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Dual Cytology, Cellular Death and Their Significance in the Proximal Ganglionic Complex: an Investigation Through the Ontogeny of the Chick Gallus Gallus Domesticus

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Chick embryos at different stages of development till hatching, as well as the adult tissues were fixed in formol-saline. Serial sections of 8-10 microns thickness were stained by cresyl fast violet to analyse the results in the proximal ganglionic complex of the cranial nerves IX and X. The dark cells are considered as active and the light cells are considered as inactive, resting, dying or degenerating ones. Cell death is most prominent and common among the small and medium sized ones; possibly it is during these stages of cellular growth, peripheral and central processes (of axons) begin to grow from the cell body and get established in their projection fields. The tiny cells are always dark; the very-small cells are also usually dark during the embryonic development till E18. The light cells have appeared among this group of cells just on the day of hatching. When cells fail to establish functional connections and are no more needed, they tend to become inactive, begin to die and disappear from the ganglion. It is assumed that the time of appearance of the light cells might be related to the onset of establishment of functional connections of neurons and to the functional importance of the organs that it supplies.

Key words: Proximal ganglionic complex of nerves IX and X, development, apoptosis

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Introduction

Fascinating changes have been described in different organs during embryonic development in different animals. Several changes in neural structures have been described in many animal species including primates (Carmel and Stein, 1969). This includes the studies relating to the size and density of neural elements (Ptacek and Fagan-Duban, 1974), difference in sensory function (Spassova, 1982), different histogenetic characteristics (Meyer *et al.*, 1973), neuronal death (Pillay, 2000).

Distinction of neurons as dark and light ones based on staining properties has been documented in many vertebrates. The significance of these two populations of neurons has been the subject of considerable speculations. Dual embryonic origin (of epidermal placode and of neural crest origin) (Hamburger, 1961 in birds), fixation artefacts (Cammermeyer, 1962), difference in central and peripheral projections, difference in the distribution of cytoplasmic organelles and relative density of cytoplasm (Carmel and Stein, 1969), stage of activity (Pillay, 2000) etc. have been offered as different hypothesis. However, most workers have confined their studies to certain selected stages during embryonic development or postnatal growth. There is no systematic study performed in the whole life of any one animal species and therefore, no proper interpretation about the significance of their occurrence could be postulated. Therefore, this study is aimed at analysing the complete structural organisation occurring in the proximal ganglionic complex in the chick during embryonic development through adult so as to formulate the significance of these changes.

Materials and Methods

The chicks Gallus gallus domesticus, White Leghorn breed were used in this study. Fertilised eggs were incubated at 37.5 degree centigrade. After every 24 hours, it was considered as Embryonic Day 1 (E1), Embryonic Day 2 (E2) etc. till hatching (H). Embryos till hatching were removed carefully and fixed in 10 % formaldehyde solution at least for two weeks. Larger embryos were cut transversely into suitable smaller pieces and labelled serially for future orientation. The tissues of older embryos (i.e., E15 and onwards till adult) were usually decalcified after fixation. Serial sections of 8 - 10μ were stained by cresyl-fast-violet for Nissl granules. These include E6, E8, E10, E13, E15, E18, chicks on the day of hatching (H) and adult (A). Three animals in each group, having a total of twenty-four animals were used. Ganglia of both sides in each animal (i.e., 6 ganglia for each stage) and therefore 48 ganglia for the whole study were used.

Every section of the ganglion was observed and drawn. The cells are plotted in a diagram with the help of a light microscope. Different categories of neurones were classified into dark and light neurones based on the difference in the intensity of cytoplasmic stain. Each of these types is again subdivided into various subclasses on the basis of size difference and represented in the diagram by a symbol. Only those cells having a clear nucleus and a nucleolus were counted and measured with the help of an eye- piece graticule. The dimension of every cell was determined by calculating the average of the two measurements: one measurement taken on its long axis at the place where the nucleus and its nucleolus were very clearly observed, and another taken at an angle perpendicular to this long axis. However, thepossible error in calculating the size of the cells is considered to be very minimal or negligible. The following categories of cells were classified: Tiny (< 5μ), very small (6-10μ), small (11 - 15μ), medium sized (16 - 20μ), big (21- 25μ), very big (26 - 30μ) and large (31 - 35μ), and very large (36 – 40μ) types. The categorisation of cells on the basis of size with a uniform difference of 5μ was initially maintained just for the sake of convenience. The behaviour of cells, especially that of very-small cells, is very interesting on the day of hatching (uniformly) in all the ganglia studied.

Results

The proximal ganglionic complex of cranial nerves IX and X could be recognized on E6 while it had a rostro-caudal length of 0.328 mm and a volume of 0.0078 mm^3. The ganglion had 17905 cells (P. D = 2295513) all of which were dark type. In all, there were 785 (4.38 %) tiny cells (P. D = 100641), 8222 (45.92 %) very small type (P. D = 1054103), 8444 (47.16 %) small cells (highest P. D = 1082564) and 454 (2.54 %) medium sized ones (P. D = 58205). In many sections larger cells were observed in the periphery while smaller cells were observed in the centre. During the following days, several changes in the composition and volume of the ganglion, distribution and density of different types of cells etc. took place. The ganglion showed great difference in different age groups of animals and in different areas in the same ganglion. The most striking changes are as follows. On E8, the ganglion had a length of 0.360 mm, a volume of 0.0236 mm^3 and contained 31016 cells (P. D = 1314237) all of which were dark type. In all, there were 492 (1.59 %) tiny cells (P. D = 20847), 22987 (74.11 %) very small type (highest P. D = 974025), 7342 (23.67 %) small ones (P. D = 311102) and 195 (0.63 %) medium sized ones (P. D = 8263). In some places the ganglion was divisible into 2 - 3 lobules by means of transverse or oblique partitions formed of connective tissue. On E10, the ganglion had a length of 0.612 mm, a volume of 0.0445 mm^3 and 28813 cells (P. D = 647483) all of which were dark type. In all, there were 2810 (9.75 %) tiny cells (P. D = 63146), 18610 (64.59 %) very small type (highest P. D = 418202), 7019 (24.36 %) small ones (P. D = 157730) and 374 (1.3 %) medium sized ones (P. D = 8405). In some sections of the nerve trunk near the ganglion, neurones were observed in longitudinal rows between nerve fibres. On E13, the ganglion had a length of 0.660 mm, a volume of 0.0676 mm^3 and 26208 cells (P. D = 388843). Among these cells, 19277 (73.55 %) were dark type (P. D = 286009) and 6931 (26.45 %) were light ones (P. D = 102834). In all, there were 302 (1.15 %) tiny cells (P. D = 4481), 13166 (50.24 %) very small type (highest P. D = 195341), 6342 (D = 2956 + L = 3386) (24.2 %) small ones (P. D = 94095), 5142 (D = 2274 + L = 2868) (19.62 %) medium sized ones (P. D = 76291), 1161(D = 537 + L = 624) (4.43 %) big ones (P. D = 17225) and 95 (D = 42 + L = 53) (0.36 %) very big ones (P. D = 1409). In some of the caudally placed sections the ganglion cells were observed mainly in the middle part of the lateral half of the sections, thereby these cells were surrounded on all sides by nerve fibers. On E15, the ganglion had a length of 0.660 mm, a volume of 0.0505 mm^3 and 24677 cells (P. D = 488653). Among these cells, 16770 (67.96 %) were dark type (P. D = 332079) and 7907 (32.04 %) were light ones (P. D = 156574). In all, there were 281 (1.14 %) tiny cells (P. D = 5564), 7451 (30.19 %) very small type (P. D=

147544), 5435 (D= 4221 + L= 1214) (22.02 %) small cells (P. D= 107624), 8131 (D= 3905 + L= 4226) (32.95 %) medium sized ones (highest P. D= 161010), 3282 (D= 867 + L= 2415) (13.3 %) big ones (P. D= 64990), 82 (D= 38 + L= 44) (0.33 %) very big ones (P. D= 1623), 9 (D= 5 + L= 4) (0.04 %) large ones (P. D= 178) and 6 (0.02 %) very large ones (P. D= 119). In most of the sections, the cells were distributed at random without any organized pattern. On E18, the ganglion had a length of 0.900 mm, a volume of 0.2469 mm^3 and 106131 cells (P. D= 429854). Among these cells, 103947 (97.94 %) were dark type (P. D= 421008) and 2184 (2.06 %) were light ones (P. D= 8846).). In all, there were 37646 (35.47 %) tiny cells (highest P. D= 152475), 36189 (34.1 %) very small ones (P. D= 146573), 17228 (D= 16518 + L= 710) (16.23 %) small ones (P. D= 69778), 12344 (D= 11356 + L= 988) medium sized ones (P. D= 49996), 2018 (D= 1657 + L= 361) (1.9 %) big ones (P. D= 8173) and 706 (D=581+L=125) (0.67%) very big ones (P. D= 2859). On the day of hatching, the ganglion had a length of 0.980 mm, a volume of 0.2480 mm^3 and 17536 cells (P. D= 70710). Among these cells, 10354 (59.04 %) were dark type (P. D= 41750) and 7182 (40.96 %) were light ones (P. D= 28960). In all, there were 182 (1.04 %) tiny cells (P. D= 734), 2136 (D= 945 + L= 1191) (12.18 %) very small type (P. D= 8612), 4620 (D= 2251 + L= 2369) (26.35 %) small ones (P. D= 18629), 6380 (D= 3942 + L= 2438) (36.38 %) medium sized ones (highest P. D= 25726), 3142 (D= 2185 + L= 957) (17.92 %) big ones (P. D= 12669), 1013 (D= 799 L= 214) (5.78 %) very big ones (P. D= 4085) and 63 (D= 50 + L= 13) (0.36 %) large ones (P. D= 254). In some of the sections the proximal part of the ganglion contained comparatively more light cells of smaller size. In the adult situation, the ganglion had a length of 1.300 mm, a volume of 0.6408 mm^3 and 13105 cells (P. D= 20451). Among these cells, 10757 (82.08 %) were dark type (P. D= 16787) and 2348 (17.92 %) were light ones (P. D= 3664). In all, there were 509 (3.88 %) tiny cells (P. D= 794), 3192 (D= 2517 + L= 675) (24.36 %) very small type (highest P. D= 4981), 2658 (D= 2047 + L= 611) (20.28 %) small ones (P. D= 4148), 2641 (D= 2052 + L= 589) (20.15 %) medium sized ones (P. D= 4121), 1052 (D= 1004 + L= 48) (8.03 %) big ones P. D= 1642), 1664 (D= 1494 + L= 170) (12.7 %) very big ones (P. D= 2597), 1196 (D= 987 + L= 209) (9.13 %) large ones (P. D= 1866) and 193 (D= 147 + L= 46) (1.47 %) very large ones (P. D= 301).

Discussion

The results show s that the changes taking place in the size of the cell population and the volume of proximal ganglionic complex of cranial nerves IX and X, through whole ontogeny of the chick. The greatest cell population, considered as 100 %, is observed on E18, on the basis of percentage of cellular population for other age groups of animals is calculated. The size of the cellular population in the ganglion on E6 is 16.87 % that rise to 29.23 % on E8, but gradually decreases through E10 and E13 to reach 23.25 % cells on E15. However, there is a sharp increase to reach its greatest value of 100 % on E18. Such increase in the cell population of the ganglion mainly concerns with the tiny and very small type of cells. But this value drops down to 16.52 % on the day of hatching, with a loss of 83.48 % cells that again drops to 12.35 % in the adult situation that seems to be the lowest value through the whole ontogeny. The first increase in the size of cellular population observed on E8 might be mainly concerned with nerve cells, whereas the second increase observed on E18 possibly concerns with phagocytic cells which helps to remove the unsuccessful neuronal elements and fail to establish functional connections therefore die, and later these phagocytic cells themselves disappear from the ganglion. There is a fluctuation in the volume of ganglion that does not seem to correspond with the fluctuation in cell population. The volume of ganglion on E6 is 0.0078 mm^3 that shows a gradual increase through E8, E10 and E13, but drops down on E15. In embryonic stages, the volume of ganglion reaches its maximum 0.2469 mm^3 on E18 while cellular population and its density are also the greatest. The volume remains to be almost same (0.2480 mm^3, with a fractional increase) on the day of hatching while cell population has greatly reduced. However, the ganglionic volume increases by 2.6 times to reach 0.6408 mm^3 in the adult situation while the population size shows further reduction.

The largest cells observed in the proximal ganglionic complex of cranial nerves IX and X on E6 are medium sized ones that continue to be the same through E8 and E10. The big and very big cells have appeared for the first time on E13. The large and very large cells have also appeared on E15 and both disappeared on E18. But once more, the large cells are found in the ganglion on the day of hatching and the very large cells have appeared in the adult situation.

All the cells observed in this ganglion on E6 are dark ones and this continue to be the same through E8 and E10. The light cells have appeared in the ganglion for the first time on E13 whose presence becomes a constant feature through the whole ontogeny. The dark and light cells are dispersed at random throughout the ganglion as from E13 through rest of the ontogeny, unlike the observations of Noden (1978) who found such random distribution only in mature ganglia (i.e., as from 18th day of incubation to adult situation) i.e., from shortly before hatching up to the adult, and not in the younger stages. The very small type of cells are also found to be dark through the whole embryonic stages from E6 to E18; however, on the day of hatching, light cells of this category have appeared and they continue to be present even in the adult situation.

The results in the proximal ganglionic complex of cranial nerves IX and X show that its volume is continuously increasing through the whole ontogeny of the chick till adulthood except on E15 where there is a small reduction due to reduced average cross-sectional diameter. Similarly, length of the ganglion is continuously increasing through the whole ontogeny till adulthood except on E15 where the length remains same as that on E13. In contrast, the number of cells in the ganglion shows fluctuations at many stages: a gradual raise up to E8 and a fall up to E15, then again a sharp increase on E18 a sharp fall on the day of hatching and later again a reduction in adult situation. The reduction in the ganglionic volume during E13 and E15 coincides with comparatively a greater loss of cells within the ganglion. During E 18 greatly increased ganglionic volume there is a tremendous increase in the number of cells but the cellular packing density is reduced, because the volume increase is proportionately greater (4.89 times) than the rate of population increase (4.3 times). Even though large and very large cells on E15 have been removed from the ganglion on E18, the additional space available by this loss is minimal

Table: Illustrates The Distribution Of Dark And Light Cells In The Proximal Ganglionic Complex In The Ontogeny Of The Chick

SIZE AGE	Tiny <5u	Very small 6-10u	Small 11-15u	Medium 16-20u	Big 21-25u	Very big 26-30u	Large 31-35u	Very large 36-40u	Giant >40u	Total Number	Grand Total
E6-D	785	8222	8444	454	0	0	0	0	0	17905	
E6-L	0	0	0	0	0	0	0	0	0	0	17905
E8-D	492	22987	7342	195	0	0	0	0	0	31016	
E8-L	0	0	0	0	0	0	0	0	0	0	31016
E10-D	2810	18610	7019	374	0	0	0	0	0	28813	
E10-L	0	0	0	0	0	0	0	0	0	0	28813
E13-D	302	13166	2956	2274	537	42	0	0	0	19277	
E13-L	0	0	3386	2868	624	53	0	0	0	6931	26208
E15-D	281	7451	4221	3905	867	38	5	2	0	16770	
E15-L	0	0	1214	4226	2415	44	4	4	0	7907	24677
E18-D	37646	36189	16518	11356	1657	581	0	0	0	103947	
E18-L	0	0	710	988	361	125	0	0	0	2184	106131
H-D	182	945	2251	3942	2185	799	50	0	0	10354	
H-L	0	1191	2369	2438	957	214	13	0	0	7182	17536
A-D	509	2517	2047	2052	1004	1494	987	147	0	10757	
A-L	0	675	611	589	48	170	209	46	0	2348	13105

D = DARK CELL, L = LIGHT CELL, E = EMBRYONIC DAY, H = DAY OF HATCHING, A = ADULT

because there are only a 9 large + 6 very large cells. This reduction in the number of cells along with the increased ganglionic volume on the day of hatching suggests availability of the greater space (cell-space) for every cell in the ganglion. The ganglionic volume increase due to several factors: recruitment of additional migratory cells moving away from the neural crest / placode, proliferation of cells which have reached their final position in the ganglion, considerable growth of cell body of the individual cells, increase in number of satellite cells, the development of interstitial spaces and blood vessels etc. Even though there is a fluctuation in the ganglionic volume and in the number of cells at different stages of development, the relationship between size of the cell body, cell number and ganglionic volume provides an indication that it is the expansion of cellular processes which dominates in resulting an increased size of the ganglion. Although there is some degree of fluctuation in the ganglionic volume from time to time, the ganglion-size increase is minimal during early stages of development, i.e., up to E10 whereas from E13 onwards the ganglion shows a rapid and greater increase in volume. There is a reduction in the number of cells during E8 to E15 whereas this had a sharp increase on E18. This suggests that the increase in ganglionic volume during this period is basically due to increased size of the neuropil and that of the cell. The results also show that the ganglionic volume begins to increase greatly around E13 while the cell death comes to an end around E13-E15 so as to reach its maximum size on E18 during embryonic development.

Because of fluctuations observed in the ganglionic volume and in the number of cells available within the ganglion and change in the space (cell-space) available for every cell within the ganglion at different stages of development and growth. This cell-space includes size of each cell along with the tissue (neuropil) available around every cell in the ganglion. Thus the cell-space is increased by 6 times on the day of hatching from that available on E18, by 3.46 times in the adult situation from that available on the day of hatching and by 21 times in adult from available on E18.

Even though the total number of cells has increased on E8, the proliferation rate has reduced during this period as indicated by the presence of reduced number of tiny cells. The increased number of cells observed on E8 might have been produced as a result of a proliferative activity occurring before probably around E7 but has come to an end by E8. The number of very small cells have increased on E8 suggesting cellular growth and maturation process, the reduction in other small and medium sized ones classes might imply that loss was due to cell death, degeneration and phagocytosis. The light cell stage is not observed on E8 possibly due to active and rapid phagocytosis as to remove the inactive or dead cells as soon as they are formed. The reduced number of tiny cells on E8 is indicative of a reduced proliferative activity even though growth and maturation of cells continue among other larger classes. The largest cells remains medium sized ones irrespective of such continued growth and maturation. The cellular death and degeneration taking place at the same time. It could be assumed that this medium sized cells is one of the critical stages in development of the ganglion where an attempt to develop proper projection taking place among the neuronal elements. When this attempt fails, the cells could not grow further and so become inactive and die, and on staining change to a light-colored cell (so-called light cell). This resembles suggested by earlier investigator (Pillay, 2000) that cell degeneration is possibly influenced by the peripheral field of innervation and also the cell-death can be influenced by target cells of a neuron population. It is also possible that cells might undergo exhaustion due to some defects in inherent capacity (endogenous factor) or in the microenvironment (exogenous factor) at any stage of development and growth as advocated by Pillay (2000) even though establishment of proper connections might play an important role in this process.

The presence of greater number of tiny cells on E10 might be suggestive of accelerated proliferative activity. Since there is reduction in the total number of cells and a corresponding reduction among very small and small cells this represent a stage where cellular death is active, even slightly increased number of medium sized cells represent cellular growth and maturation attempting to establish functional connections. The few cells have reached this medium sized stage and assumed that the period of accelerated degeneration (E10-E15) is the period of active establishment of proper connections of ganglion cells (Pillay, 2000). In our results the major cell-death occurs for a longer period, as from E10 to E15.

There is again a reduction in the total number of cells on E13 where large number of light cells have appeared first time

while dark cells have greatly reduced, a greater cellular death and degeneration during this period. The proliferation has stopped or greatly reduced as evidenced by presence of a reduced number of tiny cells, even though continued growth and maturation are represented by appearance of big and very big cells. The light cells have made their appearance among all categories of cells beginning from the stage of small cells onwards. This suggests that presence of cellular inactivity, death and degeneration among these classes of cells. This could have occurred either as a result of unfavorable conditions in the micro environment or in the inherent capacity of cells themselves or by their failure to establish functional projections. It could be assumed that period E13 is a critical stage in the development while an attempt to establish proper projection is taking place among the neuronal elements. When this attempt fails, the cells could not live longer and die. The most active period of cell death and degeneration is observed around E10-E15. The light cells have appeared only as from E13 onwards. The period E10-E15 having accelerated degeneration of cells might be considered as the period of active establishment of functional connections in the proximal ganglionic complex of nerves IX and X. This is in agreement with the findings of earlier investigator (Pillay, 2000).

The total number of cells on E15 is reduced, along with reduction of dark cells but having a greatly increased number of light cells which is suggestive of a greater incidence of cellular inactivity, death and degeneration (Pillay 1999, 2000). In spite of tendency to lose the cells in all categories starting from tiny ones, the appearance of large and very large type of cells indicates the process of cellular growth and maturation, their success in establishing a functional projection. These categories have light cells greater in number indicating an active cellular death and degeneration. It is possible that at least some of these cells are not capable of performing proper function due to some adverse factors, even though the peripheral connection is established. The presence of this defective factor may be confirmed by the results observed on E18 where large and very large classes of cells have again totally disappeared. The reduced total number of cells, but, with the appearance of larger categories on E15 suggests that cellular death and degeneration are taking place at a time while cellular growth and maturation are also continuous. Cell death and degeneration takes place in all stages of development and growth. This process is more accentuated around E10 – E15 with certain amount of fluctuation (cellular increase). This period appears to be one of the critical stages in the development of ganglion while an attempt to develop a functional projection is being continued among the neuronal elements. This emphasizes the suggestion that the period of accelerated degeneration E10 – E 15 is the period of active establishment of functional connections of ganglion cells. This is somewhat similar to the suggestion of Yip and Johnston (1984) that survival of mature neurons of dorsal root ganglion in the new born rats is partially dependent on the availability of nerves growth factor (NGF) transported from the CNS via dorsal root fibres. Those cells have established functional connections will survive because of supply of NGF. However, when this attempt fails, the cells are unable to live longer, become inactive and die.

On E18, there is a great increase in total number of cells and also in all categories starting from the tiny ones suggesting a greatly increased proliferation rate and an increased rate of growth and maturation. In spite of such enormous multiplication of cells, there are only 2184 light cells in the ganglion which indicates that cellular degeneration and removal are very fast and the cells become inactive or die, they are being removed by an active phagocytosis (Pillay, 2000). A comparative an alysis of number of different types of cells found on E18 and those found on the day of hatching shows that there is a great loss of cells between these periods. This huge cellular loss concerns mainly with the large number of tiny, very small, small and medium sized cells. This clearly indicates that multiplication of the smaller classes of cells concerns with a new category with a capacity for phagocytosis to help remove the unsuccessful neuronal elements that have failed to establish functional connections. This process of cell degeneration and removal take place at different stages of their cellular growth and activity (Pillay, 2000). The active phagocytosis may be aimed at removing the remnants of all dead cells that might generate toxic substances within the ganglion; otherwise the toxic substances might prove fatal to the animal.

There is a great loss of cells in the ganglion on day of hatching with the appearance of a greater number of light cells suggesting either a greater cellular death or a reduced rate of removal of dead cells by a reduced number of phagocytes. The large cells have once again made their appearance on the day of hatching which continue to be present even in adult stage. In addition, other larger classes of cells have appeared in greater numbers in adult situation, but with a reduced number of light cells. On the day of hatching, almost all classes of cells the tiny, very small, small and medium sized ones have reduced in number while other classes, the big and very big types have increased. This clearly suggests that there is a continuous cellular growth and maturation during this period, probably as a result of successful establishment of central and peripheral connections. All these suggest that major changes in cell size, cell number etc. occur between E13 and the day of hatching. This observation is similar to that Pillay (2000)

In the adult situation, the total number of cells has further reduced, having 10757 dark cells and 2348 light ones, along with new appearance of very large type of cells in the ganglion. The tiny and very small type of cells have increased in number indicating the new proliferative young cells, probably representing the continued presence of phagocytic cells and some reserve neuroblasts. The increased number of very big and large cells along with the new appearance of 193 very large type in adult situation is suggestive of a continued cellular growth and maturation during post-hatching period. The enormous loss of cells in the ganglion in adult from that found on the day of hatching may be a sign of functional reduction due to ageing process. It is reasonable to assume that the optimum number of cells and neuronal organization are present when the new animal is ready for an independent living, i.e., on the day of hatching, leaving a chance only for further growth and maturation in later life and for other changes due to ageing process. Similar programmed cellular death (Clarke, 1982). Some of the earlier investigators (Hamburger 1975, Pillay 1999, 2000) have suggested that 40 % or more of the neurons that are initially generated fail to survive to maturity.

The details concerning the changes taking place in the cell size, cell number, ganglion size, rate of cell proliferation, rate

of cell death etc. in the ganglion at different stages during development could be noticed or calculated from our results. This varies greatly from stage to stage and among different types of cells without falling under any defined pattern or regulation, having irregular fluctuation in the cell death and volume of ganglion that does not fall under any defined pattern. Therefore, it might be agreed that a non-overlapping temporal, rosrocaudal, ventrodorsal and proximodistal sequence or gradient of neuron proliferation and death occurs in this ganglion. In many situations through the whole ontogeny, it is found that new cells are being formed or added by active proliferation of tiny cells in the ganglion with some interrupted periods of rest or reduced proliferation, while the total number of cells is actually not declining.

The results show that there are more than one period of active proliferation (on E6, E10 and E18) and active degeneration (on E10-E15, on the day of hatching and in the adult) which are indicated by the sharp fluctuations in the number of cells in the ganglion at different stages through the ontogeny of the chick. This ganglion, there is one slow degeneration around E10-E15 reaching its lowest population on E15, then the population increases sharply to reach its maximum level on E18 and later it again decreases to leave a much reduced number of cells on the day of hatching which later during post-hatching period continues to decrease further towards adult situation.

The percentage or rate of cell proliferation and cell loss may be calculated for every stage and for every type of cell during development; however, the results of this calculation does not seem to be constant by this method because proliferation, growth, cell death, degeneration, loss etc occur within different types of cells at different stages of development.

The superior ganglionic complex of cranial nerves IX and X, the dark and the light neurons are dispersed at random within the ganglion even from E13 onwards through the rest of ontogeny. Only dark cells are found in the ganglion up to E10. The light cells appear for first time on E13 and continue to be present afterwards through whole ontogeny of the chick. The tiny cells and very small cells are found to be dark, the whole embryonic period till E18. The very small type of cells keep themselves to be an active till the day of the hatching, cell death and degeneration begin among these cells also as from the day of hatching. There may not be, normally, any need for the establishment of new functional projection at this stage because these connections might have been completed by the day of hatching while the animal is ready for an independent living. There is no need for the growth and maturation of the smaller class of cells since there is no need to replace larger classes of cells that have well-established functional connections. The behaviour of cells in ganglion is almost similar to other ganglion studied (Pillay, 2000). On the day of hatching, there are 10354 dark cells and 7182 light ones in adult situation, there are 10757 dark type and 2348 light ones. The number of dark cells remain almost the same both on the day of hatching and in the adult situation. The reduction in the total number of cells observed in the adult animal is mainly due to the disappearance of light cells. This behaviour again confirms the role of the light cells as representing inactive or degenerating cells in the ganglion.

D'Amico-Martel (1982) suggested that neurons in the ganglia associated with cranial nerves VII, IX and X have a dual embryonic origin. The proximal crest and distal placodal relations occur in these ganglia; however, cells from two sources are not just apposed in a single ganglion but form separate ganglia. This might mean that the proximal (superior) ganglia of nerve IX and of nerve X (jugular ganglion) might have derived from neural crest cells and the distal ganglia (petrous ganglion of nerve IX and nodose ganglion of nerve X) might have developed from placodal cells and that in any particular ganglion, either small or large type must alone be present. The proximal ganglionic complex (combined proximal ganglion of nerves IX and X) all the cells must be of the small type (crest derived) and those found either in the petrous or nodose ganglion must be larger cells (placode derived). The superior ganglionic complex of these cranial nerves IX and X itself the distinct segregation of small (proximal and core regions) and larger (distal and peripheral regions) cells is found at least in a few sections on E6 during development. According to the descriptions of the above investigator, such condition must not occur and all the cells found in the ganglion must be of similar size, if they are segregated in different ganglia as stated by him. Again that all three ganglia (proximal ganglionic complex, petrous and nodose ganglia) exhibit both smaller as well as larger classes of cells almost in equal capacity throughout the ganglia, having almost all categories of cells which again seems to contradict his statements.

It might be agreed that several convergent intrinsic and extrinsic factors might play a role on the regulative and/or stimulative control of the behaviour of cells in this ganglion during cellular proliferation, growth, maturation, establishment of projection, cell death, degeneration and ageing process.

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