940; Shioda and Nishida, 1966). Tingari and Lake (1972) extended the list of species investigated to birds. Unsicker (1973) observed adrenergic nerve fibres in the testicular interstitial tissue in reptiles. However, the knowledge regarding the innervation of the entire reproductive system of reptiles is incomplete. The present investigation is intended to add to the existing knowledge on this aspect with a study on the distribution of nerve fibres in the testis and spermatic duct of the male freshwater turtle *Trionyx gangeticus* during its reproductive cycles.

MATERIAL AND METHODS

Ten adult male turtles were purchased in every month during 1979-1981 from a local market, which were captured from the Betwa river (22° 32′ N, 77° 51′ E) located at 45 km north of Bhopal, India. They were immediately killed by chloroform anesthesia after being brought from the market. The reproductive organs were removed and small pieces of the tissues were fixed in Bouin's fluid and of the three reproductive organs in 10 per cent formalin for both histological and neurohistological studies. Routine paraffin sections were obtained and stained with haematoxylin and eosin for histology. Bielschowsky (1902) staining method modified by Davenport *et al* (1934) was followed to study the neurohistology.

To study the cholinesterase activity, 30-35 μm fresh frozen sections of the testis and spermatic duct of two breeding turtles were taken and were stained according to the method of Koelle and Friedenwald (1949), modified by Coupland and Holmes (1957). In order to stain selectively for specific (Ache) and non-specific (Che) cholinesterase, butyrylthiocholine was substituted for acetylthiocholine as a substrate and eserine was used as inhibitor.

RESULTS

The testis of turtle *Trionyx gangeticus* seems to be richly innervated as is evident by the presence of many thick and thin fibres. In the stretch preparation the covering of the testis, the tunica albuginea, is provided with a good number of nerve fibres extending for a considerable distance (Fig. 1). The blood vessels of the testis are also traversed by a few fine nerve fibres. The majority of the nerve fibres, as they encircle the testis, are accompanied by arteries. Many fine branches of testicular nerves are located in the interstitial tissue. Some of the nerve fibres are associated with blood vessels. Nerve endings are observed in close association with interstitial cells (Fig. 2). However, penetration of nerve fibres to the individual Leydig cells could not be traced.

Acetylcholinesterase-positive nerve fibres around the seminiferous tubule can be seen (Fig. 3). The cholinesterase-positive activity is seen in the intertubular spaces (Fig. 4). There is no evidence, however, of any nerve penetration into the tubules. Some of these nerve fibres terminate near the Leydig cells.

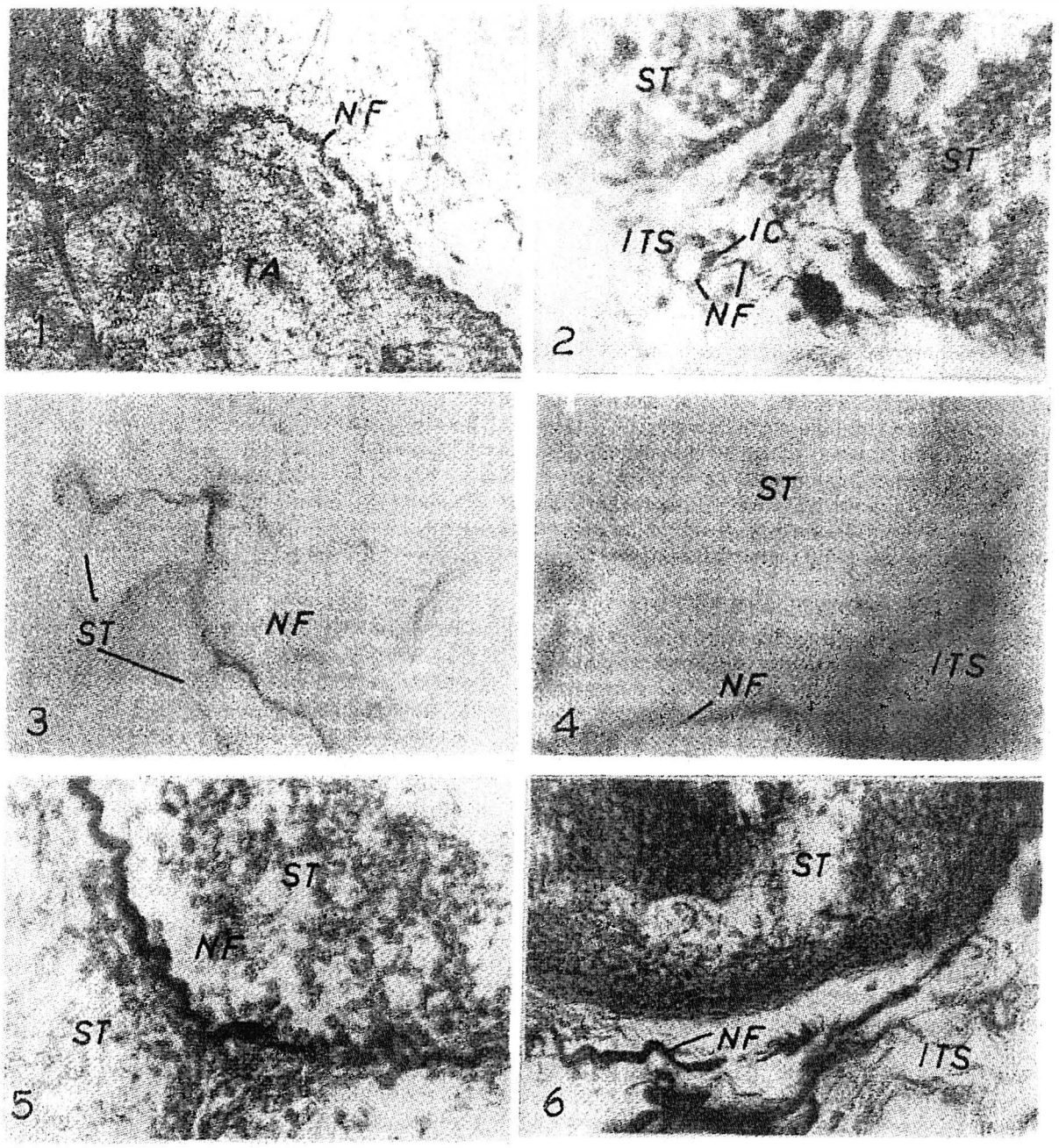

PLATE 1

Fig. 1 Stretched preparation of the tunica albugenia (TA) showing nerve fibres (NF) passing on the surface of tunica albuginea.

Fig. 2 Nerve fibres (NF) in the intertubular space (ITS). Note the nerve endings in association with the intertitial cells (IC). Seminiferous tubule (ST).

Fig. 3 Acetylcholinesterase positive nerve fibres (NF) passing around the seminiferous tubules (ST).

Fig. 4 Acetylcholinesterase positive activity in the interstitial tissue (ITS). Note the nerve fibre (NF) in between the seminiferous tubules (ST).

Fig. 5 Nerve fibres (NF) passing around the enlarged seminiferous tubule (ST).

Fig. 6 Nerve fibres (NF) around the seminiferous tubule (ST). Note the nerve fibres in the intertubular space (ITS).

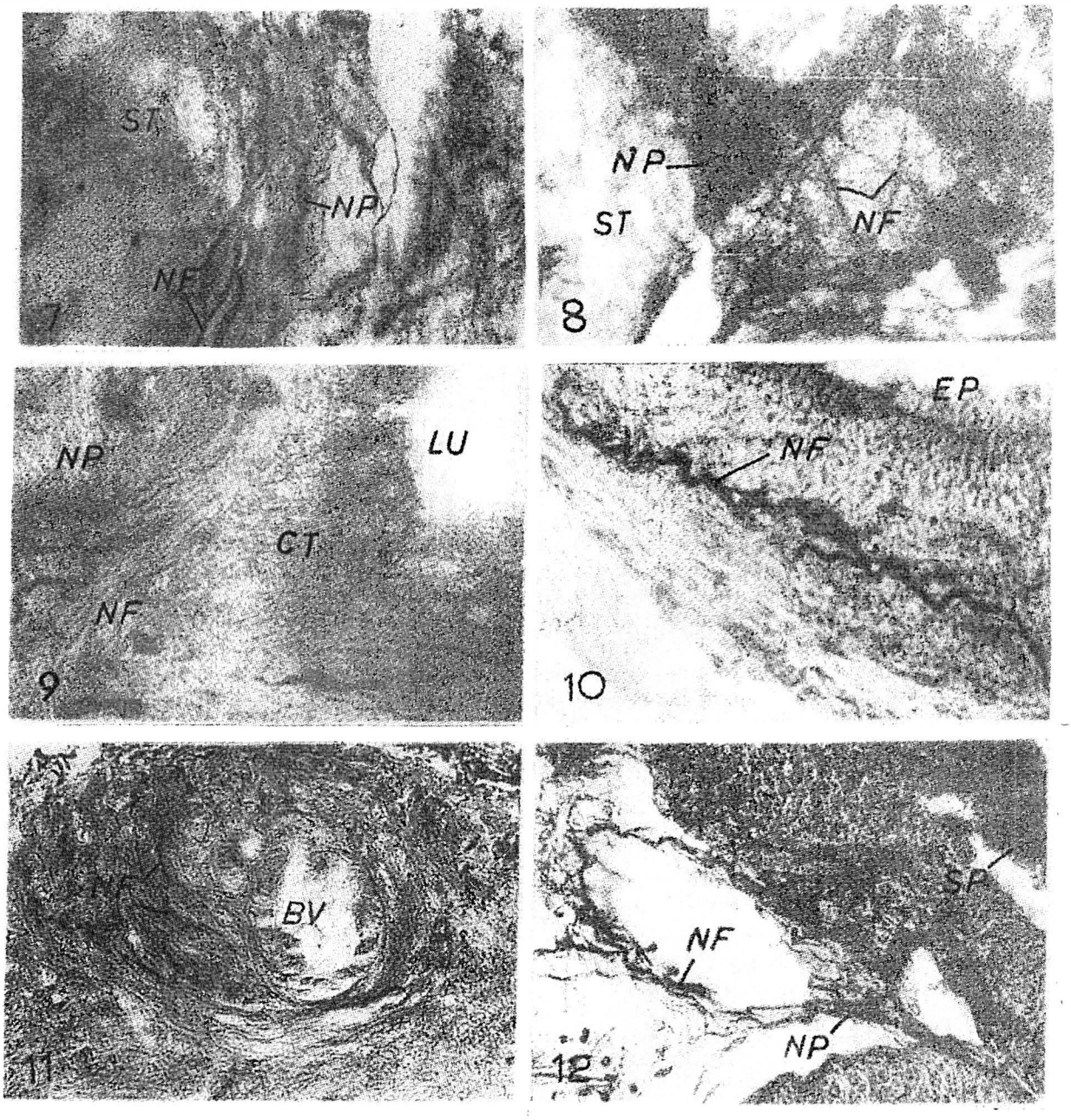

PLATE 2

Fig. 7 Thick nerve fibres (NF) penetrating the tunica propria. Nerve plexuses (NP) are also present. Note the inactive stage of the seminiferous tubule (ST).

Fig. 8 Network of nerve fibres (NF) in the intertubular tissue. Note nerve plexuses (NP) around the seminiferous tubules (ST).

Fig. 9 Nerve plexuses (NP) and many thick nerve fibres (NF) in the connective tissue (CT) of epididymis. Lumen (LU).

Fig. 10 Nerve fibres (NF) near the epithelium (EP) of epididymis.

Fig. 11 Short, thin nerve fibres (NF) around the blood vessel (BV) in the epididymis.

Fig. 12 Nerve fibres (NF) and nerve plexuses (NP) in between the ductules of epididymis. Note the sperm (SP) present in the luman of the ductule.

There was no positive staining of the nerve fibres when the butyrylthiocholine was used as the substrate.

The intrinsic nerve supply to the testis changes according to the changes that occur in the histology of the testis. During August, in the testis of the spermatogenically active stage, shrunken intertubular spaces are present. During this period, a clear-cut association of nerve fibres either with vessels or with Leydig cells can not be established. An individual fibre occasionally passes for some distance within the tunica propria, but it always emerges without penetrating the full thickness and without branching (Fig. 5). However, in the months of October-November, after the release of sperms into the epididymis, the intertubular spaces widen and the Leydig cells along with nerve fibres are observed. Most of the nerve fibres are present at the periphery of the tubule (Fig. 6). Rich innervation is observed throughout the winter months. In the month of June, thick nerve fibres are seen penetrating the tunica propria (Fig. 7). In July, a network of nerve fibres can be traced in between the seminiferous tubules (Fig. 8) and some nerve fibres are found penetrating the walls of seminiferous tubules occasionally.

The epididymis of the turtle *T. gangeticus* is found to be richly innervated throughout its length. Denser innervation is observed in the proximal part of the epididymis than in other parts of the genital tract. Prominent nerve plexus are found in the thick muscular wall of the epididymis (Fig. 9). Some nerve fibres are seen close to the epithelial lining of some ductules in the epididymis (Fig. 10), but there is no evidence of fine branches, or of endings taking a course between the epithelial cells. Thick and thin nerve fibres are observed in the other musculature of the ductules and also around the blood vessels (Fig. 11).

The density of nerve fibres vary from the anterior to the posterior extremity of the vas deferens and the number of these nerve fibres is extremely high in the muscular coats and the blood vessels of the organ.

Acetylcholinesterase-positive activity is seen in the interductular tissue of the epididymal region and in the muscular walls of the vas deferens. Many short cholinergic nerve fibres are observed in the close vicinity of the epithelium of epididymis and vas deferens.

Seasonally, the intervation of the epididymis varies in relation with the breeding cycles. In the breeding season, when the sperm is present in the lumen of the ductule, a rich innervation is noted (Fig. 12). In the months of November and December the innervation of the spermatic duct is sparse and is further reduced during January and February when the ductule is devoid of sperm. But from March onwards the density of innervation increases until it reaches its maximum in July.

DISCUSSION

The autonomic nervous supply to the male reproductive system has been extensively investigated in mammals and excellent accounts are available for brief comparison with the nervous supply of the reptilian reproductive system under study. Bell and McLean (1970) reported that the outer testicular capsule of rat receives both adrenergic and cholinergic motor nerves. Other investigators have detected a number of nerves in the tunica albuginea of man (Gray, 1947) and rat (Davies *et al*, 1970). In the present investigation, the network of nerve fibres and their endings are observed in the stretch preparation of the tunica albuginea of the testis of the turtle *Trionyx gangeticus*.

The blood vessels which are innervated by nerve fibres are found to penetrate more deeply into the interior of the testis in cat (Norberg *et al*, 1967), and man, swan and rhesus monkey (Baumgarten and Holstein, 1967, 1968). As evident from the present study the testis of the turtle is also innervated by a number of nerve fibres which in turn innervate the blood vessels supplying the testis.

Nerves penetrate the tunica propria to enter the cellular portion of the seminiferous tubules (see Hodson, 1970 for review). This is further confirmed in the present investigation. Some nerve fibres occasionally penetrate the tunica propria of the turtle testis. Okkels and Sand (1947) stated that nerve fibres form a nervous contact with Leydig cells. Gray (1947) found that large groups of the interstitial cells were penetrated by one or more nerve fibres and were commonly broken-up into smaller and smaller groups until a single interstitial cell was entirely surrounded by a fibre. In the present investigation, a number of nerve fibres are noticed in the intertubular spaces of the turtle testis. However, the relationship with these cells depends on their position. Peripheral groups of Leydig cells are associated with many nerve fibres while those in the interior of the testis are associated with very few.

As the Leydig cells are the main source of the production of androgens in the testis (Lofts, 1968), these cells might be regulated directly by the nerve endings (Okkels and Sand, 1940) and indirectly through the blood vessels which were accompanied by the nerves (Kuntz, 1919; Gray, 1947). These blood vessels are particularly provacative with respect to the action of androgen in maintaining and initiating spermatogenesis (Muller, 1957). In supporting the above views, many nerve fibres in close association with the blood vessels are observed in the interstitial tissue of the turtle testis. The Leydig cells are also supplied by these nerve fibres. Nerve endings noted near the Leydig cells strongly support the idea of an involvement of the autonomic nervous system in the regulation of Leydig cell activity.

Unsicker (1975) mentioned that the nerve fibres can more easily be encountered in juvenile animals than in adults, perhaps owing to the smaller volumes of testis, the smaller diameters of seminiferous tubules and large intertubular areas of immature animals compared to mature specimens. He also stated that the amount and distribution of adrenergic nerve fibres varies considerably both in various species and in various stages of the reproduction cycle. Groups of Leydig cells supplied by sympathetic nerve fibres were described as occurring in a testis with low spermatogenic activity in the swan (Baumgarten and Holstein, 1968) in the

pigeon (Ljunggren, 1969) and in the cock (Tingari and Lake, 1972). Ljunggren (1969) considered that there were more adrenergic fibres in the regressed testis of the pigeon than in the testis actively producing spermatozoa. In the present investigation, the intertubular spaces of the turtle testis contain many nerve fibres and their distribution varies during breeding cycles. A number of nerve fibres associated with Leydig cells are observed in the intertubular spaces of the testis during the non-breeding season but during the active breeding period their number is very small. The present findings are in close conformity with those of Ljunggren (1969).

In the present study, silver staining procedures revealed richer innvervation to the inner epithelium and the muscular coats of the epididymis than the cholinesterase technique. The density of innervation varies in different regions of the entire reproductive tract. Innervation in the vas deferens is sparse compared to that in the epididymis. Nerve fibres at the base of the epithelial lining may control the secretion of epithelial cells.

El Badawi and Schenk (1967) consider that the variation in innervation of the parts of male genital ducts are due to differences in both structure and function and they consider that an innervation is necessary for rapid conduction over long distances. As passage of spermatozoa is rapid from the testis to the epididymis, an innervation is necessary in cat and dog when the ductuli efferentes are long. However, Risley (1963) stated that as transportation through the corpus epididymis takes several days an innervation is unnecessary. There is some evidence, traced from the results of a histological study, that in the turtle there is a rapid passage of spermatozoa from the testis to the vas deferens via the epididymis (Rao, 1982) and thus the innervation is considered to be essential to enable spermatozoa to reach the ductus deferens quickly during seminal emission. Rich innervation noted during peak reproductive activity also suggests that the autonomic nerves do play a key role for the propulsion of seminal contents in the excurrent ducts.

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