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CONSEQUENCE OF THERMAL RELATION (LOW TEMP): CHANGES IN THE NEUROSECRETORY PROFILE OF THE MILLIPEDE, ANOPLODESMUS TANJORICUS (POCOCK)

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Abstract: The distribution and structure of the neurosecretory cells of the brain, suboesophageal gangalion and other ventral gangalia with the associated neurohaemal organs in millipede, based on cytomorphological characteristics and trophography in the millipede, Anoplodesmes tanjoricus,their are three different types of cells A, B and C present in dense neurosecretory material in the brain. The A Cells are divided into subtypes A_1, A_2, A_3, A_4 : and C cells into C_1 and C_2.The data on changes in the neurosecretory cells of cerebral gangalion ,suboesophageal and ventral gangalion of the millipede acclimated to low temperature (12^0C and 10^0C) revels decrease in neurosecretory intensity of A, B and C types cells. They are differentiated in A_1,A_2, A_3, A_4 and C_1 , C_2 after exposure of millipede to low thermal temperature there is absence of neurosecretory material or if present,it is in traces. Nuclear diameter shows decrease and neurosecretory intensity was reduced. The present investigation experimentally verifies the course of the neurosecretory material in the depletion at lower temperature therefore neurosecretory intensity showed prominent changes, probably due to the inactivity of enzymes than exposed to upper low temperature (12^0C-10^0C).

Keywords: Millipede, Neurosecretory Cells, Cells types, Exposure , low temperature,

INTRODUCTION:

About 60-90% of terrestrial primary production is known to decomposer in soil habitats (Giller, 1996). Decomposition of organic matter and nutrient release takes place by the synergistic function of microflora and saprophagus fauna. saprophagous invertebrate particularly, millipede, woodlice and larvae of diptera. Millipede belongs to the class diplopoda (myriapoda) of phylum arthropod. They are found in moist and humid condition under fallen leaves and rotten woods. They feed on decaying vegetable matter and soil litter. Increasing concern over climate change and limited supplies of fossil fuels have resulted in a growing need for renewable energy sources They play important role in soil formation. In India millipedes received less attention as compared to other arthropod groups. Anoplodesmus tanjoricus millipede is cosmotropic they found abundantly in open ground covered with soil of Amravati district in the rainy season The neurosecretory system of myripodes is through be limited to a group of neurosecretory cells situated on either side in the protocerebrum closed to the globuli cells, the product of which in the end organ, the cerebral gland(Gabe1952,1954a,b,;1956a; Plam1956). It also believed that the cerebral glands ,

besides being a storage gland and presumably release centres also elaborate another secretion of their own ,which is distinguishable from that produced in the neurosecretory cells of the brain due to difference in its stainability. (Segal,1961)working on the American slug Limax flavus mentioned that animals acclimatized to the cold have a higher rate of oxygen consumption than those acclimatized to warm when both are measured at the same temperature The terrestrial animals have to withstand variation in temperature. This environmental factor must influence their distribution as a matter of fact terrestrial animals tend to locate themselves in the part of their environment where there is least stress,(Rising and Armitage, 1969) these animals can adapt to changes in environment temperatures. The thermal relationship of millipede are to be considerable interest not only to be ecologist, but also to the physiologist concerned with the problems of explaining the mechanism of acclimatization to temperature This present investigation was undertaken to study of effect on thermal regulation on neurosecretory Cells in the cerebral ganglion (brain) of millipede. In the cerebral ganglion there are three different types of neurosecretory cells , A , B and C types and their subtypes.

Material and Method:

Millipede were collected from Amravati region, they were brought to the laboratory and preserved in the glass container with moist soil. The weight representing size where noticed and the group of 10 - 20 millipede having weight 1.5-3.0 gms each were taken for acclimatization into glass jar covered with muslin cloths and it was kept inside the incubators for low acclimatization at 12 ± 0.5˚C & 10 ± 0.5˚C the millipede were gradually cooled until the desired acclimatization temperature was reached. Every after two days the soil in the jars were replaced with moist soil already brought up to the appropriate acclimatization temperature. After that collection, maintenance and selection of millipede for experiment and animals were dissected in fixatives or in normal saline (0.7%Nacl) .The cerebral ganglion, nerve ring and connective bodies intact and transferred and immediately fixed in aqueous Bouin's fluid for 12 hrs. Then tissue were dehydrated and paraffin section of 3-10 u were cut and strained with chrome alum haematoxylene phloxine stain (CAHP stain) (Gomori 1941).

Observation and Results:

A cells :- A cells are pyriform with abundant golgi bodies, no mitochondria and are visible with the light microscope and show reduction in the amount of the nissal substance as secretary activity progresses. They have distinct axons. The secretary granules of these cells are differentiated by Gomori's chrome alum haematoxylene-phloxine stain. The A cells are of four different pattern such as A_1, A_2, A_3 and A_4.

A_1:A_1 cells are largest of all cells, oval or pyriform in shape with axon, blue black in colour. They discharge their secretory products peripherally as well as axonally in the form of small granules.

A_2:These are smaller than the A_1 cells oval or pyriform in shape with axon, blue black in colour. They discharge their secretory products axonally and have no peripheral discharge.

A_3: A_3 cells are smaller than A_1 and A_2, pyriform in shape . They discharge their secretory products axonally and have no peripheral discharge.

A_4:A_4 cell are smaller than A_1, A_2 , A_3,oval in shape and their secretary product form large discrete irregular clumps in the cytoplasm which stain deep blue with CAHP.

B Cell: B cells are smaller than A cells. These are pyriform and have never been observed to exceed 16µm - 17µm in diameter. The cell membrane is not especially stainable. B cell have small nucleus with deeply staining chromatin network of red color. Their oval nucleus measures about 7 to 8µm in diameter.

C Cells:-The 'C' cells are one of the three types of neurosecretory cells of millipede, Anoplodesmus tanjoricus found distributedin the brain. In general they are small.15 µm to 17 µm in diameter, pyriform or spherical in shape differing from other neurosecretory cells by their totally different chromatin rich nuclei. The nucleus, measuring about 5µm to 6µm diameter,is either spherical or oval usually dense chromatin material.The secretary granules of the cytoplasm are stainble, deep blue with chrome alum haematoxylene- phloxine. These granules thus represent cystrine/ cysteine material, according to the presence or absence of axonal transport of neurosecretory material. The C cells could be distinguished into two subtypes C_1 and C_2 cells.

C_1 cell: They are distributed along the ventral and mesial side of the brain, small in size. They are situated along the middle line and either sides of the brain. The axons of the majority of the C1 neurosecretory cells are directed towards the lateral oesophageal connectives and they end in a pair of neurohaemal organs called connective bodies. C_2 cell: They are distributed at randam in different region of the brain. They have no distinct cellular processes to name as axons. They scattered uniformly in the brain. Their discharge in peripheral ,evidently they have no end organs. They also scattered irregularly among other neurons and neuroglia of the brain and VNC. They also occur through in frequently, in the neurophile and liberate secretory products probably from the cell periphery.

Millipede Anoplodesmus tanjoricus the effect of low temperature showed greater amount of discharge of neurosecretory material in comparison with control. Nuclei of neurosecretory cells of treated millipede displayed reduced over those of control and scanty neurosecretory material was scanty in their perikarya and axon. The (C/N) ratio showed pronounced alteration, staining property and neurosecretory activity were also affected. Thermal stress, severally affected the pyriform cells as compare to oval cells in brain, when compared with of control. Changes in histomorphology of subtypes of A ,B and C cells are found to be more or less similar. The cell size and nuclear size of all A , B and C cells was found to be decrease upto 12^0C &10 ^{0}C hours Alteration in A_1,A_2,A_3 and A_4 neurosecretory cells of millipede Anoplodesmus tanjoricus exposed to low temperature was observed. The C/N ratio increased and it is inversely proportional to rate of synthesis. In C_1 and C_2 cells, there was a significant decrease in cell as well nuclear diameter. The C/N ratio increased and it is inversely proportional to rate of synthesis. The neurosecretory activity which includes synthesis as well as transport of neurosecretory material was thus accelerated under the influence of temperature. The chromatin material inside the nucleus was intact but the cell wall and neuropile region was slightly distorted.

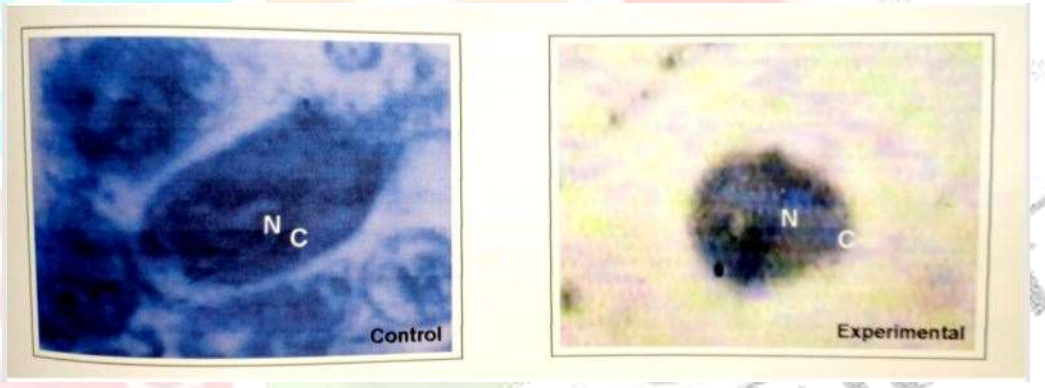


Figure 1: Photograph of A1 neurosecretory cells of cerebral ganglion of control and experimental millipede after exposure to low temperature(10^0C); 1000x; C-Cytoplasm, N-Nucleus

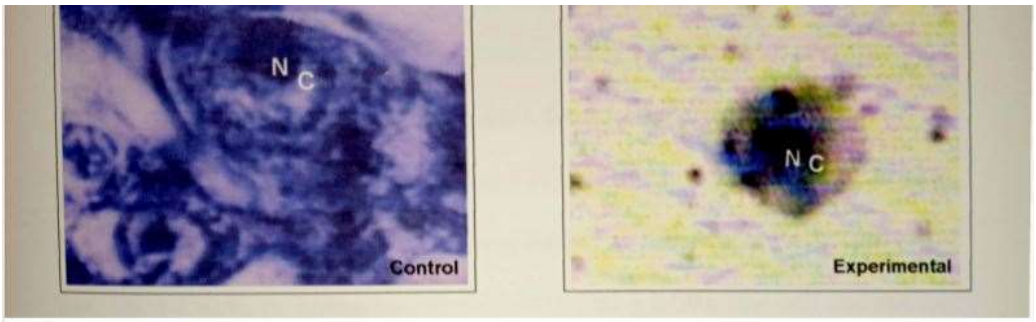


Figure 2: Photograph of A2 neurosecretory cells of cerebral ganglion of control and experimental millipede after exposure to low temperature(10^0C); 1000x; C-Cytoplasm, N-Nucleus

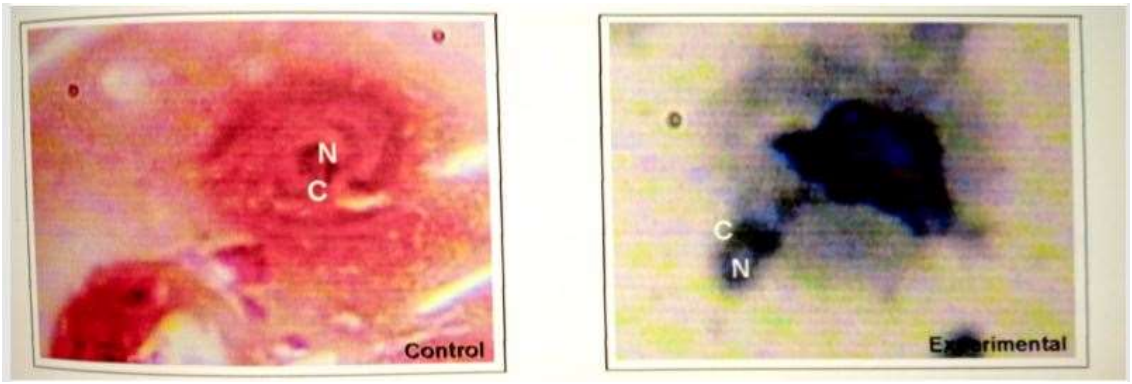


Figure 3: Photograph of A3 neurosecretory cells of cerebral ganglion of control and experimental millipede after exposure to low temperature(10^0C); 1000x; C-Cytoplasm, N-Nucleus

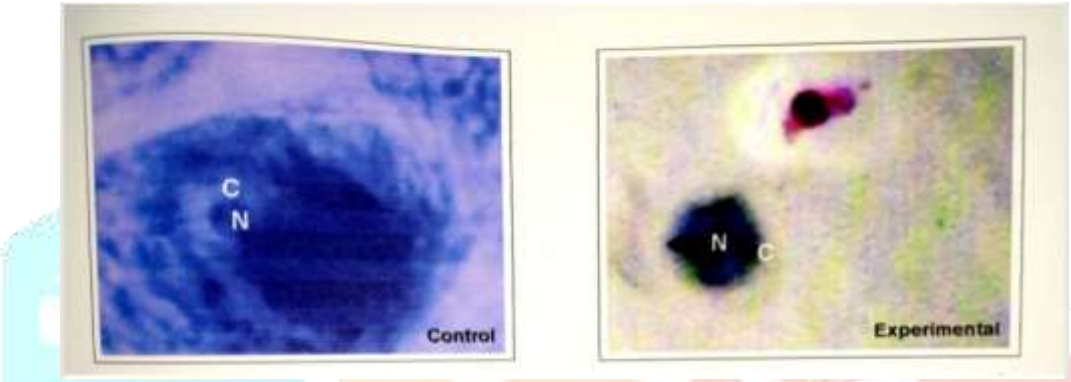


Figure 4: Photograph of A4 neurosecretory cells of cerebral ganglion of control and experimental millipede after exposure to low temperature(10^0C); 1000x; C-Cytoplasm, N-Nucleus

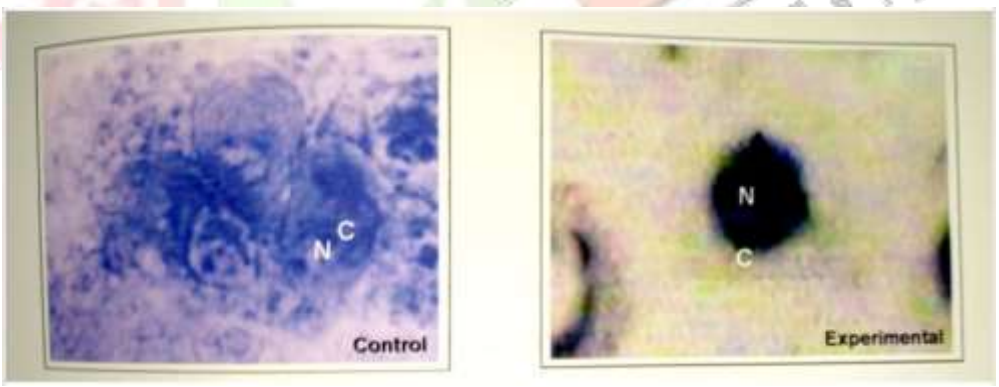


Figure 5: Photograph of B neurosecretory cells of cerebral ganglion of control and experimental millipede after exposure to low temperature(10^0C); 1000x; C-Cytoplasm, N-Nucleus

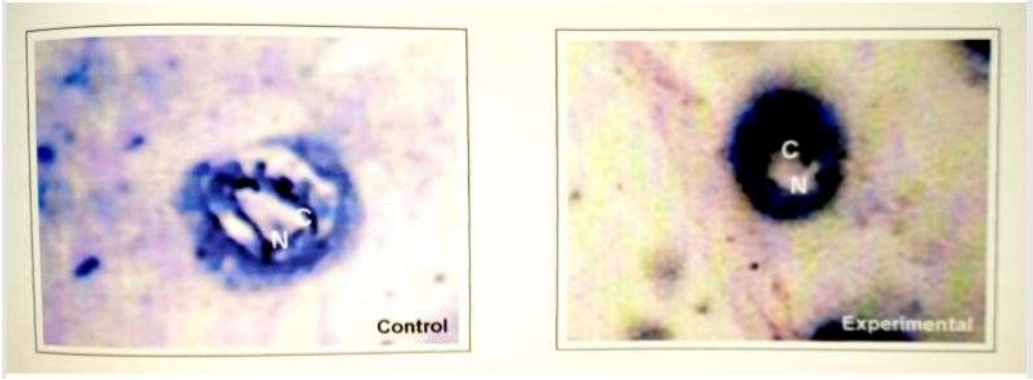


Figure 6: Photograph of C1 neurosecretory cells of cerebral ganglion of control and experimental millipede after exposure to low temperature(10^0C); 1000x; C-Cytoplasm, N-Nucleus

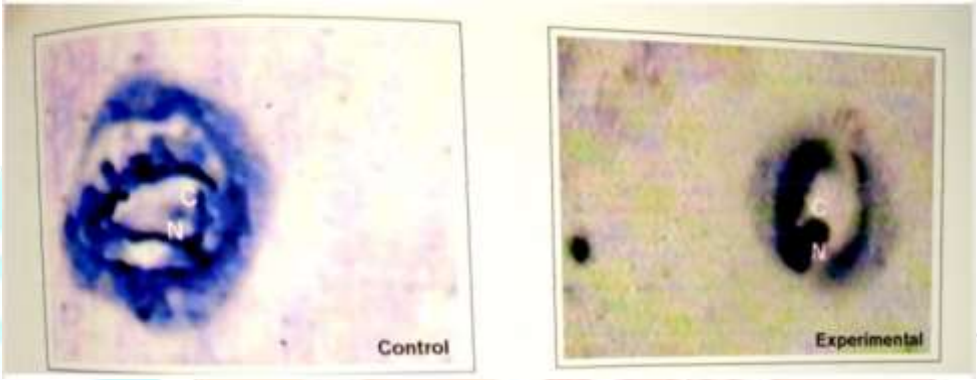
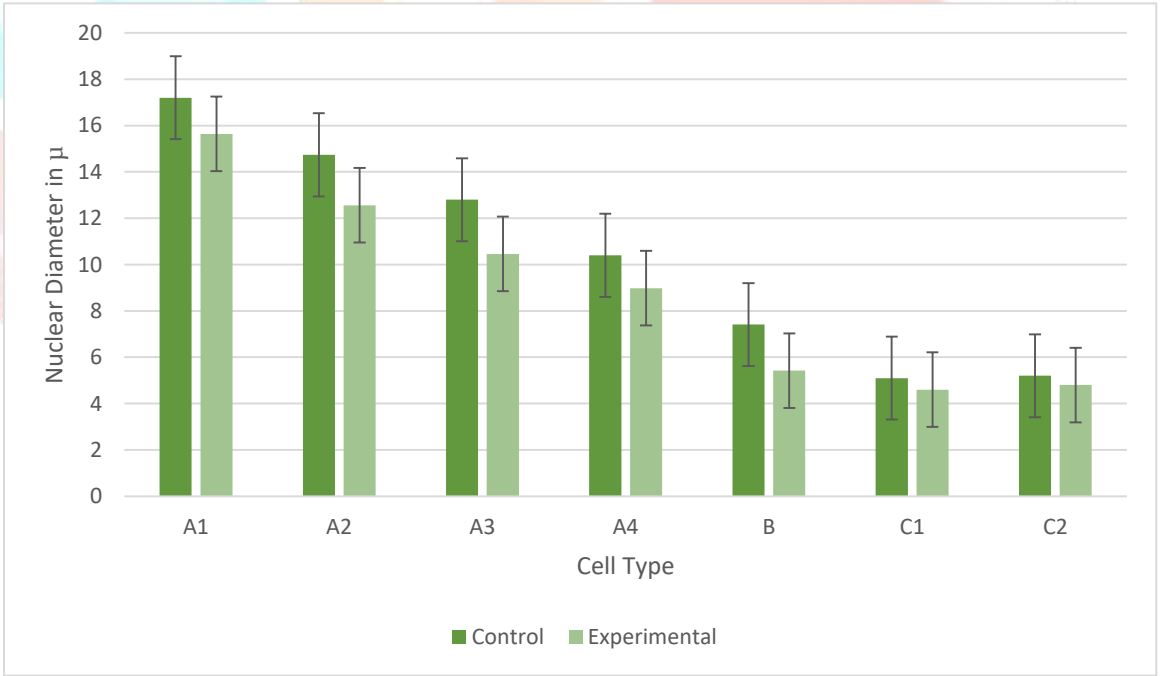


Figure 7: Photograph of C2 neurosecretory cells of cerebral ganglion of control and experimental millipede after exposure to low temperature(10^0C); 1000x; C-Cytoplasm, N-Nucleus

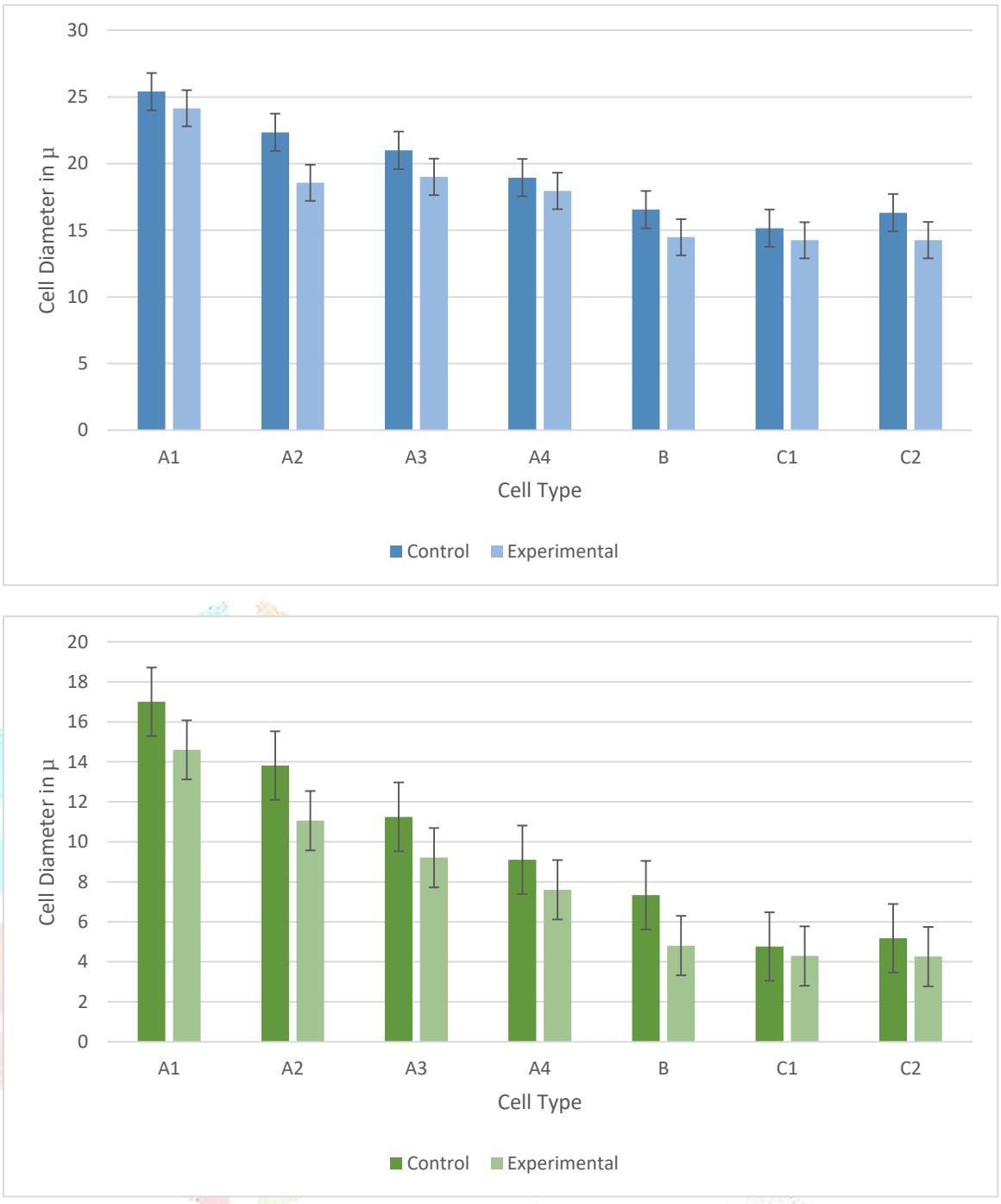
		12 ± 0.5˚C			10 ± 0.5˚C		
Cell type		Cell size in u	Nuclear size in u	C/N ratio	Cell size in u	Nuclear size in u	C/N ratio
A_1	C	25.12 ± 0.53	17.20 ± 0.81	1.46	25.18 ± 0.45	17.00 ± 0.20	1.47
	E	24.72 ± 0.24*	15.64 ± 0.21	1.58	24.15 ± 0.40*	14.60 ± 0.18*	1.65
	% change	(-1.59)	(-9.06)		(-4.92)	(-14.11)	
A_2	C	22.30 ± 0.41	14.74 ± 0.50	1.51	22.35 ± 0.40	13.81 ± 0.50	1.61
	E	19.32 ± 0.15	12.56 ±0.27*	1.53	18.56 ± 0.42	11.06 ± 0.18*	1.67
	% change	(-13.36)	(-14.78)		(-16.79)	(-19.91)	
A_3	C	20.80 ± 0.31	12.80 ± 0.35	1.62	21.00 ± 0.38	11,25 ± 0.56	1.86
	E	19.32 ± 0.14	10.46 ± 0.19	1.84	19.00 ± 0.88*	9.21 ± 0.76*	2.06
	% change	(-7.11)	(9.06)		(-9.52)	(-18.13)	
A_4	C	18.80 ± 0.29	10.40 ± 0.33	1.80	18.95 ± 0.21	9.10 ± 0.25	2.08

	E % change	17.82 ± 0.13* (-5.21)	8.98± 0.18** (-13.65)	1.98	17.95 ± 0.33 (-5.27)	7.60 ± 0.35 (-15.48)	2.36
B	C E % change	16.50 ± 0.59 14.76 ± 0.44 (-10.54)	7.41 ± 0.39 5.42 ± 0.23* (-26.85)	2.22 2.72	16.55 ± 0.39 14.48 ± 0.30* (-12.50)	7.34 ± 0.50 4.81 ± 0.22 (-34.46)	2.25 3.01
C_1	C E % change	15.10 ± 0.43 14.40 ± 0.23* (-4.63)	5.10 ± 0.61 4.60 ± 0.23 (-9.80)	2.96 3.13	15.16 ± 0.40 14.25 ± 0.22 (-6.00)	4.74 ± 0.23 4.29 ± 0.19* (9.87)	3.18 3.32
C_2	C	16.30 ± 0.35 15.66 ± 0.27 (-3.92)	5.20 ± 0.59 4.80 ± 0.24* (-7.69)	3.13 3.26	16.32 ± 0.40 14.26 ± 0.30* (-12.62)	5.18 ± 0.40 4.26 ± 0.35* (17.76)	3.12 2.28

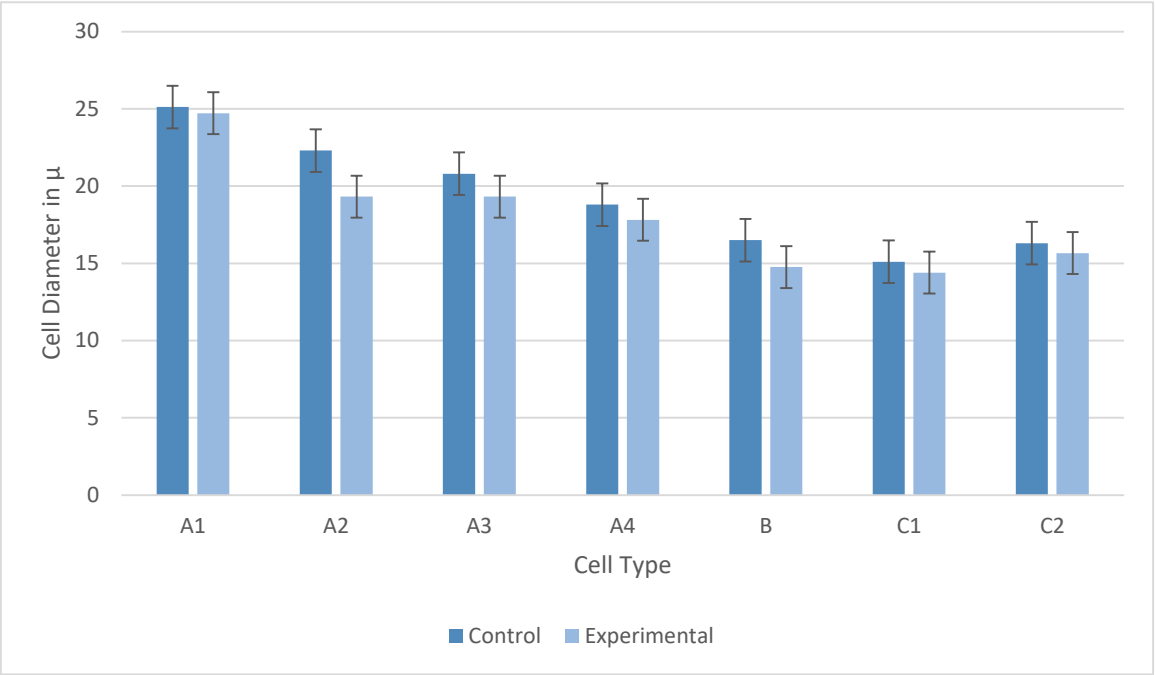
Table 1: Effect of low Temperature on Cell, Nuclear Diameter and C/N ratio of neurosecretory cell of cerebral ganglion of the Anaplodesmus tanjoricus



Graph 1: Effect of low Temperature(12^{0}C) on Cell diameter of neurosecretory cell of cerebral ganglion of the Anaplodesmus tanjoricus.



Graph 3: Effect of low Temperature(10^0C) on Cell diameter of neurosecretory cell of cerebral ganglion of the Anaplodesmus tanjoricus.



Graph 4: Effect of low Temperature(10^0C) on Nuclear diameter of neurosecretory cell of cerebral ganglion of the Anaplodesmus tanjoricus.

Discussion:

In the present investigation the effect of thermal regulation of millipedes to low temperature 12 ± 0.5˚C & 10 ± 0.5˚C on the A, B and C types and their subtypes oval, pyriform, spherical cells from cerebral, visceral, suboesophageal, ventral gangalion were studied. As there cells and nuclei displayed condensed over those of control millipede and scanty neuroseceation was not spotted in their perikarya and axons .(Shukla and Tripathi.,1979) have observed various histopathological changes or cytomorphological character, distribution and mode of discharge of the neurosecretory material into axons or directly through the cell surface into the surrounding blood. An enhanced synthetic activity may be correlated to extent of pollution stress and to maintenance of homeostasis in the internal environment. However,as exposure period was decreased , the C/N ratio was increased over the control of neurosecretory cells. This indicates that long duration of tempreature stress hampared the synthetic activity of neurosecretory cells. The functional status of neurosecretory element is linked with changes in the size of the nucleus and nucleolus and may be considered as the index of cell activity. In the present investigation it was noted that the areas of nucleus and nucleolusis altered. The chromatin material in the nuclei of the neurosecretory cells treated with low temperature, these neurosecretory cells and material must be result of the cessation of the axonal transport and release are extremely not active, therefore transport and release of neurosecretory material is probably faster than the synthesis get decreased in cell size and the cytoplasm nucleus ratio was increased probably due to increase metabolic activity, and due to inactivity of enzymes and number of vacuoles also decreases. At the low temperature there is absence of neurosecretory material or if present, it is in traces. there is reduction in neurosecretory material of the cells when temperature is gradually lowered. The depletion in neurosecretory material at lowered temperature is probably due to the inactivity of enzymes.

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