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Cellular Death as a Regulative Mechanism in the Control of Cell-Number towards Normal Establishment of Orderly Structure and Function in the Petrous Ganglion during Development and Aging: An Investigation in the Chick

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Abstract: On the evidence available from the present study in the Petrous ganglion, the dark cells are considered as active ones and the light cells are considered as resting, inactive, dying or degenerating cells. Cell death is most prominent and common among the small and medium sized ones. Probably, it is during these developmental stages, 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 embryonic development till E18. The light cells have appeared among this cell group just on the day of hatching.

During the late stages of development (around E15 - E18). the phagocytosis is too fast that the inactive, dying or dead cells (I. e., so-called light cells) are removed immediately as soon as they are formed so as not to leave such a light-cell stage for clear observation. The light-cell stage becomes clearly observable only when the phagocytic process is slow, and this becomes prominent at a time when some of the important connections are being actively established.

The fluctuation in the number of cells during embryonic development may be considered as a normal process for the purpose of re-arrangement and better organization to perform an orderly function most efficiently. The period of accelerated degeneration or loss of cells is the period of active establishment of proper connections of ganglion cells. The reduction or loss in the number of neurons in the adult ganglion might indicate a functional reduction probably as a result of aging process.

Key words: Petrous ganglion, apoptosis, development, aging

Introduction

Distinction of dark and light neurons, based on staining properties has been documented in vertebrates (Peach, 1972; Meyer *et al*., 1973) including primates (Kerr, 1967; Carmel and Stein, 1969). The significance of these two population of neurons has been the subject of considerable speculations. Dual embryonic origin (of epidermal placode and of neural crest origin) (Hamburger, 1961), as fixation artifacts (Cammermeyer, 1962), difference in central and peripheral projections (Preto Parvis, 1954), different sensory functions (Noden, 1980; Spassova, 1982), different histogenetic characteristics (Meyer *et al*., 1973), difference in distribution of cytoplasmic organelles and relative density of cytoplasm (Carmel and Stein, 1969; Matsuura *et al*., 1969), fluid shift between cells and the surrounding extracellular spaces (Moses, 1967), difference in functional activity have been offered as different hypotheses. Kalina and Wolman (1970), Peach (1972) and Silbermann and Finkelbrand (1978) found differences in chemical constituents in these two types of neurons in sensory ganglia of rodents. Similar observations have been reported in mammals (Cauna and Naik, 1963; Kalina and Bubis, 1969; Peach, 1972; Kishida *et al*., 1982).

There is no report of a study of this kind in the whole life cycle of any one animal species so as to infer a conclusive significance of this dual cytology of neurons. All these works have been done in adult animals or in certain stages of development or growth. Therefore it is thought useful to study this subject matter in the Petrous ganglion through the whole life cycle of the chick (during embryonic development through adult) so as to form a conclusive significance and hypothesis for the occurrence of these two types of neurons.

Materials and Methods

The chicks *Gallus gallus domesticus,* White Leghorn breed were used in this study. Fertilized eggs were collected in groups of 25 - 30, and incubated at a temperature of 37.5 degree Centigrade. The date and time while beginning the incubation were recorded every time when a new set of eggs was used. After every 24 hours from this time, it was considered as Embryonic Day I (El), Embryonic Day 2 (E2) etc till hatching (H). Embryos from E3 till hatching were removed carefully without causing damage and fixed in 10% formaldehyde solution at least for two weeks. Larger (older) embryos were cut transversely into suitable smaller pieces and labeled serially for future orientation. The tissues of older embryos (i.e., El 5 and onwards till adult) were usually decalcified after fixation. In the adult, and in those belonging to later stages of development, the head at the level of C6 was separated and the brain was exposed by a longitudinal cut on the skull by means of a thin bone-saw, to facilitate proper fixation of the brain tissue. Serial sections of 8 - 10 microns were stained by Cresyl Fast Violet for Nissl granules.

Only a few selected stages which showed some remarkable changes are described in this study. These include E6, E8, E10, E13, E15, E18, chick on the day of hatching and adult. In all, three animals in each group, having a total of twenty four animals were used. Ganglia of both sides (right and left sides) in each animal were used for observation. Every section of the ganglion was observed, drawn and the cells were plotted in diagram with the help of light microscope having a camera Weida attachment. Neurons were classified into Dark and Light neurons according to the

Table 1: Illustrates the Total Number of Dark and Light Cells in the Petrous Ganglion in Different Age-groups of Animals in the Ontogeny of the Chick

SIZE	Tiny	Very	small	Small	Medium	Big	Very big	Large	Very large	Giant	Total	Grand
AGE	<5u	6-10u	11.15u	1 6-20u	21-25u	26-30u	31-35u	36-40u	>40u	Number	Total	
E6-D	205	3681	3484	408	0	0	0	0	0	7778		
E6-,L		0	0	0	0	0	0	0	0	0	7778	
E8-D	106	3446	4579	235	13	0	0	0		8379		
E8-L	0	0	0	0	0	0	0	0	0	0	8379	
E10-D	839	3244	2211	475	89	0	0	0	0	6858		
E10-L	0	0	7	1	0	0	0	0	0	8	6866	
E13-D	128	6191	853	440	326	22	0	0	0	7960		
El 3-L	0	0	990	575	463	34	0	0	0	2062	10022	
El 5-0	399	3097	1556	1521	93	7	0	0	0	6673		
E15-L	0	0	474	845	128	6	0	0	0	1453	8126	
E18-D	10948	9937	6136	3577	635	229	0	0	0	31462		
E18-L	0	0	197	340	121	83	0	0	0	741	32203	
H-D	42	50	288	992	258	81		0	0	1711		
H-L	0	409	799	880	55	5	0	0	0	2148	3859	
A-D	52	85	304	1053	291	85	0	0	0	1870		
A-L	0	150	445	463	58	6	0	0	0	1122	2992	

D = Dark Cells, L = Light Cells. E Embryonic Day, H = Day of Hatching, a = Adult

difference in intensity of cytoplasmic stain. Each of these types is again subdivided into various subclasses represented in the diagram by a symbol. The dark cells were represented by a pen (ink) and the light cells by a lead pencil. Only those cells having a clear nucleus and a nucleolus were counted and measured with the help of an eye piece graticule. The following categories of cells were classified. Tiny (<5 microns), very small (6 - 10 microns), small (11 - 15 microns), medium sized (16 - 20 microns), big (21 - 25 microns), very big (26 - 30 microns) (Table 1).

Results

Population Density (P. D = in mm^3) for certain types of cells is given in parenthesis, to avoid repetition. When dark neurons alone are present in a ganglion, they are represented just by numbers; however, when they are mixed with light neurons, D = dark neurons, and L = light neurons. The Petrous ganglion could be recognized on E6 while it had a rostro-caudal length of 0.336 mm, a volume of 0.0077 mm^3 and had 7778 cells (P. D = 1010130) all of which were dark type. In all, there were 205 (2.64 %) tiny cells, 3681 (47.33 %) very small ones (greatest P. D = 478052), 3484 (44.79 %) small ones (P. D = 452468) and 408 (5.25 %) medium sized ones (P. D = 52987). 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. On E8, the ganglion had a length of 0.360 mm, a volume of 0.0118 mm^3 and had 8379 cells (P. D = 719085), all of which were dark type. In all, there were 106 (1.27 %) tiny cells (P. D = 8983), 3446 (41.13 %) very small ones, 4579 (54.65 %) small ones (greatest P. D = 38805), 235 (2.8 %) medium sized ones (P. ID = 19915) and 13 (0.16 %) big ones (P. D = 1102). On E10, the ganglion had a length of 0.360 mm, a volume of 0.0157 mm^3 and had 68866 cells (P. D. 437325). Among these cells, 6858 (99.88 %) cells were dark type (P. D = 436815) and 8 (0.12 %) were light ones (P. D = 510). In all, there were 839 (12.22 %) tiny cells (P. D = 53439), 3244 (47.25 %) very small ones (greatest P. D = 206624), 2218 (D = 2211 + L = 7) (32.3 %) small ones (P. D = 141274), 476 (D = 475 + L = 1) (6.93 %) medium sized ones (P. D = 30319) and 89 (1.3 %) big ones (P. D = 5669). On E13, the ganglion had a length of 0.370 mm, a volume of 0.0243 mm^3 and had 10022 cells (P. D = 412428). Among these cells, 7960 479.43 %) were dark type (P. D = 327572) and 2062 (20.57 %) were light ones (P. D = 84856). In all, there were 128 (1.28 %) tiny cells (P. D = 5267), 6191 (61.78 %) very small ones (greatest P. D = 254774), 1843 (ID = 853 + L = 990) (18.39 %) small ones (P. D = 75844), 1015 (D = 440 + L = 575) (10.13 %) medium sized ones (P. D = 41770), 789 (D = 326 + L = 463) (7.87 %) big ones (P. D = 32469) and 56 (D = 22 + I = 34) (0.56 %) very big ones (P. D = 2304). On E15, the ganglion had a length of 0.450 mm, a volume of 0.0425 mm^3 and had 8126 cells (P. D = 191200). Among these cells, 6673 (82.12 %) were dark type (P. D = 15702) and 1453 (17.88 %) were light ones (P. D = 34188). In all, there were 399 (4.91 %) tiny cells (P.D = 9388), 3097 (38.11%) very small ones (greatest P. D = 72871), 2030 (D = 1556 + L = 474) (24.98 %) small ones (P.D = 47765), 2366 (D = 1521 + L = 845) (29.12 %) medium sized ones (P. D = 55670), 221 (D = 93 + L = 128) (2.72 %) big ones (P. D = 5200) and 13 (D = 7 + L = 6) (0.16 %) very big ones (P. D = 306). On E18, the ganglion had a length of 0.470 mm, a volume of 0.0561 mm^3 and had 32203 cells (P.D = 574029). Among these cells, 31462 (97.7 %) were dark type (P. D = 560820) and 741 (2.3 %) were light ones (P.D = 13209). In all, there were 10948 (34 %) tiny cells (greatest P. D = 195152), 9937 (30.86 %) very small ones (P. D = 177130), 6333 (D = 6136 + L = 197) (19.67 %) small ones (P. 0 = 112888), 3917 (D = 3577 + L = 340) (12.16%) mediumsized ones (P.D = 69822), 756 (D = 635 + L = 121) (2.35%) big ones (P. D = 13476) and 312 (D = 229 + L = 83) (0.97%) very big ones (P. D = 5561).

On the day of hatching, the ganglion had a length of 0.540 mm, a volume of 0.0548 mm^3 and had 3859 cells (P. D = 70420). Among these cells, 1711 (44.34 %) were dark type (P. D = 31223) and 2148 (55.66 %) were light ones. In all, there were 42 (1.09 %) tiny cells (P. D = 766), 459 (D = 50 + L = 409) (11.89 %) very small ones (P. 8376), 1087 (D = 288 + L = 799) (28.17 %) small ones (P. D = 19835), 1872 (D = 992 + L = 880) (48.51 %) medium sized ones (greatest P. D = 34160), 313 (D = 258 + L = 55) (8.11 96) big ones (P. D. 5712) and 86 (D . 81 +

L = 5) (2.23 %) very big ones (P.D = 1569). In the volume of 0.2390 mm^3 and had 2992 cells (P. D = 12519). Among these cells, 1870 (62.5 %) were dark type (P.D = 7824) and 1122 (37.5 %) were light ones (P. D = 4695). In all, there were 52 (1.74 %) tiny cells (P. D = 218), 235 (D = 85 + L = 150) (7.85 %) very small ones (P. D = 984), 749 (D = 304 + L = 445) (25.03 %) small ones (P. D = 3134), 1516 (D = 1053 + L = 463) (50.67 %) medium sized ones (greatest P. D = 6343), 349 (D = 291 + L = 58) (11.66 %) big ones (P. D = 1461) and 91 (D = 85 + L = 6) (3.04 %) very big ones (P. D = 381).

Discussion

In the present study the two types of cells, the dark and light neurons are dispersed at random throughout the ganglion from E10 onwards through the rest of the ontogeny unlike the observations of Gaik and Farbman (1973), Ciani *et al*. (1973), Finkelbrand and Silberman (1977) and Noden (1980) who found such random distribution only in mature (from 18th day of incubation to adult) ganglia, i.e., from shortly before hatching to adult and not in the younger stages. It may be noticed that only dark cells are observed in the ganglion up to E8. The light cells appear for the first time on E10 and continue to be present afterwards through the whole ontogeny of the chick. The tiny cells are found to be always dark. The very small cells are also dark through the whole embryonic period till E18. Later however, the light cells have appeared among the very-small type of cells as well on the day of hatching and continue to be present even in the adult situation. This might imply that even though the very-small type of cells appear to keep themselves to be an active group of cells till the day of hatching and be ready to replace the dead cells (occurring as a result of several adverse factors), cell death and degeneration begin among these cells also as from the day of hatching. It might be assumed that normally there cannot be any more necessity for the establishment of new functional projection after the day of hatching since all these connections might have been already complete by this time while the animal is ready to lead an independent living. Therefore, there is no need for further growth and maturation of these smaller classes of cells and, the cell death begins even among these very-small cells as from the day of hatching in order to prevent further growth of these cells which seems unnecessary. This phenomenon is clearly evidenced by the occurrence of dark and light neurons.

All the cells observed in the ganglion on E6 are dark type which continue to be present through E8. The light cells make their first appearance on E10 which continue to be a constant feature through the whole ontogeny. However, their number, proportion and density vary greatly within different parts of the ganglion and through different age groups of animals. The tiny cells are always found to be dark type. The very small type of cells are also found to be dark on E6 and through the whole embryonic stages till E18; however, some light cells of this category have appeared on the day of hatching which continue to be present in the adult situation as well.

On a comparative analysis of the results in the Petrous ganglion on E6 and E8, the total number of cells has increased to 8379 cells on ES even though the proliferation rate is reduced (on E8) as indicated by the reduced number of 106 tiny cells from that (205 cells) observed on E6. This increase in the total number of cells might be due to the proliferative activity which might have occurred between these two stages, i.e., some-where around E7. Since the largest class of cells observed on E6 is medium sized ones and the big cells have made their appearance for the first time on E8, it might be assumed that normal growth and maturation process is progressing well while the proliferative activity has reduced or nearly stopped on E8 as indicated by the reduced number of tiny cells as explained above. However, the results on E10 show a reduced number of cells (to 6866) having 6858 dark type and 8 light ones, and showing an increased proliferative activity as evidenced by an increased number of 839 tiny cells. However the largest class of cells remains to be the same big ones as that observed on E8. It is to be noticed that the appearance of a small number of 8 light cells is associated with the reduction in the total number of cells even though proliferative activity is found to be increased than that observed on E8. This might imply that the degeneration process is greater than the proliferative activity. However, this in turn might suggest that a greater number of phagocytic cells are also produced in addition to a greater number of neurons by this proliferation in order to remove those degenerating cells.

At this juncture, it is worth recalling the observation of Hamburger and Narayanan (1969) in the trigeminal ganglion. They showed that by the 8th day of incubation, at least some of its cells have established peripheral *and* central connections as indicated by the presence of reflexogenic responses to tactile stimulus of the beak. Noden (1980) has also found that many trigeminal sensory cells have extensive peripheral projections by the end of the first week of incubation and suggested that these cells must cease dividing early in embryonic development. In the present series of investigation in the trigeminal ganglion also, it is during this period (E8) the light cells have made their first appearance as well. This coincidence of the occurrence of light cells in the trigeminal ganglion in the present series of investigation and the results of the above mentioned investigators giving evidence on the of establishment of connections during the same period (E8) as explained above might clearly suggest that those cells which fail to establish proper functional connections might become inactive, lose their functions, die and, finally degenerate and disappear. Probably the light cells observed in the present series of investigation represent the beginning stage of this process of inactivity and degeneration. From this, it might be assumed that those cells that fail to establish proper central and peripheral connections might become inactive or die and change to light colored cells on staining. The reduction in the total number of cells in the Petrous ganglion on E10 coincidental with the appearance of 8 light cells and a reduction in the number of smaller classes of cells viz, very small and small types are suggestive of cellular death and degeneration. On the other hand, the presence of increased number of tiny cells and other larger classes viz, the medium sized and big cells are suggestive of cellular proliferation, growth and maturation processes. Thus E10 represents a stage where cellular death and degeneration are taking place in some groups of cells while there are proliferation, growth and maturation processes taking place in other cell-groups to facilitate establishing proper organization and proper functional connections of these ganglion cells in an

attempt to replace the dead ones. Thus the light cells might represent a group of inactive, dead or degenerating cells in this ganglion. This resembles the suggestion of Hamburger and Levi-Montalcini (1949), Hamburger (1958), Cowan and Wenger (1967) and Cowan (1973) that cell degeneration is probably influenced by the peripheral field of innervation and that cell death can be influenced by the target cells of a neuron population. However, it might also be assumed that the cells might undergo exhaustion due to some defects in their inherent capacity (endogenous factor) or in their micro-environment (exogenous factor) at any stage of development and growth as advocated by Ernst (1926). The establishment of proper connections might also play an important role in this process. However, it is assumed that the period of accelerated degeneration is the period of active establishment of proper connections of the ganglion cells. This is some-what similar to the suggestion of Rubel *et al*. (1976) about cell death which they observed among the cells in the brain stem auditory nuclei where most cell death occur during the period between E11 and E13. The behavior of petrous ganglion in the present study is somewhat similar to their observation. However, the period of active degeneration of cells and the period of active establishment of proper projections continue for a prolonged and extended period around E10 - E15. This period shows clear cellular death with a slight fluctuation (raise) in the cell population on E13.

The results on E13 show that the total number of cells has increased to 10022 having 7960 dark cells and 2052 light ones. Here the increase in the total number of cells concerns mainly with the formation of greater number of light cells even though the dark cells have also slightly increased. The presence of a slightly increased number of 6191 very small cells and reduction in the number of tiny cells indicate that the proliferative activity has stopped and the cells are in the process of growth. Since the tiny cells have reduced in number at this stage (E13) and the total number of cells has increased, it could be assumed that the increased proliferative activity observed on E10 might have been very fast and that it has continued for a longer period so as to increase the total number of cells (to 10022 cells) on E13, but this action might have reduced or stopped later so that the number of tiny cells (on E13) has reduced. Again it is noteworthy that even the very big type of cells have formed on El3 in spite of the presence of many (2062 cells) light cells (which represent a greater cellular inactivity, death and / or degeneration) which in turn might explain that an increased number of cells have failed to establish proper peripheral projection and have become inactive and began to die. The presence of greater number of light cells during this period might indicate that establishment of peripheral projection is active around E10-E13. It has been shown by Yip and Johnson (1984) that survival of mature neurons of the dorsal root ganglion in new born rats is partially dependent on the availability of nerve growth factor (NGF) transported from the CNS via the dorsal root fibers. This also supports the suggestion that those cells which have established proper projection will survive because of adequate supply of NGF and that others will die. However, it is possible to assume that even after establishment of connections to proper projection-fields, if these fibers are unable to transport NGF, or if the NGF available for transport through these fibers is inadequate to keep the optimum amount, these cells might die. This again might imply that the cellular death can occour at any stage of their growth, probably even after a peripheral connection is established. But however, this peripheral projection might not be in the proper field and could not succeed in establishing a functional projection and so they become inactive or die. This might be possible that when the peripheral projection established by these cells are in the proper field or when there is a defect in the micro-environment which. probably has developed later (secondarily) either in the projection field or in their passage, these connections might lose their proper functions. Therefore, these cells might be destined to -die by an adverse stimulus (such as inadequate supply of NGF) coming from the CNS las it has been explained by Yip and Johnson (1984) as being responsible to promote nerve growth in the dorsal root ganglion in the new born rail. The results on E15 show that there is a reduction in the total number of cells (to 8126 cells) having 6673 dark type and 1453 light ones. This might Adicate that the degeneration process has been ;active in order to remove the unsuccessful neuronal elements which have failed to establish proper functional projection. It is found that on E15 there is an increased number of 399 tiny cells which continues to increase further (to 10948 cells) on E18. it is also observed that on E18 other larger classes of cells have increased in number resulting in a total of 32203 cells. The increased number of all types of cells (including tiny ones) indicate that all activities of cells including proliferation, growth and maturation processes are active during this short period between E15-E18. At the same time it also becomes clear that the reduced number of 741 light cells on E18 indicates that the phagocytic activity also is greatly increased so that most of the light cells which have occurred (on their failure to establish proper functional projection) have been actively (and quickly) digested and removed from the vicinity of the ganglion. The results on E15 also reveals the same trend as that observed on El3 in that the cellular death can occur at any stage of their development and growth, probably even after a peripheral connection is established.

On a comparative analysis of the results on E 18 and that on the day of hatching, it might be noticed that there is a tremendous reduction in the number of cells leaving just 3859 cells in the ganglion having 1711 dark cells and 2148 light ones. Also all classes of cells have greatly reduced in number. There are only 42 tiny cells in the ganglion indicating a greatly reduced or complete stoppage of proliferative activity. It might be interesting to note that there is a great loss or reduction in the number of every category of cells indicating that the degeneration takes place at all stages of cell growth and maturation. The appearance of light cells even among the very small type of cells on the day of hatching shows that the degeneration takes place even at this early stage of cellular growth once they are no more needed to grow further because the young animal is already prepared for an independent living with all functional connections of its neurons. However, even on the day of hatching, the largest class of cells continues to be the very big type only. This might suggest that a functional projection is established at a stage even before this class of cells (very big cells) is formed. The enormous production and tremendous increase in the number of cells on E18 and their disappearance on the day of hatching resulting in a greatly reduced number of cells indicate that

the purpose of such proliferation is mainly to remove the cells which are unnecessary for proper functioning of the ganglion. That is to say that these newly formed cells around E18 are phagocytic cells which help remove the unsuccessful cellular elements which fail to establish proper functional projection and therefore become inactive and die. Similar findings of the presence of enormous number of phagocytic cells at similar stages have been reported by several investigators. Levi-Montalcini (1950) suggests that phagocytic cells derive from macrophages and serve to digest neuronal debris during cell death. Tennyson (1970) says that satellite cells act as phagocytes to remove the cellular debris in the embryonic ganglia. O'Connor and Wyttenbach (1974) and Pilar and Landmesser (1976) have reported that phagocytosis is accomplished entirely by glial cells. Chu-Wang and Oppenheim (1978) have observed blood borne mononuclear leukocytes acting as phagocytes. The report in the CNS by Levi-Montalcini (1949) that major changes in cell size, nuclear (neuron-group) size and cell number occur between E13 and the day of hatching and that the time of ganglionic volume-increase commences at the end of the period of cell death which begins around E13-E17. This report is similar to the present results as well, the major changes in the ganglion size etc starting around E15, even though the fluctuation in the number of cells etc begins as early as E10. The cellular death and degeneration could be one of the factors that regulate the size of cell population in the ganglion as it has been suggested by Hamburger and Levi-Montalcini (1949), Glucksmann (1951) and Saunders (1966) in their studies. They attributed that the cell death was influenced by the peripheral field of innervation and thus point out the role of this phenomenon in morphogenesis. Michaels *et al*. (1971) suggested that degenerating cells produce hydrolytic enzyme for their own degeneration and digestion. However, the present results have no evidence for this suggestion because the phagocytic cells produced during this period disappear on the day of hatching. However, this suggestion might be reasonable or suitable for the phagocytic cells themselves which appear around E15 - E18 and are thought to be responsible to remove the remnants of the degenerating neuronal elements. If the above suggestion is to be taken for the neuronal elements themselves, then no explanation or meaning could be attributed for the tremendous production and flooding of the tiny cells which appear around E15-E18 and disappear before the day of hatching.

The presence of quite a number of (i.e., 2148) light cells on the day of hatching represents that the process of degeneration and removal of dead cells still continues so that there will be a continuous reduction or loss of cells in the ganglion, during post-hatching period. It might be assumed that most of the toxic substances and other remnants of dead cells have been cleared away from the tissue by the active phagocytic cells which have appeared around E15-E18 as described above. But since the smaller categories of cells have reduced in number it may be assumed that phagocytic cells are also reduced so that the process of removal of the inactive or dead cells is a slow process. However, in the adult situation there is a total of 2992 cells having 1870 dark cells and 1122 light ones. This, in turn shows a continuous removal of dead cells and that the cells are lost among every size-category, although all classes of cells continue to be present. This shows that some of these cells must be phagocytes or cells which have the capacity to digest and remove the dead cells. Probably the continued presence of such phagocytic cells is a safety procedure for a better, effective and complete removal of any residue of the dead cells left behind in the ganglion (the presence of which would prove toxic or fatal to the tissue and to the animal itself), thereby helping the performance of efficient function of neuronal elements. It is reasonable to assume that the optimum number of neurons must be present when the new animal is ready for an independent living, i.e., on the day of hatching. On the day of hatching, one might think of a condition with normal establishment of all organs and systems in the body including that of nervous action and sensibility. This should leave a chance only for further growth and maturation of these structure in later life, and finally to changes as a result of aging process. The enormous loss of cells in the adult from that observed on the day of hatching might probably be due to changes as a result of increasing age (functional reduction) and not due to normal developmental or growth changes.

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