Interrelations of glial cells and axons in the postembryonic development of the nervous system of *Tubifex tubifex*

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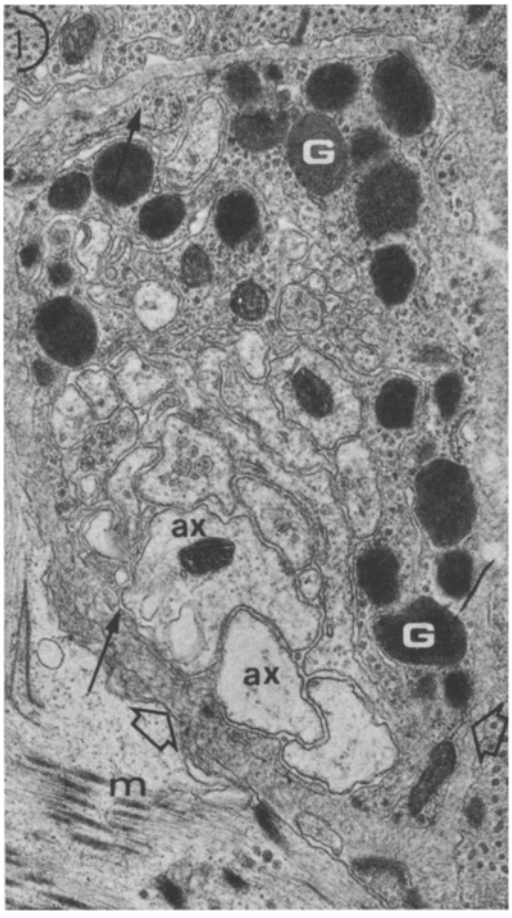
Summary. Transmission electron microscopy was used to study the role of gliosomes in the postembryonic development of the central nervous system in Tubifex (Oligochaetes).

Chemical and electrophysiological studies[1-4] have contributed to the understanding of the trophic interaction of glia and neurons, but morphological techniques, and especially ultramorphology, constitute the basis for the study of the neuron-glia relationships, mainly by preserving normal topographical relations between these 2 structures. We observed that during postembryonic development of Tubifex, the spatial relations between glia and neurons and the ultrastructural aspect and size of the gliosomes[5] were significantly different from those of mature worms. In this note we describe these differences, together with their quantitative aspect, and interprete our findings in terms of the significance of gliosomes for the maturation of the neurons.

Materials and methods. Tubifex tubifex specimens (Müll) were cultivated in laboratory conditions[6]. Postembryonic forms ranging from 1 mm to 8 mm length and mature worms (ca. 60 mm) were prefixed in toto with a mixture of 2.5% glutaraldehyde, 1% acrolein and 1% TAPO for 1 h, and postfixed with 4% unbuffered OsO_4 for 3 h. During embedding, specimens were appropriately oriented so as to facilitate identification of analogous nerves in all groups studied.

Results and discussion. We have chosen the metameric nerves that innervate body musculature for our analysis, comparing their ultrastructure at the last stage of postembryonic development (figure 1) with the ultrastructure of analogous nerves of mature worms (figure 2).



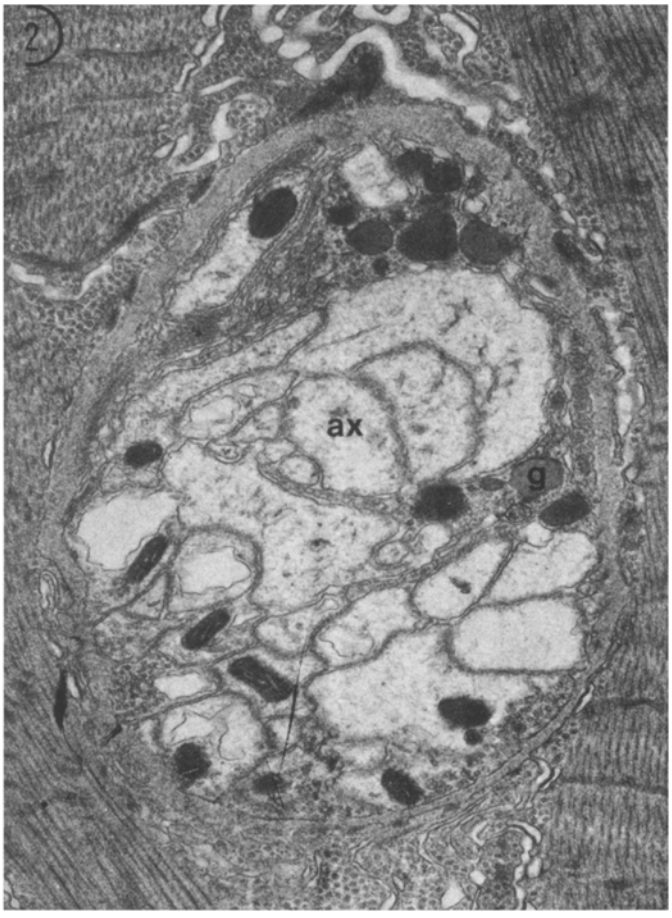


Fig. 1. Cross-section of metameric nerve of Tubifex at the last stage of postembryonic development. The nerve is surrounded by the basement lamina (empty arrows). Glial processes are closely applied to the basement lamina, sometimes only as thin cytoplasmic sheets (full arrows). Groups of maturing axons (ax) are separated by glial processes tightly packed with glycogen granules. Note the large number of electron-opaque gliosomes (G). m, Muscle cells. × 24,000.

Fig. 2. Cross-section of metameric nerve of adult Tubifex. The electron micrograph is reproduced at lower final magnification because the overall dimensions of the nerve have increased relationship to the stage represented in figure 1. The percentage of the cross-sectional area of glial processes and the number of gliosomes have greatly diminished. Some gliosomes have low electron opacity (g). Mature axons are marked 'ax'. ×16,000.

At the last stage of postembryonic development (figure 1), the glial cell processes interpose between the developing nervous cell processes and the basement lamina, forming a complete peripheral sheet around the nerve. Glial cell processes are also seen in the interior of the nerve, where they separate the nervous cell processes into several distinct groups. Unlike nervous cell processes, glial processes are tightly packed with glycogen granules; one of the prominent morphological features of glial processes is the large number of electron-opaque granules of variable size i.e. gliosomes. Mitochondria are seen in both glial and nervous cell processes.

During the maturation of the nervous system, the overall diameter of the nerve increases. The percentage of the cross-sectional area of glial cells gradually decreases, while that of nervous cell processes gradually increases. The number of gliosomes diminishes. These modifications lead to the following relations between glial cells and nervous cells in mature worms (figure 2): glial cells form a fairly uniform peripheral layer only 1 or 2 cells thick; a patch of glial processes is occasionally found in the interior of the nerve; the gliosomes are fewer than in earlier stages of development, and are less electron-opaque. The cross-sectional area of the whole nerve is occupied almost completely by nervous cell processes. For the calculation of the cross-sectional area of glial and nervous cells in the nerve, we used a grid of regularly intersecting lines[7].

In the postembryonic nerve, the mean over-all cross-sectional area of the nerve is 60 $\mu m^2 \pm 0.8\ \mu m^2$, of which: 41.56% ($\pm$ 0.26%) glial cells, 17% ($\pm$ 0.6%) gliosomes, 58.44% nervous cells. In the mature worm nerve, the mean overall cross-sectional area of the nerve is 213 μm^2, of which: 11.8% ($\pm$ 0.31%) glial cells, 2.1% ($\pm$ 0.31%) gliosomes, 88.2% nervous cells.

The present data reveal an orderly sequence of events in the postembryonic development and during the maturation of the nervous system in Tubifex. During the maturation of the nervous system, the percentage of the cross-sectional area of glial cells diminishes parallel to a drastic fall in the number of gliosomes, and it may be reasonably concluded that the gliosomes accumulate at a biologically significant period to carry out a specific biological function. This period may be considered as a transition from the stage of formation of the nerve to its maturation. It is, then, reasenable to conclude that gliosomes in Tubifex are involved in the maturation of the nerve cells.

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The effect of prostaglandin E_1 on cyclic AMP production in the salivary glands of *Calliphora erythrocephala*

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Summary. Prostaglandin E_1 noncompetitively inhibits 5-hydroxytryptamine- and theophylline-stimulated cyclic AMP production in the salivary glands of Calliphora erythrocephala by an inhibitory effect on adenyl cyclase. Phosphodiesterase is not affected.

Prostaglandins have been implicated in the regulation of the action of a variety of hormones particularly those whose effects are thought to be mediated by the secondary messenger cyclic adenosine monophosphate (cyclic AMP)[1]. Depending upon the tissue involved prostaglandins either increase or decrease the synthesis of cyclic AMP by adenyl cyclase or its degradation by phosphodiesterase. Prostaglandins appear to inhibit adenyl cyclase in adipose tissue where they inhibit the lipolytic effect of several hormones[2] and in toad bladder inhibit an adenyl cyclase associated with osmotic water flow (although stimulating an adenyl cyclase associated with sodium transport) where they noncompetitively inhibit vasopressin- and theophylline- but not cyclic AMP-induced osmotic water flow[3-5]. In most other tissues studied prostaglandins effect an increase in cyclic AMP and hence mimic many of the hormonal responses. Fluid secretion by the isolated salivary glands of Calliphora erythrocephala is stimulated by 5-hydroxytryptamine (5-HT) and analogues[6,7] the action of which appears to be mediated by cyclic AMP by activation of adenyl cyclase. Intracellular levels of cyclic AMP are elevated by the application of 5-HT[8] and the application of exogenous cyclic AMP and theophylline (an inhibitor of phosphodiesterase) mimic the effects of 5-HT on fluid secretion[9]. Pharmacological studies[7] have shown that prostaglandin E_1 (PGE_1) is an inhibitor of stimulated fluid secretion by isolated salivary glands acting via a different receptor to 5-HT and analogues but one that is functionally connected with cyclic AMP production. In this study an investigation was made into the modulation of cyclic AMP levels by PGE_1 – specifically into whether PGE_1 acts directly on adenyl cyclase, phosphodiesterase, both or neither by comparing the effects of PGE_1 on the response of salivary glands to theophylline-cyclic AMP combinations (response independent of adenyl cyclase activity but dependent upon phosphodiesterase) with the response to theophylline-5-HT combinations (adenyl cyclase dependent response).

Materials and methods. The salivary glands of 3-day-old Calliphora erythrocephala were set up for the measurement of fluid secretion using the method of Berridge and Patel[10]. Each gland was isolated in a 10 µl droplet of

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