CAUDAL NEUROSECRETORY SYSTEM OF FOUR HILL STREAM FISHES OF INDIA

Tiwari S.[1,2], A. Gopesh[4], K.Shah[3] and P. Srivastava[2]
[2]*Research Scholar, Department of Zoology, University of Allahabad, Allahabad 211 002*
[3]*P.G. Government College, Pithoragarh, Uttaranchal.*
[4]*Professor, Department of Zoology, University of Allahabad, Allahabad.*
[1]corresponding author : Tel.: 09889003012, e-mail: shailytiwari11@gmail.com

ABSTRACT

Caudal neurosecretory system is an additional neuroendocrine system found in fishes. Great variation has been observed among different groups of fishes, so far its organization is concerned. Much work has been undertaken on the caudal neurosecretory system of elasmobranchs and teleosts. Large size scattered Dahlgren cells in the posterior end of spinal cord, corresponding to last few vertebrae, with long running axon process and a neurohaemal organ the urophysis are the characteristic features of the system. Although thoroughly investigated in fresh water carps, no work is reported in hill-stream fishes. In an attempt to investigate structure and organization of caudal neurosecretory system in hill-stream fishes, present investigation was undertaken in four hill-stream fish of Indian freshwater namely, *Barilius bendelensis, Garra gotyla, Schizothorax plagiostomus* and *Tor tor.* The organization of this system in hill-stream fishes was found to be quite different from that observed in fresh water carps. It displays an organization which is more close to the organization of caudal neurosecretory system observed in elasmobranchs. The features are described and discussed.

Key words: Caudal neurosecertory system, Carps, Hill-stream, Urophysis and neuroendocrine system.

INTRODUCTION

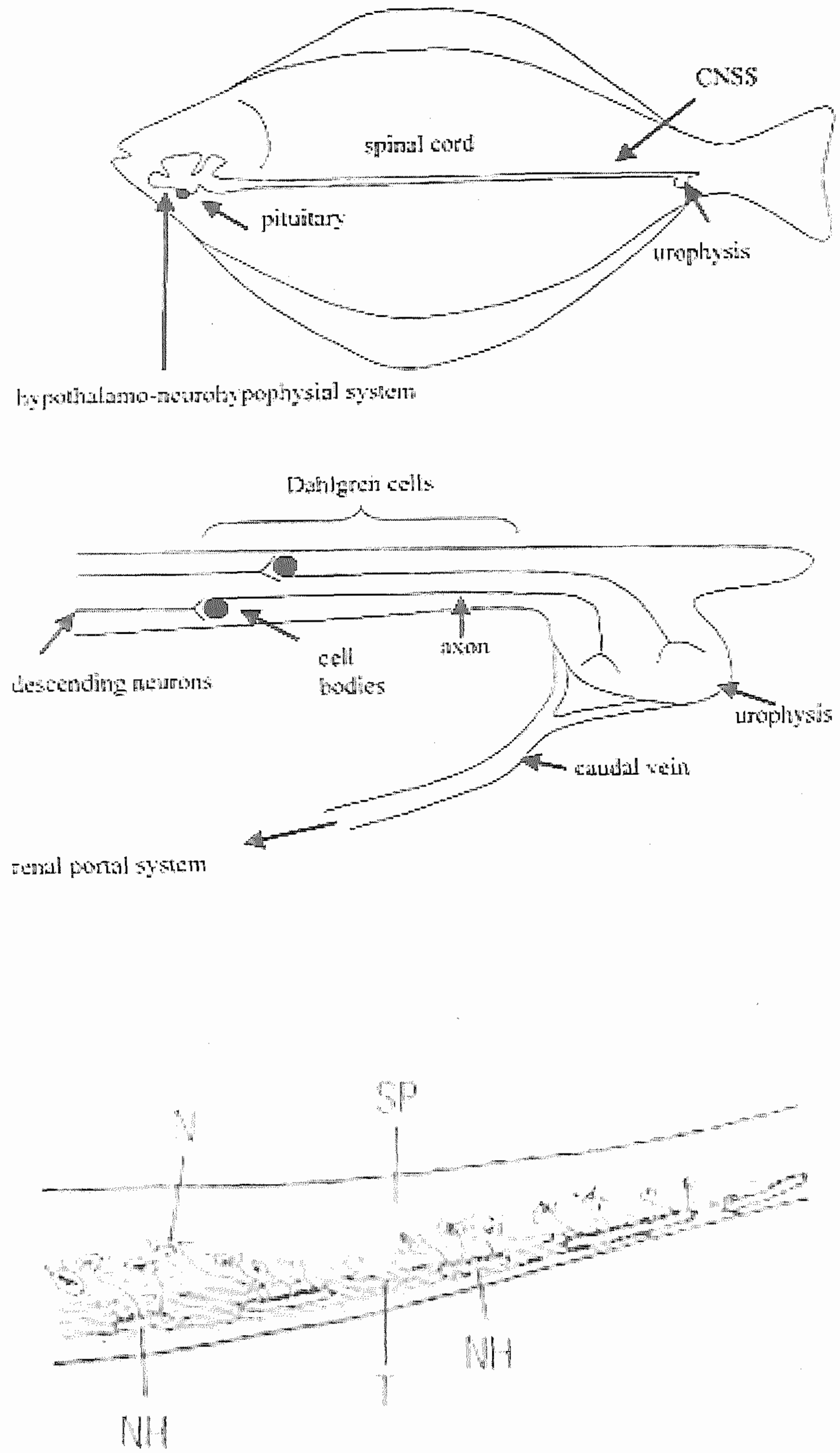
The vertebrate central nervous system has several neurosecretory centres which are concentrated in diencephalic levels of brain. Fishes are peculiar among vertebrates, to possess an additional system of neurosecretion at the tail end of spinal cord- the caudal neurosecretory system (Fig-1). Discovered about a century ago (Arsaky, 1813; Weber, 1927) the system has long been of interest to fish biologists and was thoroughly studied. Based on these studies, this system was paralleled to the hypothalamo-hypophyseal system of brain in its structure and organization (Bern *et. al.,* 1985). The morphological component of caudal neurosecretory system were investigated by many workers and the functional link between neurosecretory cells and storage release organ the system- the urophysis was described in detail (Enami, 1959). Extensive comparative studies are available for teleosts and elasmobranchs both (Fridbreg, 1962; Friedberg and Bern, 1968; Bern, 1969). General morphology of caudal neurosecretory system comprise of many large neurons (Dahlgren cells) in the posterior segment of the spinal cord. The long axons of these cells combine to form non-myelinated long tracts, terminating in a diffuse seceretory regions (Elasmobranchs and Chondrostean) (fig-1c) or in a discrete variously shaped neuroheamal organ, the urophysis (teleosts: fig-1a and 1b). Following these early studies, the caudal neurosecretory system and its secretory products, (the peptides, urotension I and urotension II) have been described in many species of fish. The biological action of these products are found to have involvement in osmoregulation and reproduction (Bern *et. al.,* 1985; Lederis *et. al.,* 1985; Arnold-Reed *et. al.,* 1991). It has recently been shown using homologous radioimmunoassay, that plasma concentration of urotension II is significantly elevated in sea water as compared to fresh water adapted fish (Winter *et. al.,* 1999; Arnold-Reed *et. al.,* 1991). Furthermore, the urophysial content of neurosecretory material becomes depleted in response to hyper osmotic challange. Immunohistochemical and biochemical studies indicate adrenergic, cholinergic, serotonergic and peptidergic inputs to the brain (Cohen and Kriebel, 1989; Audet and Chevalier, 1981; McKeon *et. al.,* 1988; Miller and Kriebel, 1986; Yulis *et. al.,* 1990 and Oka *et. al.,* 1997). Action of specific neurotransmitter / modulators on these caudal neurosecretory cells, in an *in vitro* caudal neurosecretory system preparation has been investigated on euryhaline flounder *Platichthys flesus*. Ultrastructure of caudal neurosecretory cells has been studied on Japanese eel *Anguilla, Tinca vulgaris* and *Fundulus heteroclitus*. Following these investigations, many studies have been undertaken to reveal the ultra structural morphology of the caudal neurosecretory cells which have revealed characteristic of neurosecretory cells.

Although a detailed description of morphological characteristic of the caudal neurosecretory system in Indian fishes is available (Srivastava, 1982), no information is available on hill-stream fishes so far. In an attempt to reveal the structure and organization of this system in certain fishes of hill-stream of India, present investigation was undertaken in four hill-stream fish species of U.P., namely *Barilius bendelensis, Garra gotyla, Schizothorax plagiostomus and Tor tor.* The observations are reported for the first time.

MATERIALS AND METHODS

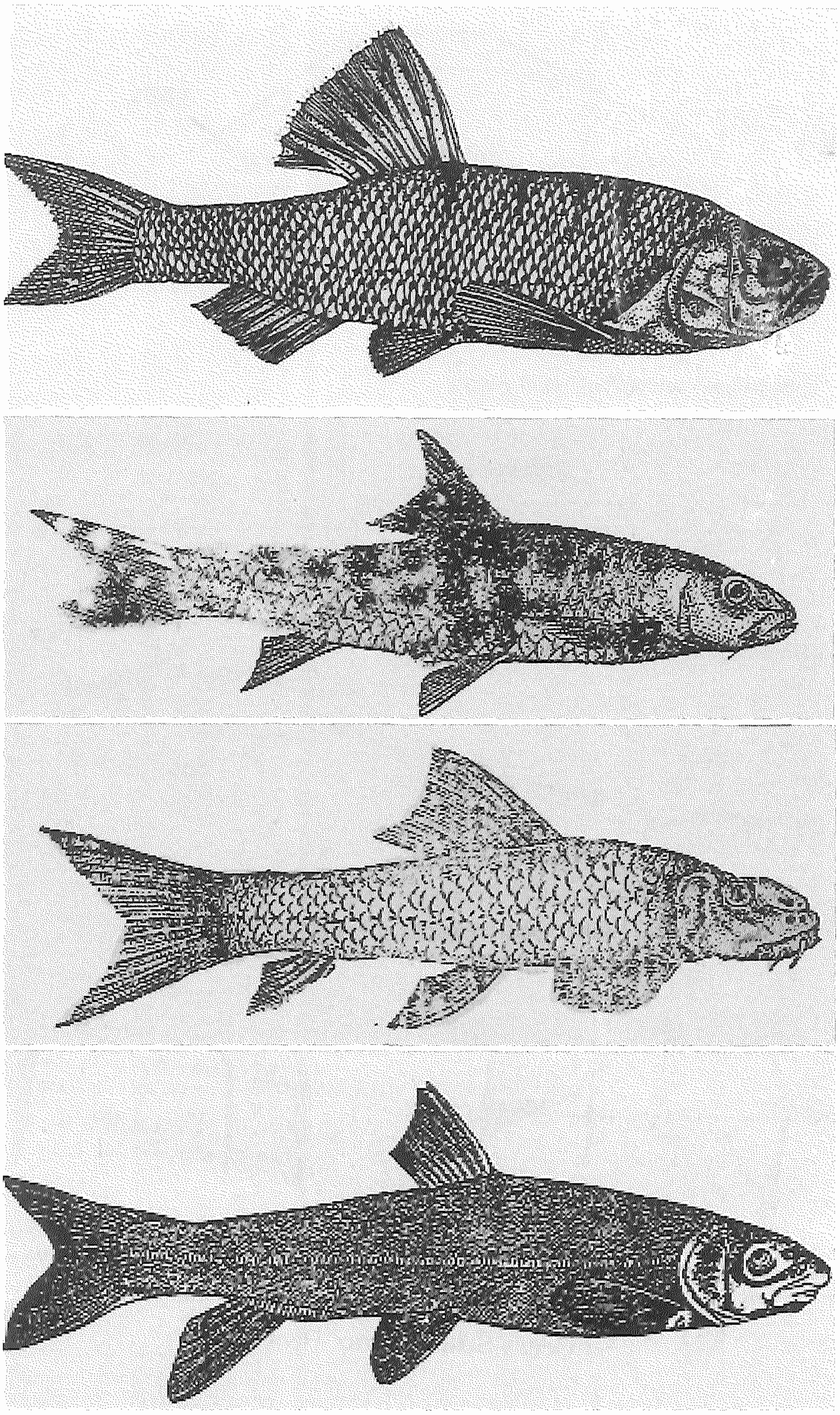
The live specimens of *Barilius bendelensis, Garra gotyla,* Schizothorax plagiostomus and *Tor tor* (fig 2) were collected from places around Pithoragarh. The specimens were identified with the help of keys, (Day, 1958; Mishra, 1965). The fish were anaesthetized with MS 222 and a portion of spinal cord from caudal region containing about 10-12 vertebrae from the end was removed. The spinal cord and the filum terminale was exposed and fixed in situ with aqueous Bouin's fluids for 18 hrs, and finally removed and processed for routine histology. 10μm thick paraffin sections were cut and stained by Acid violet stain (Takasugi and Bern, 1972) and Argyrophil silver staining techniques (Grimelius and Wilander, 1978).

Fig-1



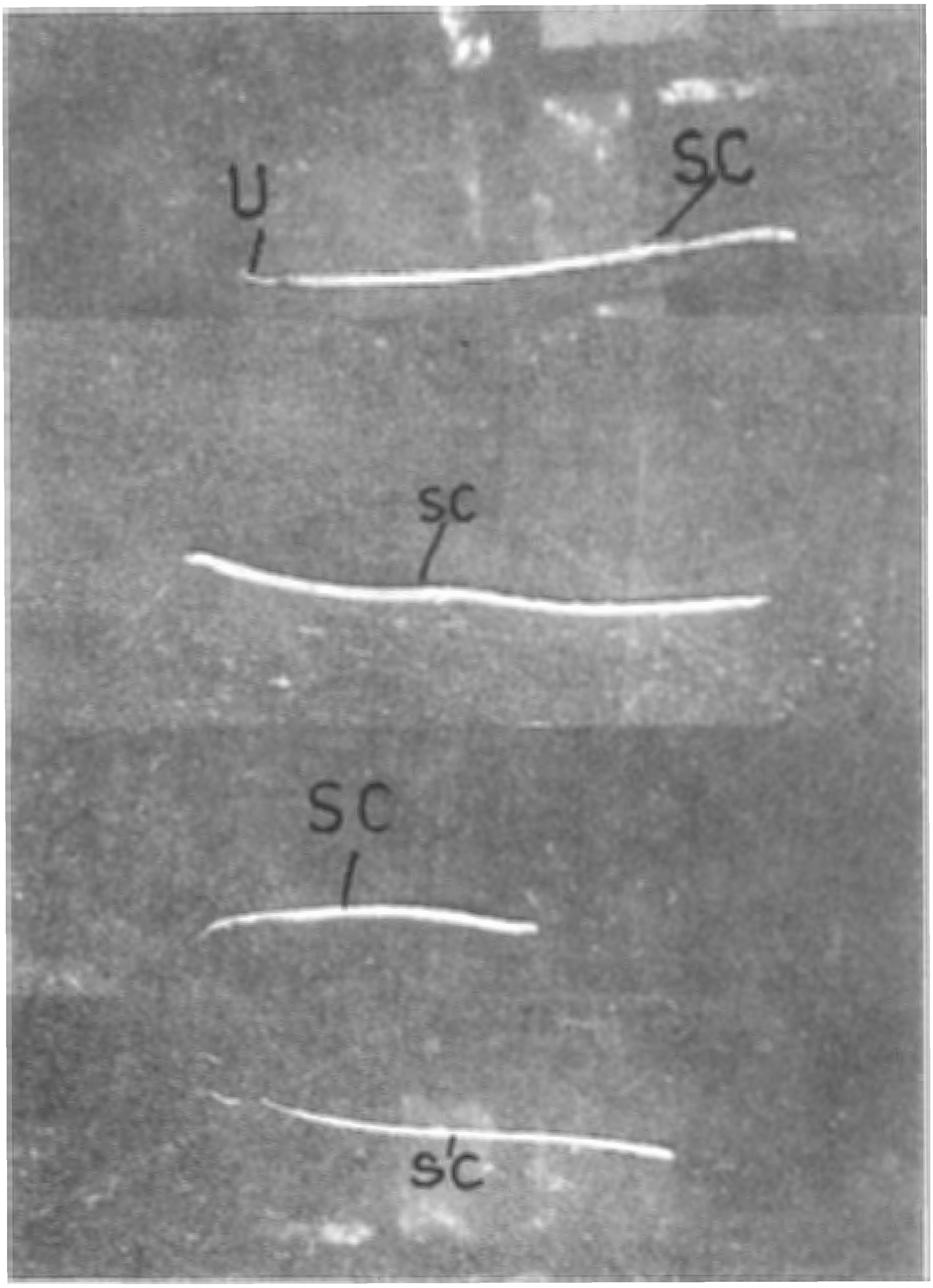
Diagrammatic representation of the caudal neurosecretory system (CNSS)
(a) Posterior end of spinal cord (b) in Teleosts (c) Posterior end of spinal cord in Elasmobranches

Fig-2



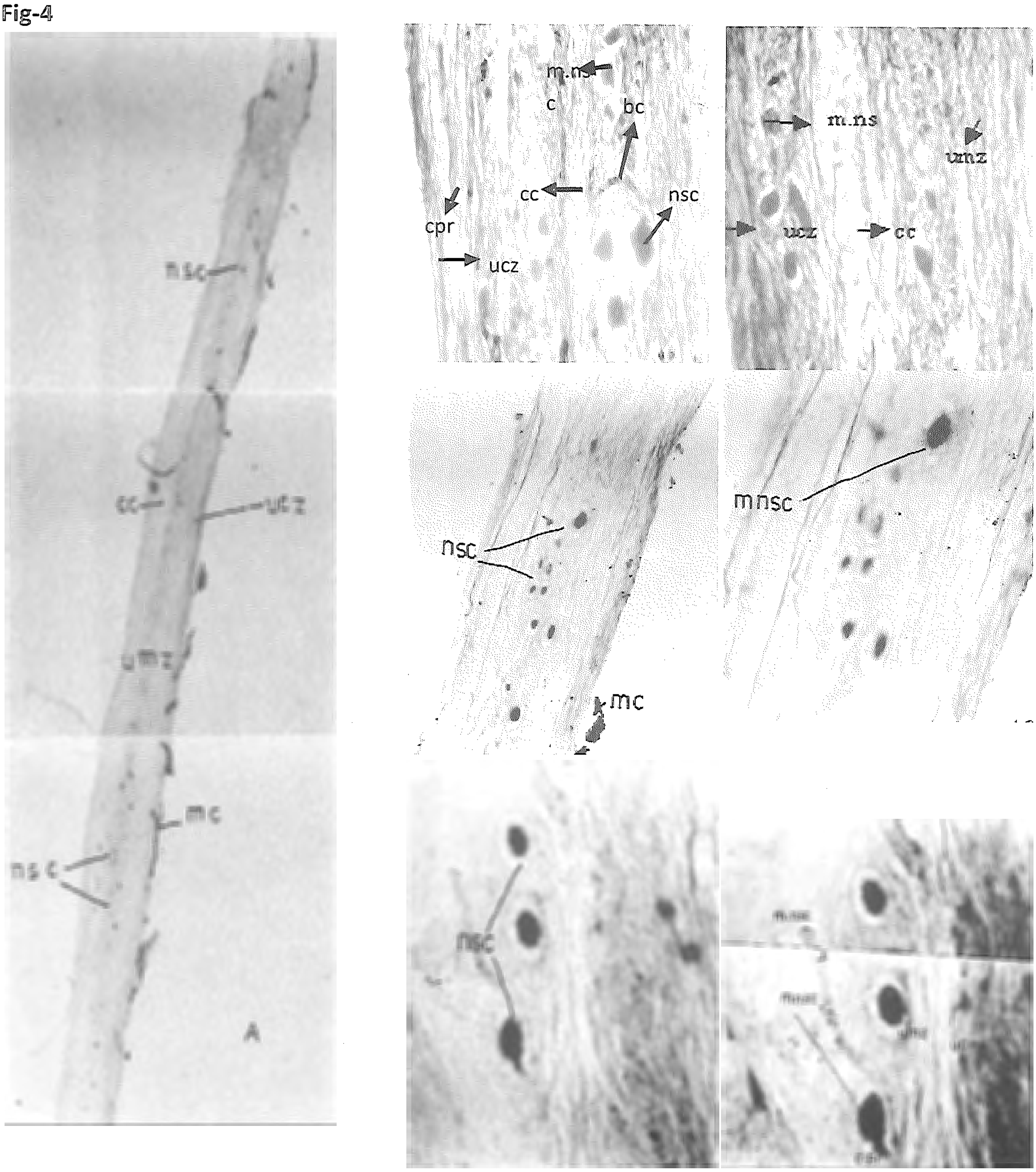
Lined diagram of external feature of hill stream fishes Lined diagram of external feature of hill stream fishes examined.
a- *Barilius bendelisis* b- *Garra gotyla* c- *Shizothorax plagiostomu* d-*Tor tor*

Fig-3



a- photograph of a dissected caudal peduncle of _Barilius bendelisis_ showing spinal cord ending (without urophysis).
b- photograph of a dissected caudal peduncle of _Garra gotyla_ showing spinal cord ending (without urophysis).
c- photograph of a dissected caudal peduncle of _Shizothorax plagiostomus_ showing spinal cord ending in blindly urophysis.
d- photograph of a dissected caudal peduncle of _Tor tor_ showing spinal cord ending

Fig-4



Caudal neurosecretory system of *Barilius bendelisis*

a- photomicrographs of sagital section of spinal cord at the tail end of*Barilius bendelisis* showing neurosecretoy cells(nsc) close to center , coritical ,and medullary zones and meninx. **X137**

b- sagittal section of spinal cord showing monopolar neurosecretory cells (m.nsc) cell processes (c.pr), nurosecretory cells(nsc), blood capillaries (bc), central canal(cc), urophysial cortical zone(ucz) and urophysial medullary zone(umz) and meninx (m). **X100.**

c- sagittal section of spinal cord showing monopolar neurosecretory cells (m.nsc), axon processes, capillaries and meninx (m). **X137.**

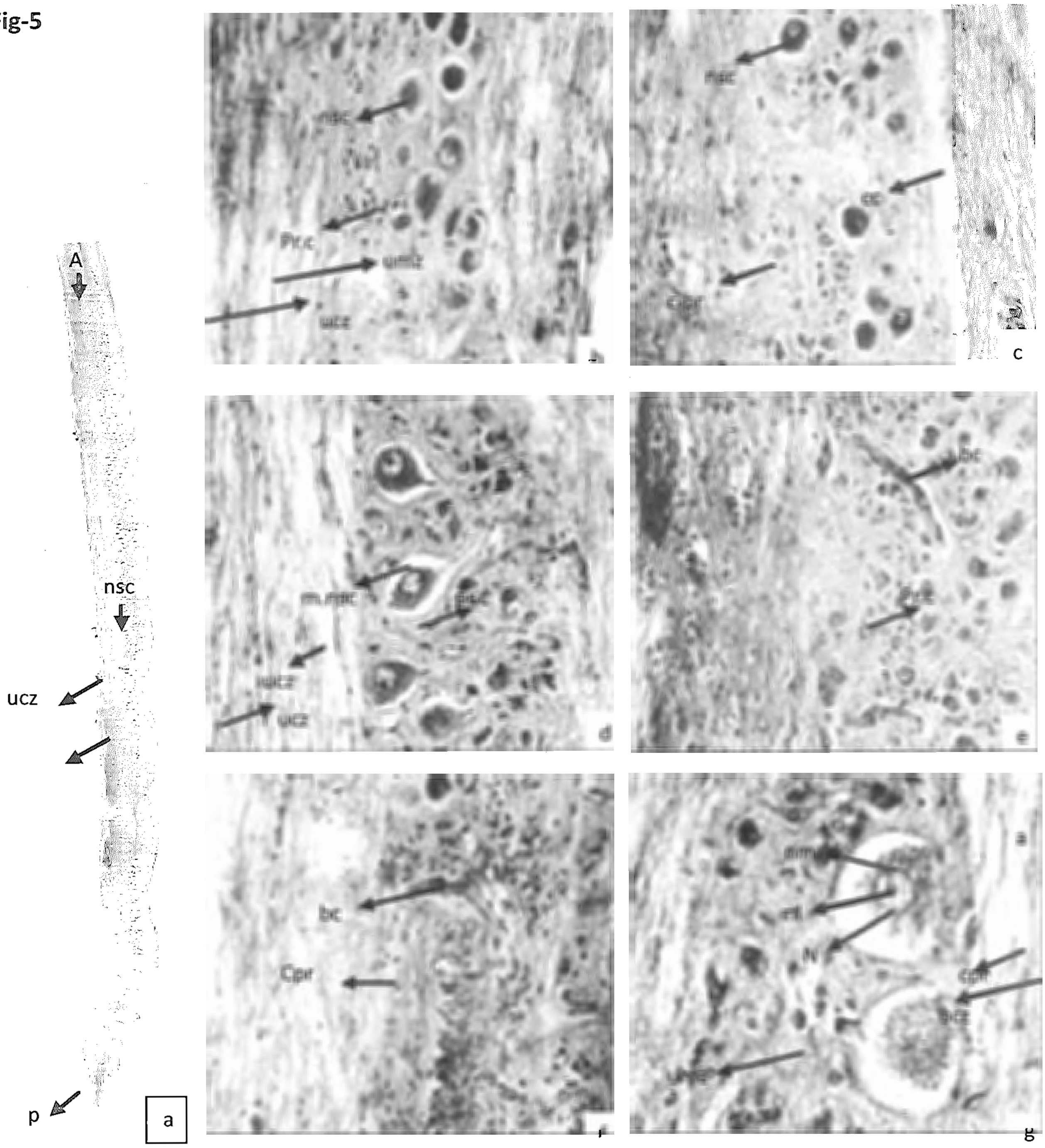
d- sagittal section of spinal cord showing monopolar neurosecretory cells (m.nsc), urophysial cortical zone(ucz) and urophysial medullary zone(umz) and meninx (m). X100.

e- Higher magnification of sagittal section of the spinal cord showing monopolar neurosecretory cells (m.nsc) bipolar neurosecretory cells (b.nsc) axons processes, capillaries and meninx(m).). **X350.**

f- sagittal section of spinal cord showing axons processes, neurosecretory cells bundle and meninx (m). **X350.**

g- A magnified view of Fig-4e .**x520** .

Fig-5



Caudal neurosecretory system of *Garra gotyla*

a- cross-section of the spinal cord showing neurosecretory cells (b.nsm) urophysial cortical zone(ucz) and urophysial medullary zone(umz) and meninx (m). **X100**

b- sagittal section of spinal cord showing neurosecretory cells (nsc) cell processes (c.pr) and urophysial cortical zone(ucz) and urophysial medullary zone(umz) and meninx (m). **X100.**

c- sagittal section of spinal cord showing central canal (cc) and primodial cells (pr.c). **X100**

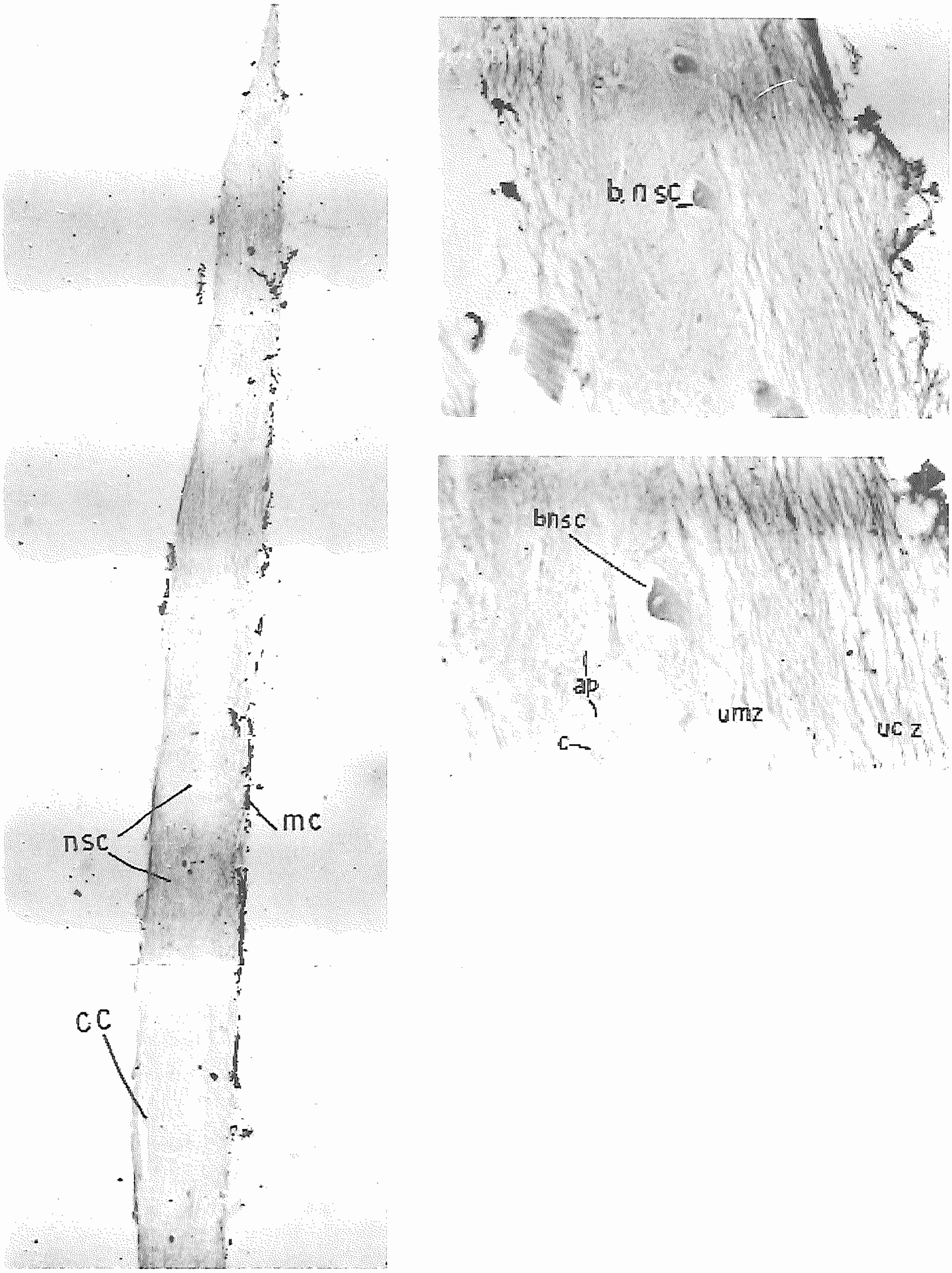
d - sagittal section of spinal cord showing monopolar neurosecretory cells (m.nsc).**x100.**

e- sagittal section of spinal cord showing blood capillaries (bc) primodial cells (pr.c). **X100.**

f - sagittal section of spinal cord showing blood cells(b) and cell processes(c.pr). **X400.**

g - sagittal section of spinal cord showing nucleus (N) nuclear membrane and nucleolus(N). **x400**

Fig-6



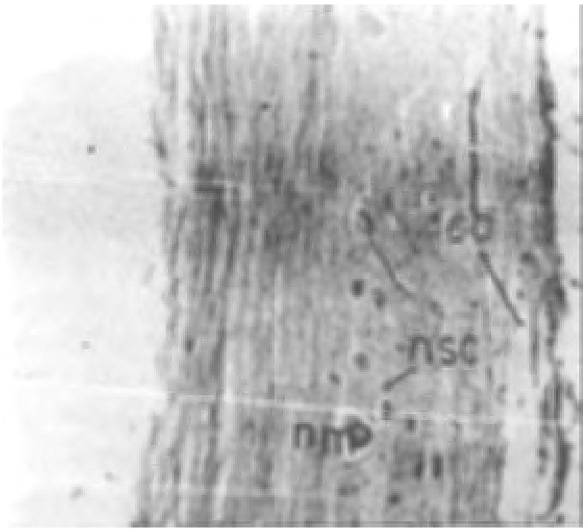
Caudal neurosecretory system of ***Schizothorax plagiostomus.***

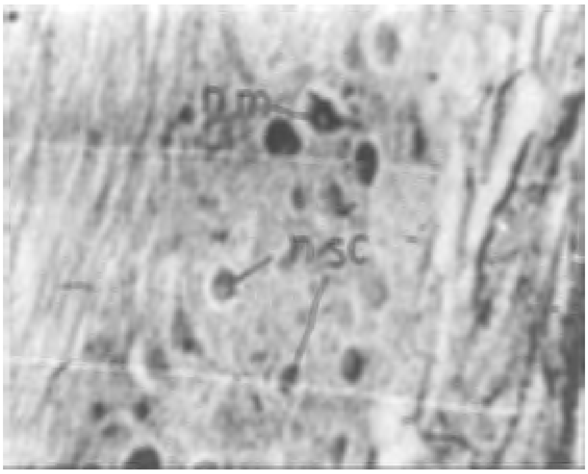
a. photomicrograph of the spinal cord showing neurosecretoy cells(nsc) axon processes, meningeal covering. **X137**

b. sagittal section of the spinal cord showing bipolar neurosecretory cells (b.nsm) and meninx (m). **x280.**

c. Higher magnification of fig-6b showing bipolar neurosecretory cells (b.nsm) urophysial cortical zone (ucz) and urophysial medullary zone(umz) and meninx (m). **X430**

Fig-7





Caudal neurosecretory system of *Tor tor.*

a. Higher magnification of sagittal section of the spinal cord showing neurosecretory cells (nsc) of different sizes. **X360.**

b. sagittal section in the posterior end of spinal cord showing various sizes of neurosecretory cells(nsc). **X510**

OBSERVATIONS

Four species namely, *Barilius bendelensis, Garra gotyla, Schizothorax plogiostomus and Tor tor* (fig 2), displayed presence of large sized Dahlgren cells with their long axon bundles running towards end of spinal cord (Fig 2) forming the caudal neurosecretory system. The cells are found to be present relatively more cephalad counting from caudal extremity, as compared to other teleosts, in which cells are found to be present up to the region corresponding to 8^{th}-10^{th} vertebrae from posterior to anterior side. The spinal cord is lodged in the neural canal, running gradually upto the level of the last but one vertebra in all the hill-stream fishes investigated. Well developed urophysis, as found in most of the teleosts, is lacking in these fishes (Fig 3).The absence is evidenced, both externally as well as internally. The examination of serial longitudinal, sagittal and transverse sections revealed internal structure forming the system. Complete spinal cord is covered with pigmented meninges. The staining intensity clearly divides the spinal cord into two region namely lightly stained cortical zone (ucz) and darkly stained medullary zone (umz). A prominent central canal found to be present in other teleosts in the fishes was not observed.The cells are found to be clustered more towards the lightly stained medullary zone. The neurosecretory cells encountered (fig 4, 5, 6 and 7) are of different sizes (6µm- 20µm) and observed to be in different stages of secretory cycle (fig- 4, 5, 6 and 7). Young, mature, spent and primodial cells are clearly visible in *Barilius bendelensis* (Fig 4), *Garra Gotyla* (fig 5) *Schizothorax plogiostomus* (Fig 6) *and Tor tor* (Fig 7). Cells are commonly bipolar; (b.nsc) but monopolar (m.nsc) and multipolar (ml.nsc) cells are also observed. Cells display large prominent nucleus (N), nucleolus (nl) and granular cytoplasm (c. pr). It is interesting to note that transitional stages between a typical ependymal cell and mature neurosecretory cells are also observed throughout the length of the spinal cord in the tail region in all four species examined. Cell processes run as long bundles and are prominent in the cortical zone. All along the course thin walled capillaries are seen transversing between the axons (fig 5 and 6). It seems that the fine capillaries are in direct contact with cell processes of neurosecretory cells, suggesting "a diffuse type of neuroheamal contact, compensating lack of definite neuroheamal organ- 'The urophysis".

DISCUSSION

Gross morphology of caudal neurosecretory system observed in four hill-stream fishes namely *Barilius bendelensis, Garra gotyla, Schizothorax plagiostomus and Tor tor,* revealed altogether a different organization of caudal neurosecretory system as compared to other teleosts, including cyprinids and silurids. Urophysis is a significant structure which needs to be emphasized in case of hill-stream fishes.It has been found to be present invariably in all the teleosts (Pearson *et. al.,* 1985, Bern *et. al.,* 1985, Srivastava, 1984), where as it is lacking in elasmobranches, holocephalan and chondrostean. A typical urophysis has been found to be absent in all the hill-stream fish examined. In their organization they seem to be parallel to elasmobranch, holocephalan and chondrostean, which lack a well defined neuroheamal organ and the non myelinated axonal tracts terminate in a diffused pattern in the cortical zones. The primary determinate of the morphological variations was accepted to be vertebral column and shape of caudal fins. Urophysis is present in all the species with indistinct caudal fin (Hamana, 1962; Friedbreg, 1962; Sano, 1966; Fridberg and Bern, 1968; Bern and Saenko, 1970; and Bern, 1969). Isospondylous fishes have gradually diminishing vertebral column and indistinct caudal fin and hence have inconspicuous urophysis. Urophysis is undifferentiated externally in *Notopterus* and *Mastacembalus* which have distinct caudal fin. Capillaries are found to be traversing all along the length of spinal cord making contact with axon process at places. Lobate urophysis of other teleosts is associated with more advance type of caudal skeleton in which urostylar centrum offers a concavity, - the urophysial fossa, to accommodate it (Bern, 1969). Comparative investigations on the urophysis (Srivastava, 1984) has revealed a wide range of variation among Cyprinids, from unpaired median condition like that found in other teleosts, to paired condition of *Cirrhinus mrigala* and intermediate condition *Labeo rohita, Puntius ticto and Rohtee cotio.* It has been suggested that paired condition is an adaptive feature of cyprinid urophysis, to provide more surface area for vacularization to obtain more effective functioning of the neuroheamal area. Thus, it seems possible that the caudal neuroscretory system is poorly developed in hill-stream cyprinids, as compared to fresh water cyprinids and silurids, as water of hill-streams is seldom deficient in oxygen content. The hill stream fishes possess well developed heterocercal caudal fin, similar to Acipenser, Chondrostean and Elasmobranchs. Absence of urophysis in some of the advanced teleosts like *Syngnathidae and Mollidae*, has been considered to be a secondary feature (Bern, 1969). However, more in-depth investigations are needed to ascertain as to whether the absence or presence of poorly developed urophysis in hill-stream fishes has any adaptive and/or phylogenetic significance and also to reach to a definite conclusion for better understanding of this system in the biology of fish, especially with reference to the ecological condition of hill-streams.

REFERENCES

Arsaky, A., 1813. De piscium cerebro it medulla spinally, Disssertalic in anguralis, Halae.

Audet, C and Chevalier, G., 1981. Monoaminergic innervations of the caudal neurosecretory system of the brook, trout Salvelinus fontinalis in relation to osmotic stimulation. Gen. Comp. Endocrinol. 45,189-203.

Arnold Reed, D. E. and Balment, R. J.,1991. The caudal neurosecretory system of *Platicthys flesus* general morphology and responses to altered salinity, Comp. Biochem.Physiol.99a 137-143.

Bern, H. A., 1968. Urophysial and caudal neurosecretory chapter 8. In fish physiology Hoar W.S.and Randell eds. D.J., Vol.2, New York Academic Press. P.399.

Bern, H. A., 1969.A reference preparation for the study of active substances in the caudal neurosecretory system in teleosts. J. Endocrinol, 45, xi-xii.

Bern, H. A., and Saenko, I. I., 1970. Caudal neurosecretory system in sturgeons, Doklady. Akad. Nauk, SSSR, otd.Biol.194,218-221.

Bern, H. A., 1972. Some question on the nature and function of cranial and caudal neurosecretory system. Prog. Brain. Res. 38,85-96.

Bern, H. A., Pearson D., Larson B. A., and Nishioka R. S., 1985. Neuro-hormones from fish tails the caudal neurosecretory system. Recent prog. Horm. Res.41, 533-552.

Cohen, S. L. and Kriebel,R. M., 1989. Brainstem location of serotonin neurons projecting to the Caudal neurosecretory complex. Brain Res.Bull.

Day, F., 1958. The fishes of India I and II William Dawson and sons Ltd.London.

Enami, M., 1959. The morphology and functional significance of the caudal neurosecretory system of fishes. In Comparative Endocrinology. 697-724.

Fridberg, G., 1962. Studies on the caudal neurosecretory system in teleost. Acta Zool. (stockh) 43,1-77.

Fridberg, G. And Bern, H. A., 1968. The urophysis and the caudal neurosecretory system in fishes Biol .Rex . 43,175-199.

Gopesh, A. and Srivastava, P., 2003. Caudal neurosecretory system in an exotic species of catfish *Clarias gariepinus*. Science Letters, Vol. 26,1-2.

Hamana, K., 1962. Uber die neurophysis spinalis caudalis bei fishen. J., Kyoto prefect. Univ. Med. 71, 478-490.

Lederis. K., Fryer, J. N., Rivier, J., Mc Cannell, K. L., Kobayashi, Y., Woo, N., and Wong, K.L.,1985. Neurohormone from fish tails: caudal neurosecretory system. II Action of urotensins I in mammals and fishes. Rec. Prog. Horm. Res.41, 553-576.

Miller, K. E. and Kriebel, R. M., 1986. Cytology of brainstem neurons projecting to the Caudal neurosecretory complex. An HRP-Electron microscopic study. Brain Res.Bull. 16,191-199.

Mckeon, T.W., DCohen, S. L., Black, E. E., Kriebel, R. M. and Parsons, R. L., 1988 Monoamines in the Caudal neurosecretory complex.biochemstry . Brain Res.Bull.21, 37-42.

Oka, S., Chiba, A. and Honma,Y., 1997. Structure immunoreactive with porcine NPY in the caudal neurosecretory system of several fishes and cyclostomes. Zool.Sci. 14,665-669.

Pearson, D, David, M., Wong, W., 1985. The caudal neurosecretory complex. Biosynthesis axonal transport and release of urotensin II. In Koobayashi, H.Bern,H.A., Urano, A.eds. Neurosecretion and the biology of neuropeptides. Tokyo.Japan .Sci. Soc. Press. 451-456.

Sano, Y., Iida, T., Takemoto, S., 1966. Weitere elecctronenmikrospische Untersuchungen am kaudalen neurosekrerischen systemvon fischen.Z.Zellforsch. 75, 328-338.

Srivastava, H. C., 1982. D.Phill. Thesis, Univ. of Alld., India.

Srivastava, H. C. and Srivastava, C. B. L., 1984. Morphological variation in teleosts urophysis. Proc. Nat. Acad. Sci. India 54(B) 4, 288-297.

Takasugi N. and Bern, H. A., 1962. Comp. Biochem. Physiol. 6, 289.

Weber, E. H.,1927. Kroten unpeurer faden, Mit demsichdes Ruckenmerit beiieinigen fischen endigt, namentlich beim Cyprinus carpio. Arch.Anat.U.Physiol.316.

Winter, M. J., Hubbard, P.c., McCrohan, C. R . and Balment, R. J., 1999. A homologous radioimmunoassay for the measurement of urotension II in the Flounder, *Platichthys flesus.* General Comparative Endocrinol.114,249-256.

Yulis , C. R., Garcia, M. E. and Rodriguez, E. M., 1990. The caudal spinal cord of coho salmon *(Oncorhynchus Kisutch):* immunocytochemical evidences of a 'caudal serotoningeric system'.Cell.Tiss.Res.259, 543-550.