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Post Embryonic Development of the Central Nervous System of the Spider *Argiope aurantia* (Lucas) [1]

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ABSTRACT Volumetric and histological changes of the central nervous system were studied during post embryonic development of a spider, *Argiope aurantia*.

The neural mass of *Argiope* grows allometrically with respect to volume of the cephalothorax and body weight. In the first instar 46% of the cephalothoracic volume constitutes the neural mass and this is reduced to 4% in the female (9th stage) and 12% in the male (7th stage) spider.

Growth curves for the cephalic ganglion, measured at all stages, represent a straight line. The neural mass of females is two and a half times larger than that of the males. The ganglion increased 24 fold in female and 10 fold in male spiders. Addition of neural mass occurs in all stages.

The brain volume is greater than that of the subesophageal ganglion in the first two instars. In subsequent stadia, the subesophageal ganglion grows faster, and in females it is finally three times and in males two times larger than the brain.

Growth of cortex and neuropile depict exponential curves. Comparison of growth patterns of these shows an inverse relationship during development. While the volume of the cortex is higher in the first two or three stages, the volume of the neuropile is higher in the remaining stadia. The causes for this growth pattern are discussed.

Counts of cell numbers show that there is a constant population of neurons throughout the post-embryonic development. The number of nerve cells in females is higher than in males, 11% in the subesophageal ganglion and 58% in the brain.

The growth of the cortex is partly accomplished by an increase in cell volume. In male and female spiders the increase in Type-B cells is 20 and 50 fold, while that of large motor neurons is 200 and 600 fold respectively. The motor neurons of 20 μ and above number 63 in male and 916 in female adult spiders.

The growth of neuropile occurs through an increase of dendritic arborization and axonal branching. The largest axons measure 1 μ in the first and 16 μ in adult stages. An increase of incoming sensory fibers is also noticed during development.

Invasion of neural lamella into cortex and neuropile increases during development. Neural lamella which are 1–2 μ in the first stage grow to 40–100 μ thickness in adult female spiders, near the origin of the main nerves. One type of astral cells, counted in neuropile, increases 10 fold.

The appearance of a central body and the beginning of web construction coincide during the second instar. The relationship between these two is discussed.

Although the spiders have been used for several kinds of experimental studies, the post embryonic development of their central nervous system has received little attention. However the nervous system of the adult spider has been the subject of several earlier investigations. Its external morphology was described by Blanchard ('59), Pocock ('02), Haller ('12), Hilton ('12), Buxton ('17), Gerhardt and Kaestner ('37), Millot ('49), Legendre ('53), Firstman ('54) and Babu ('65, '69). Its histology and

[1] This paper is dedicated to the memory of Prof. K. Pampapathi Rao on the first anniversary of his death.

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anatomy was reported by Saint-Rémy ('87). Hanstrom ('19, '21, '28, '35), Legendre ('59), Babu ('65, '69) and Meier ('67).

Studies on post embryonic development of several groups of arthropods and particularly of insects have received considerable attention. In spite of several attractive features, similar studies on spiders are lacking. Spiders pass through a long period of post embryonic development in which the pattern of growth can be measured. They adapt quickly to the laboratory conditions and this makes them easy to rear and study at all stages. The amazing behavioral repertoire of spiders is a special attraction; its understanding demands a study of the growth pattern of the central nervous system.

The aim of the present work is to study in detail the factors that contribute to the growth of the nervous system of female and male spiders of *Argiope aurantia*. Keeping this in view, attempts are made to study the growth pattern of the central nervous system in relation to body size, and the inter-relationship between cortex and neuropile, and also the organization and growth of non-nervous glial elements in all the post embryonic stages.

MATERIALS AND METHODS

Cocoons of the orbweb spider *Argiope aurantia* Lucas (family: Araneidae, Levi, '68) were collected at Raleigh, North Carolina in the field in the months of December and January. In February and March the spiderlings were released into cages by cutting open the cocoons. Each animal from the second stage ("Stage or stadium or instar," refers to an intermolt period; i.e., seventh stage after sixth molt) was kept separately in a glass jar and data sheets of the sex, age and time of molting for each animal were maintained. The younger ones, up to the fifth or sixth stage were fed with gnats and the older ones with house flies.

The first stage was brief (approximately two days), and the young ones underwent their first molt within the cocoon. The total number of molts from females varied from seven to nine whereas the number of molts for males varied from five to seven. Twenty-five percent of the population were males. The females became gravid and laid cocoons after the eighth molt. They died normally about four to six weeks after laying the last of one to three cocoons. This period is referred to in the text as post cocoon. At an early age, male and female *Argiope* look alike, feed alike and build alike, as do *Araneus diadematus* (Witt, '71).

At the fourth stage in the male, external differentiation of sex organs occurs, when the terminal pedipalpal segment begins to swell. Hence descriptions of the central nervous system for males are given only from fourth to seventh stage.

The male and female spiders were weighed before fixation. The chelicerae, legs and abdomen were cut off, and the cephalothorax was fixed in Zenker's, Helly's, or Bouin's. Granular Histowax of M.P 56–58°C was used throughout. Sections of the entire cephalothorax were cut at 8–10 μ in the three cardinal planes; very good paraffin ribbons, even of the cuticle were obtained. Some of the serial sections were stained in Palmgren ('48) silver technique and others in basic stains like Azan, Heidenhain's haematoxylin and Mallory's phosphotungstic haematoxylin.

Outlines of the entire cephalothorax, the central nervous system, the neuropile and cortex were made with a camera lucida at a known magnification on millimeter graph sheets. The areas thus enclosed were counted and actual volumes were obtained by correcting for the magnification of these outlines and for the thickness of the sections. These operations gave the volumes of the entire cephalothorax, the cephalic nerve mass, the volumes of the cortex and neuropile for supra and subesophageal ganglia. These volume measurements were made for all the stages in male and female spiders.

The total nerve cell estimates in the nerve mass were obtained in the following way: nuclei were counted in every alternate section and the total multiplied by two gave the crude count of total number of cells. The correction factor suggested by Marrable ('62) has been applied to these cell counts. Nuclei were counted at a magnification of 400 ×, and a grid was inserted into one eyepiece to facilitate counting. The cell and nuclear diameters were measured with an oil immersion lens. All cells, glial and neural, were counted in the cortex because of the difficulty in distinguishing one from the other.

TABLE 1

Mean volume and standard deviation of the central nervous system and cephalothorax of female and mean body weight of female and male spiders (N = 4) A. aurantia. The volumetric data are given in $mm^3 \times 10^{-10}$

	S 1	S 2	S 3	S 4	S 5	S 6	S 7	S 8	S 9	PC
Cephalothorax	2930	3490	11350	16619	33350	59473	121726	181998	541222	* 1
	±	±	±	±	±	±	±	±	±	
	90.07	384.3	492.3	5139	3860	9936	7236	17010	35550	
Nerve mass	1170	1659	4262	5378	7573	9337	11782	14741	19430	24523
	±	±	±	±	±	±	±	±	±	±
	47.80	32.72	419.8	957.2	464.5	561.4	970.1	825.0	737.2	1134.0
Cortex	937	1001	1355	2472	2442	3255	3887	4159	5309	6190
	±	±	±	±	±	±	±	±	±	±
	33.86	38.77	53.04	10.98	72.85	272.5	467.3	182.7	714.5	595.4
Fibrous mass	243	695	2669	3079	4922	6166	7680	9638	13822	18280
	±	±	±	±	±	±	±	±	±	±
	25.48	28.87	414.2	493.9	309.0	299.1	278.6	803.5	814.1	748.5
Female body weight (mg)	0.4	0.4	1.0	2.5	4.0	10.0	26.0	82.0	140.0	220.0
			±	±	±	±	±	±	±	±
			0.3	0.75	1.08	4.03	8.91	36.37	52.26	50.43
Male body weight (mg)				2.0	4.0	7.0	10.2			
				±	±	±	±			
				0.46	0.96	1.85	4.5			

S1-9 represent the number of stadia. PC: Post cocoon-laying period.

[1] Even though the whole cephalothorax was sectioned during this period, parts of it were removed for easy penetration of the fixative. Hence data on volume measurements PC could not be furnished.

RESULTS

The post embryonic development of the spider resembles that of heterometabolous insects like cockroaches, crickets, and locusts. The hatchling resembles an adult spider in all physical aspects except size. In *Argiope*, reared under laboratory conditions, the length of intermolt periods varies from 11 to 15 days in females (Babu, '73) and from 15 to 17 days in males. The spiders are active during the entire postnatal period and increase in size and weight after each molt. Thus spiders lack a quiescent period like that of holometabolous insects.

The total body growth in female and male spiders shows sexual dimorphism (table 1). There is no change in body weight in the first and second instars (0.4 mg). From third to sixth stage the body increases from 1.0 to 10.0 mg in female spiders. A spurt in body growth from 26.0 to 220.0 mg occurs from the seventh to the post cocoon period. Gravid females weighed 297.0 ± 11.09 mg.

The body weight of female and male spiders are nearly equal in the fourth and fifth stages. The growth rate in males, however, slows down as they attain maturity. The body weights of males are approximately one third of those of the females in the seventh stage. Such differences have been reported previously for the body weights of spiders, *Araneus diadematus*, (Witt et al., '72).

A characteristic feature of the spider central nervous system (CNS) is the high degree of cephalization. There is a single large fused cephalothoracic nerve mass at the anterior end of the animal (Babu, '65). This compound neural mass consists of a dorsally located supraesophageal ganglion or the brain, and a ventrally placed subesophageal mass (SEG). The latter includes a pair of pedipalpal and four pairs of leg ganglionic masses and several pairs of small abdominal ganglia.

The CNS of spiders conforms to the typical arthropod plan. It consists of a cellular cortex surrounding a highly complex fibrous mass or neuropile (Bullock and Horridge, '65). The neuropile forms the most important structure in invertebrates because this is the only known place of neuronal contacts where the process of functional integration takes place.

Relationship between growth of cephalothorax and central nervous system

The cephalized ganglionic mass occupies

46% of the entire cephalothorax in the hatchling. At each successive stage the percentage of nerve mass in the cephalothorax diminishes gradually. In the ninth stage only 4% of the cephalothorax constitutes the CNS.

Figures 1 and 2 represent growth curves of cephalothorax and nerve mass. The CNS shows a continuous but slow growth rate at all stages. More than 75% of the volume is added to the cephalothorax during the seventh and subsequent stages. The total body weight grows at a similar rate during developmental stages.

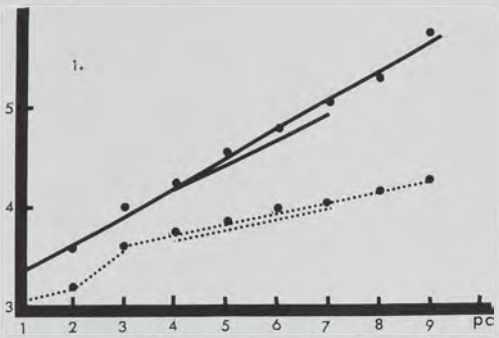

Fig. 1 Graphic representation of growth in volume of cephalothorax (solid lines) and total nerve mass (dotted lines) during post embryonic development in male (short lines) and female (long lines) *Argiope aurantia*: ordinate, volume in logarithm of mm^3; abscissa: stage in development.

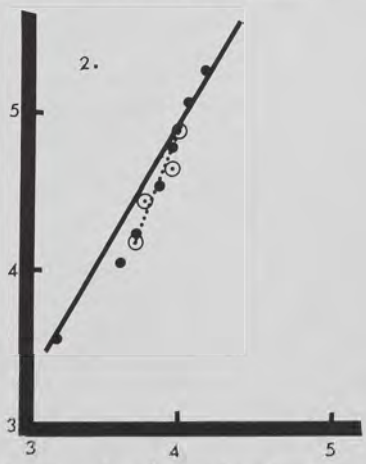

Fig. 2 Growth relationships between cephalothoraric mass, plotted as logarithm of volume in mm^3 on the ordinate, and total nerve mass, plotted in the same way on the abscissa. Full circles represent mean values for females, and the solid line indicates overall growth relationships for females; open circles and dotted line show comparable relationships for males.

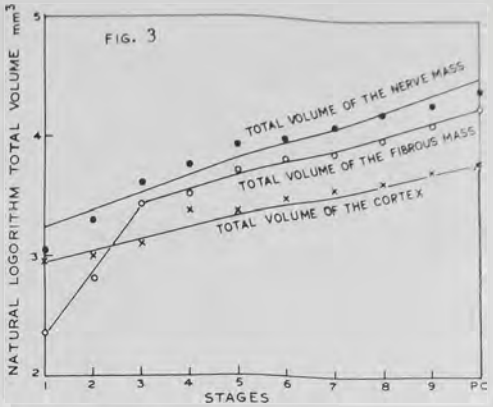

Fig. 3 Semi-logarithmic plot of volumetric growth, separately for total nerve mass, fibrous mass, and cortex in female *Argiope aurantia* spiders during post embryonic development. Note the reversal in growth trend between cortex and fibrous mass during earlier stages.

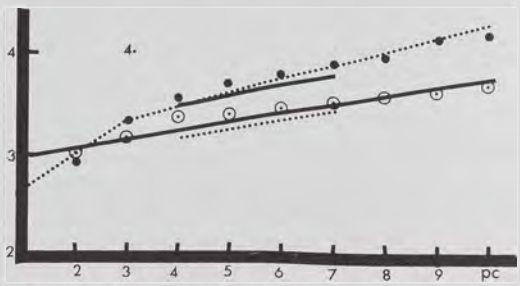

Fig. 4 Growth of total volume of brain (solid lines) and subesophageal (SEG) nerve mass (dotted lines) in male (short lines) and female (long lines) spiders, plotted in log mm^3 on the ordinate vs developmental stage on the abscissa. For females the means to which the lines have been fitted are shown as open circles for SEG and full circles for brain volume; the means for males lie all on each of the two short lines.

Growth curves are slightly flatter for male than for female spiders. In the seventh stage the total volume of the cephalothorax of male spiders forms 70% of that of female spiders. And the total nerve mass of males is slightly smaller than that of female spiders at the seventh stage (fig. 1).

Growth of the total volume of the ganglionic mass

The total mass of CNS grows in such a way that the curve derived from measurements of all stages in female and male spiders forms an almost straight line (figs. 1, 3) when plotted on a logarithmic scale.

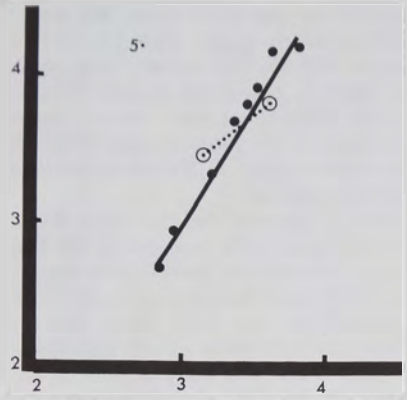

Fig. 5 Relative growth in volume of total SEG (on ordinate in log mm^3) as compared to total brain volume (abscissa in log mm^3) for males, dotted line and open circles, and females, solid line and full circles.

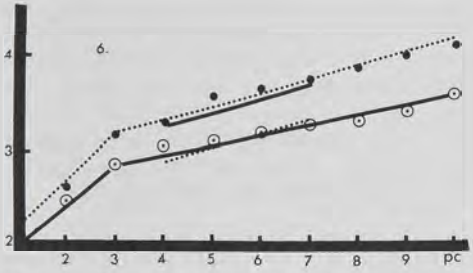

Fig. 6 This plot shows the volumes of the fibrous mass of brain (solid lines) and SEG (dotted lines) for male and female spiders during postembryonic development. Only the mean values for females are indicated.

The central nervous mass increases in volume during each larval stage. It reaches 50% of the total volume by the seventh stage. During the subsequent two molts and in the post cocoon period, the remaining 50% growth is reached. There is addition of neural mass at each successive stage, but maximal addition occurs in the last stages. The growth rate of the neural mass in males is slightly lower than that of the females at corresponding stages.

Figure 4 shows separate growth curves for brain and SEG. These ganglia show an increase in volume at each stage. The subesophageal nerve mass in females grows faster than the brain mass. Both reach 50% of the adult volume by the seventh stage. It is interesting to note that in the first and second stages the brain volume is greater than the subesophageal mass. But this trend is reversed in the third and subsequent stages when the SEG grows faster than the brain. In the seventh stage the SEG and the brain volume reach 50% of the adult neural mass. But the rate of growth differs. In the seventh stage the total brain volume is less than half of the subesophageal mass. These volume differences further increase in the post cocoon period, when the brain is only one third of the SEG (fig. 5).

Growth of the neuropile and cortex

Volumetric measurements of the fibrous mass reveal that its growth curve in brain and SEG shows an exponential curve (fig. 6). In the later stadia the rate of growth of the fibrous mass in the SEG is much greater than that of the brain.

The fibrous mass in the brain also increases rapidly in the last stages, but not

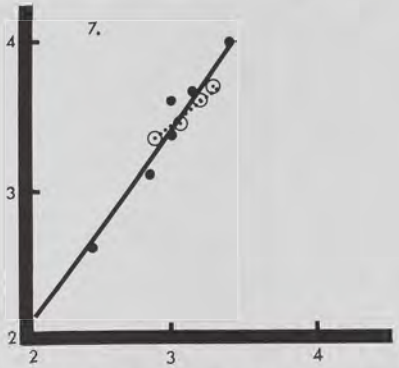

Fig. 7 Relative growth of fibrous mass of SEG and brain, plotted in the same way as in figure 5.

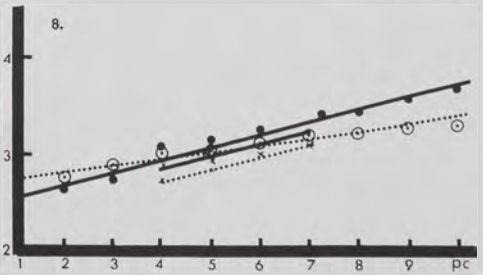

Fig. 8 Growth curve for the cortex in the SEG (solid lines and solid circles) and brain (dotted lines and open circles or X's) plotted in log volume mm^3 on the ordinate vs developmental stage on the abscissa. The shorter lines represent male growth data.

at the same rate as that of the SEG (fig. 7). In the eighth stage the volume of the fibrous mass has reached half of the mass of an adult brain. But 50% volume of the fibrous mass in the SEG is reached in the seventh stage. In hatchlings the volume of fibrous mass in the SEG and the brain is nearly equal. But during development addition of fibrous mass to the SEG occurs at a higher rate than to the brain. In the third and fourth stages the rate of addition of fibrous mass in the SEG is twice that of the brain and in subsequent stages it reaches nearly three times.

Volumetric measurements of the cortex also show a linear growth rate (fig. 8). The volume of cortex in the brain and SEG continues to increase through all stadia. An interesting aspect of cortex growth is crossing of the curves at an early stage. Up to the third stage, the cortex volume is greater in the brain than in the SEG. In the hatchling the cortex in the brain has twice the volume of that of the SEG. This difference is gradually narrowed down and the relative size is reversed in the fourth stage, after which the volume of the cortex in the SEG increases rapidly. In the fifth stage the cortex in the brain reaches 50% of the volume of an adult spider. In the SEG 50% of the cortex growth of an adult is achieved at the sixth stage. In the post cocoon stage, the cortex volume in the SEG is two and one half times greater than that of the brain.

The relationships between relative growth of cortex and fibrous mass in brain and SEG are a notable feature (figs. 6, 8). In supra and subesophageal ganglia the growth is exponential in early stages. In later stadia the rate of growth slows down, but volume continues to increase up to the post cocoon period. In brain the volume of cortex is 85% and that of the fibrous mass is 15% in the first stage. This difference narrows down to 52% for cortex and 48% for fibrous mass in the third stage. From the fourth stage on the trend is reversed: the fibrous mass represents 52% to 55% and the cortex 45–48% of the total brain mass from the fourth to the seventh stage. But in the eighth stage the neuropile starts to increase again, while the relative volume of the cortex decreases. Post-cocoon, the neuropile represents 73% and the cortex 27% of the total volume of the brain. Thus the relative percentages of neuropile and cortex in the first stage are reversed in the final adult stage. This trend of a decrease of volume for cortex and increase in neuropile in the brain is not absolute: it has been shown earlier where separate growth curves are plotted that both cortex and neuropile (figs. 5, 8) show continuous increase in volume.

A similar growth relationship between cortex and neuropile was observed for the SEG also. The cortex in the first stage represents 71% and the neuropile 29% of the total volume in the SEG. Thus the cortex constitutes a relatively small and the neuropile a relatively high percentage of the volume of the SEG as compared to the brain. Unlike in the brain the reversal trend in growth rate of neuropile and cortex takes place in the third stage. From the third to the post cocoon period, a nearly uniform growth relationship is maintained between cortex and neuropile. This ratio varies between 71–75% for neuropile and 29–25% for cortex. In the post cocoon period similar volume relationships between cortex and neuropile are present in the brain and the SEG. Thus the neuropile occupies a greater percentage of the total volume than the cortex in subadult and adult stages.

Growth of neural elements

Growth of cortex

The characteristic internal differentiation of the CNS into peripheral cortex and central fibrous mass is present from the hatchling stage. This trend becomes more marked in the subsequent stadia.

On the basis of size, staining and size of axon, three different types of cells were reported (Babu, '65). The globuli type which were classified earlier as Type-A cells are absent. The Type-B cells in the first stage, which measure 7.0 μ with a nucleus of 6.4 μ, grow to a maximum of 10.5 μ with a nucleus of 7.5 μ in the post cocoon period (fig. 9). Thus the Type-B cells show 50% growth in volume from the first to the last stage.

The Type-C are the neurosecretory cells. It was reported that these cells also increase in size from the second to the post cocoon period (Babu, '73).

The Type-D, which are mostly motor neurons (Babu, '65, '69), show continuous growth from the first to the post cocoon pe-

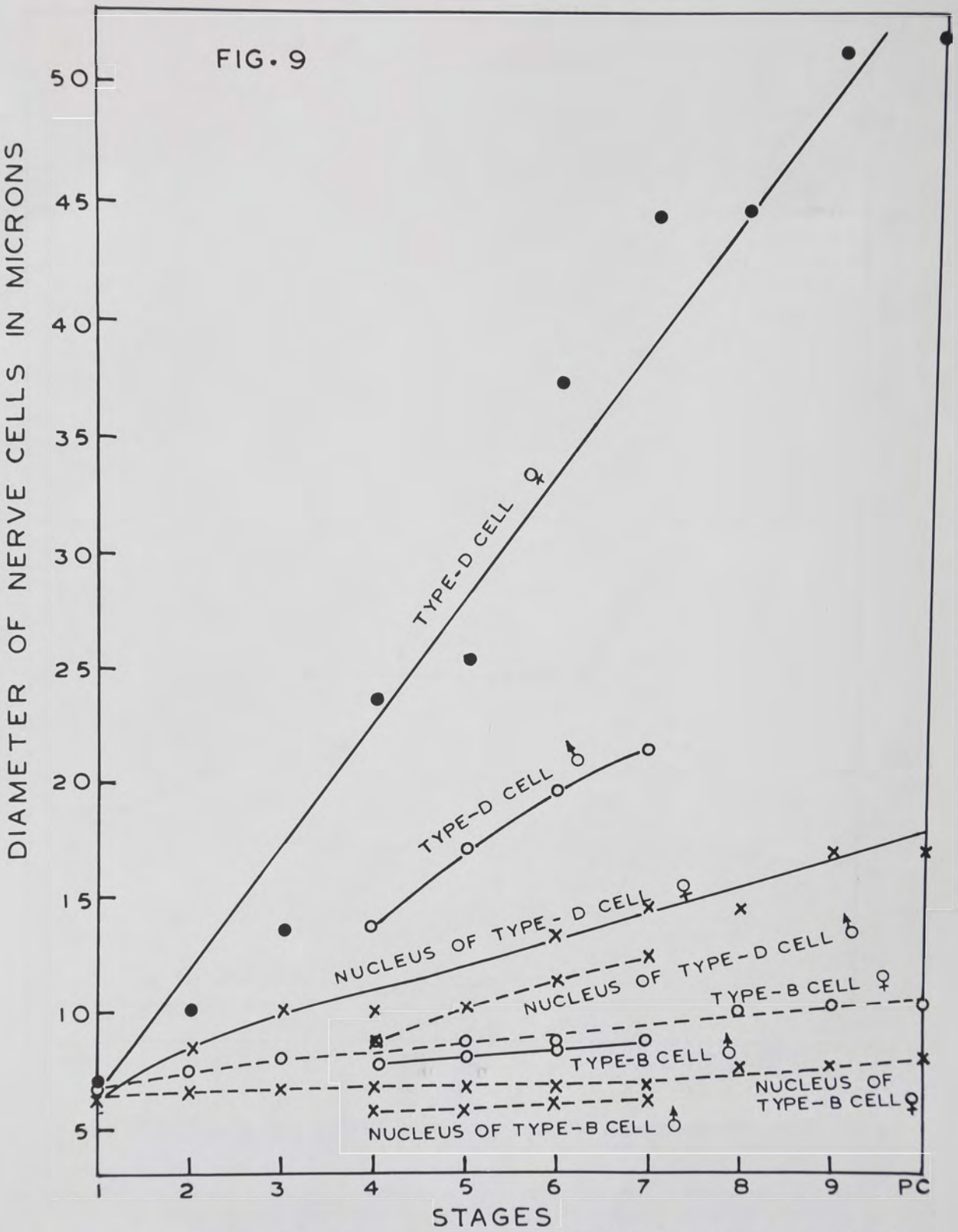

Fig. 9 The growth of Type-B and Type-D nerve cells in male and female spiders at various developmental stages.

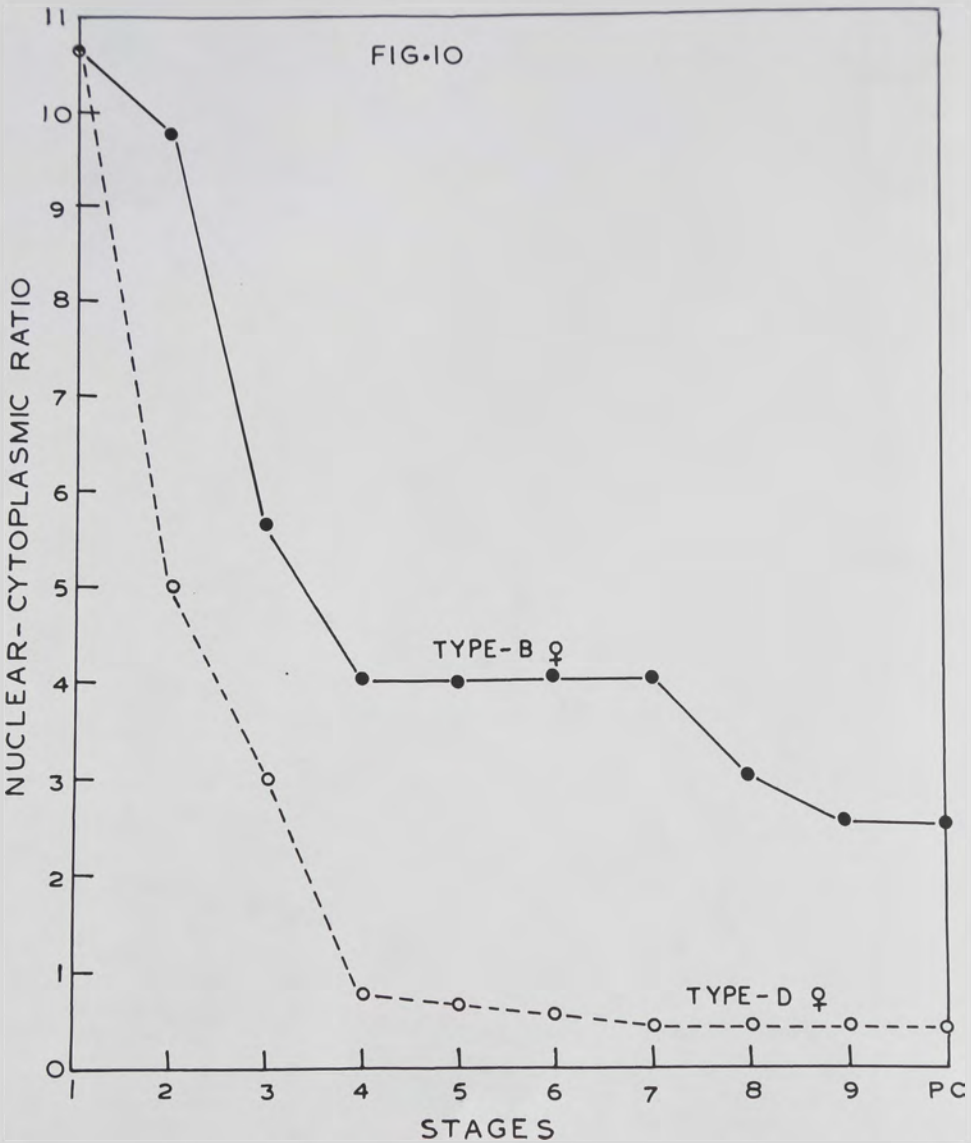

Fig. 10 Graph showing changes in nuclear-cytoplasmic ratios of Type-B and Type-D cells during postembryonic development of female spiders.

riod (fig. 9). These cells are abundant in all ganglia of the subesophageal mass. In the first stage, the average cell diameter is 7.0 μ with a nucleus of 6.4 μ. Throughout the post cocoon period, the largest cells measure 55 μ with a nucleus of 17 μ, showing a 600% increase in volume.

The Type-B cell diameters in males remain at the same level from the fourth to the seventh stage. But the growth pattern is

different for Type-D cells. In the fourth stage the Type-D cells of females measured 24 μ and that of the males measured 13 μ. In the seventh stage their diameters were 45 μ and 21 μ for female and male spiders respectively. Thus the largest motor neurons of a female in the seventh stage are twice the size of those of a male spider.

Figure 10 depicts the nuclear-cytoplasmic ratios of female spiders. The Type-B and D cells have a high ratio (10.6) in the first stage. This is reduced to 4.0 in Type-B cells in the fourth stage and is maintained at this level up to the seventh instar. But in subsequent stages the ratio falls gradually and reaches the minimum of 2.5 in the last stadia and post cocoon period. The nuclear-cytoplasmic relationship for Type-D cells shows a greater decline from early to later stadia. The initial ratio of 10.6 drops to a mere 0.73 in the fourth stage. After the fourth stage the ratio declines slowly and reaches a steady level of 0.4 in the seventh stage.

Thus nerve cell growth takes place mostly by an increase in cytoplasmic volume of the cell. The Type-B cells show a small growth rate and maintain a high nuclear-cytoplasmic ratio. But in Type-D cells there is an enormous increase of the cytoplasmic volume without a concomitant growth of the nucleus. Thus these cells are more plasmatic and bulbous, unlike the more chromatic Type-B cells.

In the first stage there is no differentiation of nerve cells into different categories in supra and subesophageal ganglia. From the second stage on the Type-B, Type-C and Type-D cells are differentiated. A greater percentage of nerve cells are Type-B, in the brain and SEG. The Type-D cells are confined to the SEG mostly, except a few cells in the tritocerebral part of the brain.

The increase in volume of cellular cortex is dependent on the growth of cell size and also on the number of large cells in each stadium. The histogram in figure 11 depicts this aspect of growth. In the post cocoon period, cells above 20 μ form 4.0% of the total number of nerve cells in the cephalic mass.

The cell volume and cell number increases at each higher stadium. The 20–30 μ size group cells appear first in the fourth stage. Their number doubles in subsequent stages and reaches a maximum level in the eighth and subsequent stages. From a mere 20 cells in the fourth stage, they increase to a maximum number of 690–724 cells in the last stages.

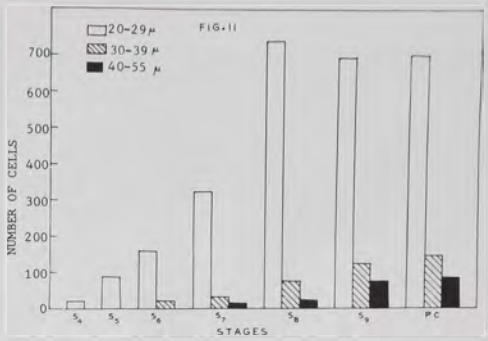

Fig. 11 The large motor neurons were arbitrarily classified into three groups based on their diameter (20–29 μ; 30–30 μ and 40–55 μ). The number of cells for each range were counted in all stadia. The nerve cells of 20–29 μ range were absent in the first, second and third instars. The histogram shows a progressive increase in volume and cell number of different diameters from the fourth to the post cocoon period. The numbers represented in the histogram are from one count.

The next size range of 30–40 μ diameter cells is found beginning with the sixth stage. The number increases from 20 cells in the sixth stage to a maximum of 140 cells throughout the post cocoon period. Unlike the earlier described group of smaller cells, these cells show a continuous increase in number up to the last post cocoon period. The increase in the number of cells from one stage to the next is one and a half to two times over that of the earlier period.

The largest cells of 40–55 μ diameter appear in the seventh stage. These cells also increase in number at each higher stadium. The cells increase from 10 in the seventh stage to a maximum of 76 in the post cocoon period. In male spiders the differentiation of cells is poor. In the seventh stage there are only 63 of the largest cells in the 20–30 μ range.

Thus, the growth of the cortex takes place by an increase in cell volume as well as by an increase in the number of large cells.

The total nerve cell population was counted in female and male spiders. Tables 2 and 3 show that there is no increase in

TABLE 2

Total number of cells counted in the female spider A. aurantia

Stadia	Subesophageal ganglion	Supra esophageal ganglion	Total
S 1	26,600	26,140	52,740
	* 14,554	* 14,520	* 29,074
S 1	25,800	24,360	50,160
	* 14,340	* 13,530	* 27,870
S 3	30,600	32,160	62,760
	* 15,300	* 17,390	* 32,690
S 5	25,600	25,340	50,940
	* 12,800	* 13,700	* 26,500
S 6	28,320	22,400	50,720
	* 14,160	* 13,333	* 27,493
S 7	25,780	27,500	53,280
	* 12,575	* 16,310	* 28,885
S 9	26,616	28,760	55,376
	* 12,674	* 16,433	* 29,107
P C	26,540	30,140	56,680
	* 12,640	* 17,223	* 29,863
P C	26,600	27,180	53,780
	* 12,666	* 15,530	* 28,196
Mean value and standard deviation	26,838 ± 1,498	27,110 ± 2,818	54,048 ± 3,694
	* 13,623 ± 997	* 15,329 ± 1,512	* 28,853 ± 1,655

The top numbers represent approximate counts of cells. The totals with an asterisk represent numbers after adjusting the correction factor.
S1–9, represents the number of stadia.
PC, post cocoon period.

TABLE 3

Total number of cells counted in the male spider A. aurantia

Stadia	Subesophageal ganglion	Supra-esophageal ganglion	Total
SM 4	24,240	16,280	40,520
	* 12,528	* 9,303	* 21,831
SM 5	23,480	15,100	38,580
	* 10,734	* 8,630	* 19,364
SM 5	23,380	17,520	40,900
	* 11,690	* 9,344	* 21,034
SM 7	24,780	18,100	42,880
	* 13,765	* 10,600	* 24,365
Mean value and standard deviation	23,970 ± 572	16,750 ± 1,156	40,720 ± 1,526
	* 12,179 ± 1,114	* 9,469 ± 871	* 21,648 ± 1,803

The top numbers represent approximate counts of cells. The totals with an asterisk represent number after adjusting the correction factor.
SM 4–7, represent the number of stadia.

total number of cells as the animal grows from young to an adult spider. Division of nerve cells was not noticed with the staining techniques used in the first stages, nor at later stages. Thus by the time an embryo is hatched the total number of nerve cells is fully formed. From the data, it is to be inferred that new nerve cells are not added during post embryonic development.

The total number of cells in the brain is only 13% more than in the SEG of a female spider. This higher number of nerve cells occurs in spite of significant differences in the number of neurons and volume of brain and SEG. The clue lies in cell size and in their compact arrangement.

In the cephalic nerve mass of the male, nuclear counts show that the total number of nerve cells is smaller than in the female. This is not surprising because the total nerve mass and the volumes of the cortex and fibrillar mass are consistently smaller than those of females from the fourth to the seventh stage. The same trend in nuclear population is reflected here.

Growth of fibrous mass

The increase in cell size with each higher stadium goes together with a corresponding increase in axon diameter. Measurements close to the cell body or on axons immediately after they enter the neuropile gave the following values:

In the first stage the largest fibre measured is 1 μ. This increases to 4 μ in the third, and to 9 μ in the seventh stage. In the ninth and other post cocoon periods the largest axon has a diameter of 16 μ. The large axons arise from the large motor cells. In adult males such large axons measuring 8 μ are comparable to those of the female in the seventh stage. But such large axons are few in number.

Observations of serial sections in the three planes from early to late stages reveal that the various processes of a neuron grow considerably. In the first stage there is very little branching of the dendritic and axonal processes. Hence volume of the fibrous mass is less than that of the cortex. At each of the higher stadia extensive branching of the dendrites and to a lesser extent the axons, takes place. The small, fine fibers which are considered sensory terminals also increase in number.

Axons from neurosecretory cells also exhibit a similar growth pattern. But the most conspicuous part of their growth is in forming pools of stainable material. Such pools increase during the peak period of secretory activity and also from early to later stadia (Babu, '73); and there is an increase in the extent of their ramification.

The only special neuropilar structure present in the orbweb spider is the central body. In the first instar a clearly recognizable and demarkated central body is absent. The central body becomes demarkated from the general neuropile by mostly astral type glial cells in the second stage, and further differentiates into lobes towards the end of the second stage. At each of the later stages there is a corresponding increase in thickness and length of the body. In many hemimetabolous insects (Panov, '59) the central body is present at the time of hatching. But in holometabolous insects the first appearance of the central body ranges from embryonic stages as in *Tenebrio*, *Antheraea*, and *Culex* (Panov, '59; Hinke, '61), through larval stages, as in *Danaus* (Nordlander and Edwards, '68b) to the pupa as in *Calliphora* (Gierying, '65).

The differentiation of the central body and the beginning of web construction have been found to occur at the same time. In the first and the earlier part of the second stage of the spiderling only single threads are formed. Towards the later part of the second stadium, when a demarkated central body is formed, the spider begins to construct its first small web with radii and spirals. Thus a time correlation between the formation of the central body and the beginning of web construction was noticed.

Growth of neuroglial elements

The entire cephalothoracic nerve mass is enveloped by a sheath called the neural lamella. The number of layers in the connective tissue normally varies from four to ten. These layers widen at irregular intervals to enclose a connective tissue cell nucleus. The thickness of the sheath varies considerably depending upon the location and stadia. From the first to the sixth stage of the spider, the thickness of the sheath varies from 1 to 2 μ all around the nerve mass. It remains at this level on the mid dorsal region of the supraesophageal ganglion throughout the life span of the spider. From the seventh to the last stage the sheath thickness gradually increased on the ventral and dorsal region of the subesophageal mass, reaching a maximum of 4 μ. The greatest increase in thickness of the neurilemmal sheath occurs at the origin of major nerves. In the seventh stage the sheath measured 8 μ in the pedipalpal and leg nerve region. In subsequent stages it rose to 20–40 μ. Maximal increase in sheath thickness was noticed in gravid females. Immediately after the eighth molt, the sheath near the root of the pedipalpal nerve measured 30 to 40 μ. This increased to 80–100 μ twenty days after the last molt, at which time the animal is full of eggs. Such differential growth of neural lamella in adults is suggestive of an important role in reproduction of the animal. In some cases the sheath serves as a neurohemal organ for storing and releasing the neurosecretory products (Coggeshall, '67; Frazier et al., '67; Rosenbluth, '63; Simpson et al., '66). Legendre ('59) suggested an endocrine role for the neurilemma of spiders.

In the seventh stage of a male spider the thickness of the neural lamella varied from 0.5 μ to 4 μ in different regions of the cephalic nerve mass.

The neural lamella is not only found as an outer covering of the nerve mass but also as a separating sheath between the fused ganglionic masses (fig. 12). Since the fusion of ganglia is complete at the time of hatching, these septal layers (fig. 12: INL) can be distinguished in all instars. The total number of these septal layers corresponds to the total number of fused ganglia.

The neural sheaths between the ganglia also fuse together enclosing a small groove or canal running in the dorso-ventral direction. In the groove and between the sheath layers, granules of different diameters are present. These are presumed to be nutritive material on its way into the ganglion. According to Heywood ('65) such granules form the permanent or semi-permanent storage of food, not food in readily available form. In mid sagittal sections where these intra-ganglionic connective sheaths are seen, it gives the appearance of blood vessels running through the ganglion. Careful

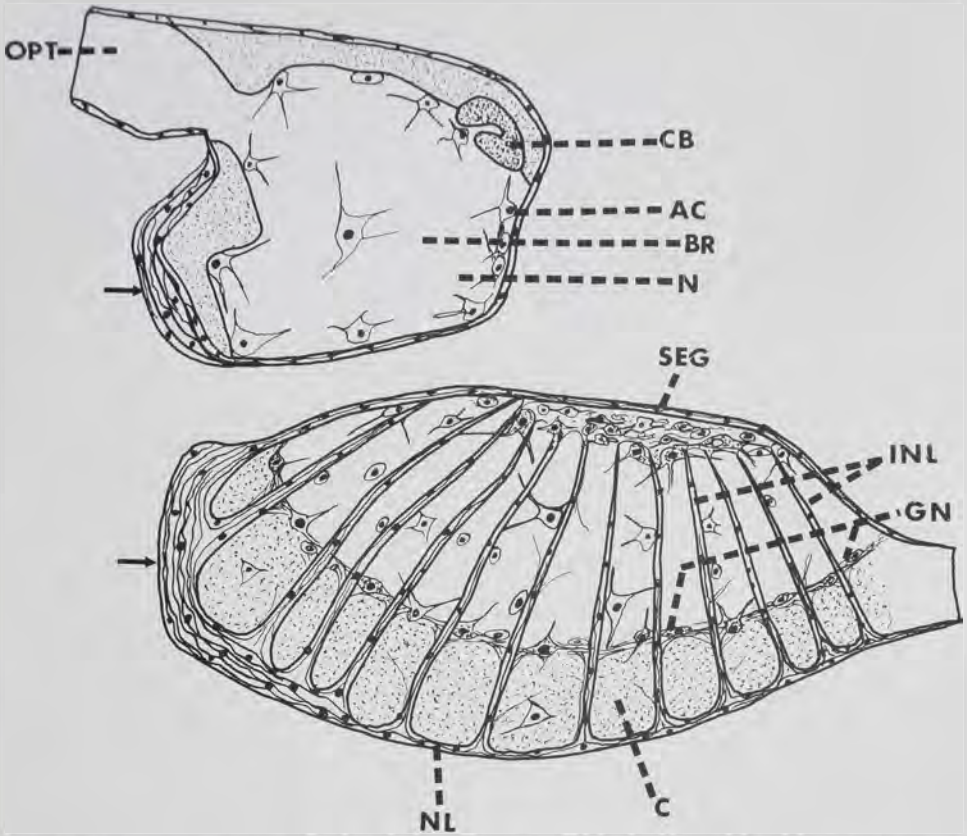

Fig. 12 Schematic drawing of cephalic nerve mass in mid sagittal section showing distribution of neural lamella and glial cells in brain and subesophageal ganglion. The intraganglionic neural lamellae represented, are only an approximate number. AC, Astral type of glial cells; BR, Brain; C, Cortex; CB, Central body; GN, Glial cell zone between cortex and neuropile; INL, Intraganglionic neural lamellae; N, Neuropile; NL, Neural lamellae; OPT, Optic nerve; SEG, Subesophageal ganglion. Arrows represent thickened areas of neural lamellae.

observations of serial sections in the three cardinal planes reveal that these are not blood vessels. Further work is necessary to confirm this observation by using special techniques for tracing blood vessels. Until then it is proposed that the cephalothoracic nerve mass of spiders is avascular.

These intraganglionic sheaths have the same characteristics as the outer neural lamella. In both, the sheath layers run parallel and nuclei are found at irregular intervals. The intra ganglionic sheaths are continuous with the inner layers of the outer neural lamella. In the central or mid sagittal region, the outer layers of the neural lamella of the two adjunct ganglia are tucked in so as to enclose the canal.

The outer neural lamella is chiefly a compact sheath. But the layers in the peripheral region of the central canal are loosely bound. These layers enter into the cortical and fibrous areas at different levels and an increase in their intrusion was noticed in higher stadia. In the leech, *Hirudo*, penetration of the neural lamella into central ganglia has been reported (Coggeshall and Fawcett, '64). Thus the neural lamella is not a mere outer investing sheath but it invades deep into the cortical and central fibrous zones.

Legendre ('59) described four types of neuroglial cells in *Tegenaria*. Similar types are noticed in the CNS of *Argiope*. The glial cells, particularly of the astral type, are ar-

ranged in such a way that they further divide each ganglion into several zones in female and male spiders.

This differentiation starts even in the first instar and becomes more and more pronounced in subsequent stages. A thick zone of glial cell layer is formed between the cortex and fibrous mass. On the dorsal side of the subesophageal mass and near the exit of major nerves, a similar concentration of glial cells was noticed. It is difficult to identify them in the cortex but they are relatively easy to identify in other places. In the thicker zones, the astrocyte type cells with denser cytoplasm are abundant. In the fibrous mass, however, the astrocyte type with clear cytoplasm is abundant. The latter is found around the large axons in the dorsal region of the neuropile where motor axons are present. The size of the glial cells shows little increase in diameter from early to later stages. The growth in a glial cell is mainly through extensions of ramifications. Unlike the nerve cells the glial cells show an increase in number during post embryonic development. The astral type cells were counted in the fibrous region because of easier identification. In the first instar the total astral type glial cells are 2,574 ± 80. Their number increased to 6,580 ± 150 in the sixth stage. In the post cocoon period the astral cells increased to 22,040 ± 235. This shows a tenfold increase in number of the astral cells from early to last stage. The other types of glial cells also increased in number.

DISCUSSION

For comparison and evaluation, studies like the present one, are lacking for most of the invertebrate groups, except insects. The developmental and anatomical organization significantly determines the integrative functions of the nervous system. Hence, knowledge of the growth pattern of neural and non-neural elements in the CNS is essential.

Volumetric changes in the growth of the insect nervous system show a wide range of patterns in brain growth (Edwards, '69). In Holometabola, the brain and whole body growth has a negative allometric relation in larval stages (Power, '52; Hinke, '61; Nordlander and Edwards, '68b). In hemimetabolous insects the growth relationship between the brain or the ganglion and the whole body bears a negative allometric relation during post embryonic development (Neder, '59; Gymer and Edwards, '67) as in the spider *Argiope aurantia*.

Gymer and Edwards ('67) had reported that the terminal ganglion of the house cricket increased 40 fold during post embryonic development. In *Argiope* the cephalic nerve mass showed a 24 fold increase in volume. Several component parts of the CNS contribute to this growth pattern. In the cricket an increase in cell volume rather than cell number was reported. In the spider the growth of the cortex is due to an increase in cell size. This increase in Type-B cells is 50% and in the largest Type-D cells 600%. The large Type-D motor neurons show further differentiation into different size categories where the number in each category increases during post embryonic development. Similar observations were made in the brain of the beetle *Popillia* which grows by increase in cell size (Abercrombie, '36). Monopolar neurons are characteristic of invertebrate nervous systems, including spiders (Babu, '69). The soma, detached from dendritic and axonal processes, is principally trophic in function and does not participate in nerve conduction (Bullock and Horridge, '65). Thus the enormous increase in cell volume and the differential growth of motor cells is presumably due to the demand of growing body organs and to an increase of dendritic and axonal processes at later stadia of female spiders.

The growth of the cortex results also from an increase in the number of glial cells during development. Such glial cell increase occurs amidst groups of neurons, in the prominent glial zone between cortex and neuropile and is due to increased intrusions of neural lamella into the cortex. The increase in axon diameters within the cortex also contributes to an increase in volume.

Much of the neural growth, especially in later stages is due to the growth of fibrous mass. The cortical volume in the total nerve mass exceeds the neuropile volume in growth rate in the first two stages in *Argiope*; in the first four instars of *Acheta* (Gymer and Edwards, '67) and in the prepupal and pupal period of *Drosophila* (Power, '52). In spiders the dendritic and axonic processes of neurons, especially of type-D cells grow extensively. The fiber diameter

of large cells increases from 1 μ in the first stage to a maximum of 16 μ in adults. As the size category of cells increases, there is a corresponding increase in size categories of axons. The glial cell proliferation and the increase in cell volume is enormous. In *Argiope* one type of astral cells counted increased ten fold.

Glial cells of all four types increase in volume and number during post embryonic development in *Danaus* (Nordlander and Edwards, '68b) and *Pieris* (Heywood, '65). In *Acheta* (Gymer and Edwards, '67) the number of glial cells increased from 3,400 to 20,000 during post embryonic development. In adult spiders the astral type glial cells with clear cytoplasm were observed to extend their processes around the larger axons which are presumably motor in the dorsal region of the fibrous mass. Glial cells are known to form sheaths around axons in invertebrates. In electron microscopic studies, Edwards ('67) had shown in cercal nerves of crickets that axons over 1 μ diameter have individual glial sheaths, and larger axons have more elaborate glial sheaths. Such relationships between glial cells and axons were also reported in *Aplysia* (Batham, '61; Coggeshall, '67) and *Hirudo* (Coggeshall and Fawcett, '64). In the spider the neural lamellae also increase enormously at the origin of main nerves while remaining static at most other places. At the intrusions of neural lamella into the fibrous mass, the thickness of the glial zone around the neuropile also increases in post embryonic development.

The growth of neuropile is also dependent upon incoming sensory fibers. At each molt, new sensory neurons are differentiated from ordinary ectodermal cells and send their axons into the CNS where they are incorporated into it (Wigglesworth, '54). The hair receptors of the abdominal cerci of the house cricket increase in number from 50 to 750 as the animal goes through successive molts (Edwards, '67). Similarly there is an increase with age in the size and the number of ommatidia in the eye of insects (Bodenstein, '53; Wigglesworth, '65) and of arachnids (Waterman, '54) and a concomitant increase in number of fibers they send to the CNS. In the major metathoracic nerve of the house cricket (Edwards, '67) the nerve growth is achieved by addition of both sensory and motor fibers and by an increase in the diameter of fibers.

The neural mass in *Argiope* is shown to grow independent of the molting cycle. A similar observation was made in *Drosophila* (Power, '52) where the CNS grows smoothly without showing any relationship to molting. But Edwards ('69) suggests to accept such conclusion with caution, since glial cells show cyclic patterns of mitosis in *Acheta* (Panov, '61) and DNA synthesis in *Danaus* (Nordlander and Edwards, '68a).

In *Argiope*, the growth patterns of the brain and of the subesophageal ganglion show an inverse relationship. From the first to the last stage in the female the brain increases 10 fold, the subesophageal mass 36 fold, and the cephalothorax 180 fold. The brain in the first stage constitutes 1/4 volume of the cephalothorax, and this is reduced to 1/90th in adult spiders. The subesophageal mass on the other hand is 1/6th in the first stage, but occupies 1/30th volume of the cephalothorax in the adult animal. The brain volume is relatively greater in the first two stages, but in the remaining stages the subesophageal volume is greater. The number of neuromeres which fuse to form the brain is two: the protocerebrum and tritocerebrum. But in the subesophageal ganglion there are five thoracic and approximately eleven abdominal segments. Besides, except for a few cells in the tritocerebrum, the rest are small cells with fine processes, which show a slow growth rate. Such neurons are considered as association fibers. The neuropile, even though of diffuse type, is compactly packed and also contains one specialized mass, the central body. In the SEG there is great diversity in cell size, and the cells increase in volume during post embryonic development. The larger cells are motor cells with well developed dendritic arborizations (Babu, '69). The size of axons in the neuropile also shows a similar growth rate. Moreover, the neuropile is of the diffuse type with loosely packed fibrous matter, and the compact special neuropilar structures are absent. A casual observation reveals that there are more glial cells and specially thickened glial areas in the subesophageal mass.

The morphology of the CNS (Babu, '65, '69) shows that the subesophageal ganglion is the major recipient of sensory input from pedipalps, legs, cephalothorax and abdomen. On the other hand, the brain receives sensory input primarily from eyes and

chelicerae. In *Argiope* the optic centers are poorly developed, while mushroom bodies, olfactory and antennal centers of other arthropods are lacking. These factors may contribute to the growth differences of brain and subesophageal mass.

Yet the breakdown of total number of nerve cells to individual neuromers shows that the protocerebrum has still the largest number (approximately 13,000 in female and 8,000 in male spiders) of cells. The number of nerve cells for the other ganglion range from 2,000–750 in females and males respectively. Due to reasons mentioned earlier and by virtue of the large number of small chromatin rich cells, with a specialized neuropilar mass like the central body, the brain functions as an important integrating center.

Post embryonic changes in number of neurons seem to vary in different invertebrate groups. In *Argiope*, the number of nerve cells remained constant at 28, 853 ± 1,655 for female and 21,648 ± 1,803 for male spiders. In the post embryonic stages, division of nerve cells was not observed with the stains employed. In hemimetabolous insects, *Acheta*, the number of neurons in the last abdominal ganglion remains relatively constant at 2,100 neurons throughout development (Gymer and Edwards, '67). In the thoracic ganglion of *Oncopeltus* a similar observation was made by Johannson ('57).

However, certain cell types in the brain of insects increase in number during post embryonic growth. In the optic lobes (Panov, '63) and the Corpora peduncula (Edwards, '69) of insects the cell number continues to increase throughout the post embryonic life. In holometabolous insects a large number of neurons is added during post embryonic development and metamorphosis in the brain (Norlander and Edwards, '68a) and in thoracic ganglia (Heywood, '65). In the earthworm the number of neurons increases in most parts of the brain and this increase is particularly prominent in ganglia which control the reproductive apparatus (Ogawa, '39). A similar differential increase in neuronal number was also reported in *Aplysia* (Coggeshall, '67; Frazier et al., '67). In the brain of *Octopus* (Packard and Albergoni, '70) estimates of cell number based on their DNA content, showed continuous increase from early to adult stages.

A correlation was suggested between the formation of the central body and beginning of web construction in *Argiope*. Ablation and stimulation of brain regions in several arthropods were done successfully. This has enabled investigators to demonstrate a variety of behavioral activities in bees (Vowles, '61, '64), locusts (Rowell, '63), grasshoppers and crickets (Huber, '67). In cricket and grasshopper (Huber, '67), if the central body was destroyed or hemisectioned, stridulation and associated behaviour disappeared. On the other hand, stimulation of the central body gave rise to songs with some change in temporal patterning. Thus the central body in insects is an important coordinating and integrating center.

The integrative functions of the nervous system depend on its anatomy and on specific connections and the relationship of cells. Anatomical studies in spider brain reveal that the central body is linked with major pathways from the legs to the subesophageal ganglion (Babu, '65; Meier, '67). Preliminary studies on the effects of laser lesions on the central nervous system were made (Witt, et al., '64; LeGuelte and Witt, '68; Witt, '69; LeGuelte and Witt, '71). Lesions in the cortex close to the central body produced disturbances in both radii and spirals of the web. Further work designed for ablation and with implanted electrodes in the brain will help to localize the functional organization of the CNS, especially with respect to web building. It was reported that motor patterns of song production and flight in the field cricket *Teleogryllus* appear in a specific sequence during the last four molts. Although neuronal cell bodies may be present at hatching, neural circuits underlying adult motor programs are not functional in early instars (Bentley and Hoy, '70). In spiders the beginning of web building in the later part of the second stage and cocoon spinning in adult animals may involve similar neural mechanisms developed during post embryonic growth.

Witt, Rawlings and Reed ('72) have reported that the fine detail of the web undergoes change throughout the life time of the spider. New behavioral patterns like spinning of the cocoon, absent in earlier stages, will develop in adult spiders. The causes for this new pattern of behavior may be the development of reproductive or-

gans. The CNS of spiders increases in volume throughout ontogeny while maintaining a constant number of nerve cells. It is suggested that specific neural contacts in the neuropile change so as to meet the new demands of the growing body organs. Neurons respond to normal events by meaningful movements of axonal or dendritic terminals (Bullock and Horridge, '65). The late emergence of motor patterns in crickets was mentioned earlier. Presumably, in the spider while sacrificing the detail in web construction without much loss in functional efficiency, new changes in the nervous system occur in order to meet the adult behavioral patterns.

The CNS of female and male spider *Argiope* show certain interesting growth patterns during post embryonic development. Even though the body weight of male and female spiders is equal in the fourth and fifth stage, the growth slows down as the male attains maturity. The male body weighs one third of the female in the seventh stage. This is largely because of poor development of body musculature.

Volumetric measurements of the neural mass and its component parts like cortex and neuropile in male spiders are consistently smaller than that of females throughout. But a considerable difference between male and female spider is noticed in cellular organization. The total number of nerve cells counted in males is smaller than that of females by 11% in the SEG and 58% in the brain. The total number of cells in the brain of males is 30% less than in the SEG. In contrast the number of cells in the brain of females is 13% more than in the SEG. In the SEG, while three different size groups and a varied number of motor neurons (325 cells of 20–29 μ; 30 cells of 30–39 μ and 10 cells of 40–55 μ) are noticed in the seventh stage of the female, only 63 cells of the small size group are present in mature males.

If animal behavior is "What an animal does," then development is an aspect of behavior (Edwards, '67). The anatomical differences between male and female spiders during development of the CNS may partly explain the behavioral peculiarities of both sexes.

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