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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Zoologia. — *On the relationship between nervous system, gonads and neurosecretion in* Dendrocoelum lacteum. Nota di GIUSEPPE GARDENGHI (*), ANTONIO QUAGLIA (**) e MARIO GRASSO (***), presentata (****) dal Corrisp. G. CHIEFFI.

RIASSUNTO. — Nel presente lavoro si cerca di fare luce sulle cause del limitato potere rigenerativo della planaria *Dendrocoelum lacteum.*

Muovendo dai risultati di precedenti ricerche che sembrano dimostrare che gli spermatogoni partecipano insieme ai neoblasti ai processi rigenerativi, gli AA. hanno studiato, col metodo dell'impregnazione argentica Cajal III, con l'osservazione al M. E. e col metodo di Gabe per la neurosecrezione, i rapporti anatomici fra sistema nervoso e follicoli testicolari. Tali tecniche hanno rilevato che la rete di fibre nervose, che in altre specie di planarie a rigenerazione totale circonda le gonadi, in *Dendrocoelum* è assai esile o addirittura assente. Lo studio della neurosecrezione col metodo di Gabe ha confermato una diffusa presenza di elementi neurosecretori prevalentemente nella regione anteriore del corpo.

Posterior stumps of *Dendrocoelum lacteum* (*Turbellaria, Tricladida, Dendrocoelidae*) resulting from a cut behind the pharynx base, are unable to regenerate a new head; in addition, they show unchanged testes after more than a month from the cut [1]. On the contrary, posterior stumps resulting from a cut before the pharynx base, regenerate the head after about ten days and at the same time a degeneration of testes takes place [1]. However, it appears that when the regeneration processes are rather limited, only the testes near the wound regress. Instead, when regeneration processes involve a large body portion, all testes disappear [1].

The above results are consistent with those obtained by Manelli and Contoli-Amante, Gremigni and Puccinelli [2] with different methods in different planarian species. They also appear to support the hypothesis that spermatogonia can dedifferentiate and take part in forming a regenerative blastema together with proper neoblasts [3]. So, if spermatogonia are assumed to play not only the role of germinal elements but also of regenerative neoblast-like cells, the relationships between regeneration and sexuality in *Dendrocoelum* appear to take on a special meaning. In this connection we mention that testes regression occurs also in posterior stumps cut behind ovaries in fully regenerating *Dugesia lugubris* and *Polycelis nigra* (*Planariidae*) during head regeneration [4].

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According to Sauzin-Monnot and Lender [5] the low regenerative power of *Dendrocoelum lacteum* would depend on a poor neurosecretory activity: this would prevent neoblasts from RNA synthesis and hence their differentiation. However, the reason for this reduced neurosecretory activity is still unknown. On the other hand, *Dendrocoelum* neoblasts could be supposed either to be unable to receive neurosecretory material or to react to it. We can in any case assume that the low regenerative power of *Dendrocoelum* may be ascribed to some feature of its nervous system, such as a functional feature like the absence or the shortage of some neurosecretory material, or a structural feature like the peculiar arrangement of the peripheral nerve fibres which would release neurosecretory product to neoblasts and to cells behaving as such.

Owing to the great difficulty in quantitatively and qualitatively analysing the neurosecretory material of *Dendrocoelum* and, by way of comparison, in fully regenerating species as *Dugesia*, in this paper we have focussed our attention on the relationship and the arrangement of *Dendrocoelum* nerve fibres especially near the testes, as compared with those of *Dugesia*. In this latter planarian, as well as in *Polycelis*, the presence of a subepidermal peripheral nerve plexus and of a nerve fibre network around the testes and ovaries has already been evidenced by one of us [6].

About twenty adult sexually mature *Dendrocoelum lacteum* and *Dugesia lugubris* were Serra-fixed and Cajal III silver impregnated. In addition several specimens of *Dendrocoelum* were treated with Gabe's fuchsin-paraldehyde method in order to detect neurosecretory cells. Finally, some other animals were processed for electron microscopy, following the method described in previous papers dealing with neurosecretion in *Dugesia lugubris* and *Polycelis nigra* [7].

Cajal III silver impregnated specimens of *Dendrocoelum lacteum* did not clearly show either the subepidermal nervous plexus or the nerve fibre network surrounding the dorsal testes. Similarly, they did not permit the detection of a direct relationship between posterior nerve ventral cords, ovaries and ventral testes. On the contrary, simultaneously prepared control specimens of *Dugesia lugubris* distinctly showed a subepidermal nerve plexus as well as a nerve fibre network surrounding both ovaries and testes (Pl. I, Figs. 1, 2).

On the other hand, Gabe-positive cells appeared to be present in the *Dendrocoelum* nervous system, prevailingly in the cephalic region.

On electron microscopy neurosecretory cells were seen to be supplied with a large nucleus and a prominent nucleolus. In addition, they showed an extensive rough endoplasmic reticulum, several mitochondria, some glycogen particles and a well-developed Golgi apparatus, associated with a number of dense-cored vesicles, 60–100 nm in diameter. Golgi cisternae appeared filled with an electron dense material, very similar to the one contained in the above vesicles (Pl. II, Figs. 3, 4).

Nerve fibres resulting from the above neurons showed many microtubules 20 nm in diameter, some irregularly-shaped vesicles, a few glycogen particles and small roundish mitochondria. In addition, a few of them contained a certain amount of dense-cored vesicles, quite similar to those described above (Pl. II, Fig. 5). These fibres, mainly located within the posterior ventral nerve cords, never came into contact with the testes because of muscle and vitelline cells interposed in between.

In our opinion the most significant result from this research is that the thin nerve fibres incapsulating testes in different planarian species [6] seem to be missing in *Dendrocoelum lacteum* at light microscope level.

On the other hand, Gabe's method and especially electron microscopy, have revealed certain nerve cells whose features are quite similar to those of neurosecretory cells described in other planarian species [7, 8] and recently in *Dendrocoelum* [9]. These cells appear to produce from their Golgi apparatus the dense-cored vesicles that are found in posterior ventral nerve cord fibres and that are possibly carried by the microtubules to the different bodily territories. However, electron microscopy also proved unsuccessful in detecting the possible presence of thin nerve endings in direct contact with the testes.

Assuming that spermatogonia are able to dedifferentiate and then take part in regeneration as neoblast-like cells, the absence of nerve endings near the testes appear to explain the low regenerative power of *Dendrocoelum lacteum*. Since neurosecretory material seems to be released by exocytosis from neurosecretory fibres and to move by diffusion inside the parenchyma toward the target-cells [10] the above distance of the neurosecretory fibres from the testes would result in a poor inflow to spermatogonia and neoblasts of neoblast-stimuline neurosecretory material. This would also explain why *Dugesia*, where the nerve network surrounds the testes, has a different regenerative behaviour.

As already reported in the introduction, posterior stumps of *Dendrocoelum* resulting from a cut before the pharynx base regenerate the head but show a regression of testes. In this case, since the regenerative blastema is closer to the cephalic nerve cord tracts and to the brain, where the neurosecretory cells are more numerous, we can assume that a more abundant neurosecretory material may reach the neoblasts and spermatogonia even with poor nerve relationships; this assumption would agree with the morphogenetic grandient theory [11].

In conclusion, the genetically determined different arrangement of peripheral nerve fibres in the various planarian species could be extremely important to these animals whose biological processes largely depend on neurosecretion-neoblast stimulation-regeneration and germ cell production.

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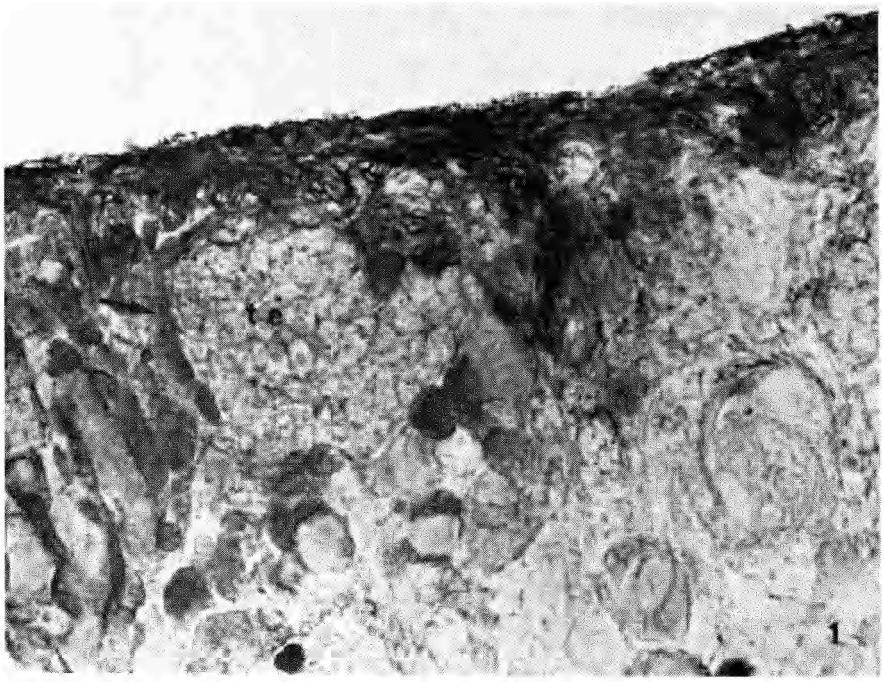
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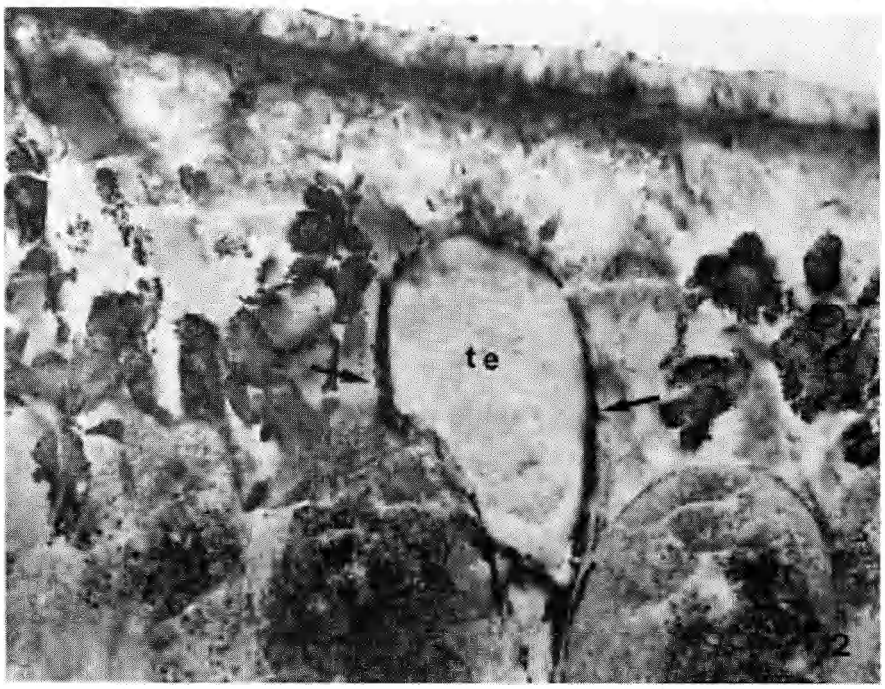
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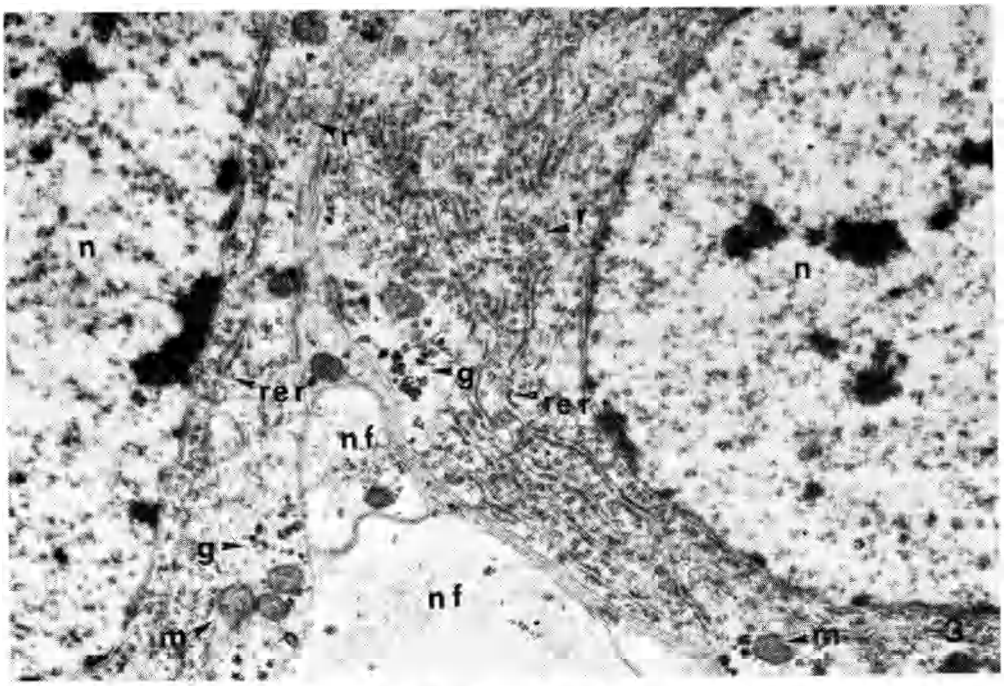
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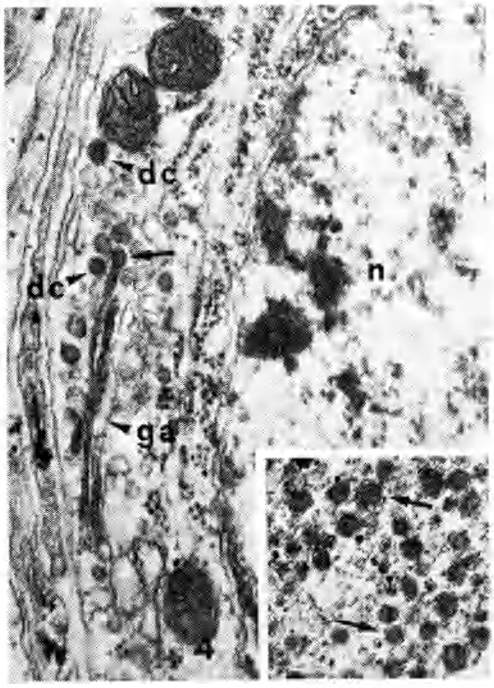
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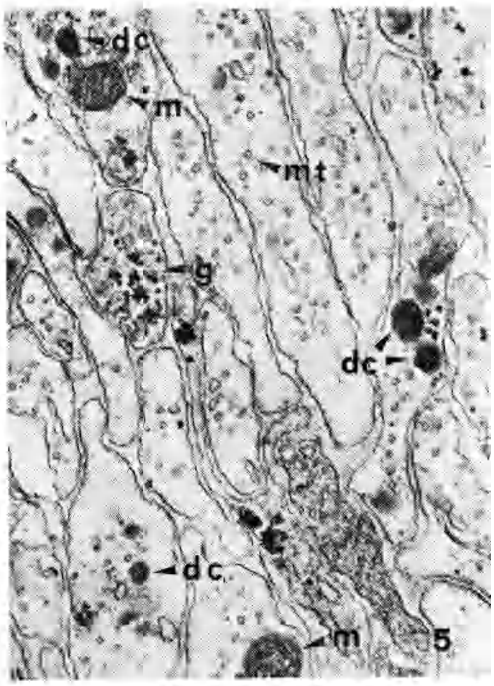
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EXPLANATION OF PLATES I-II

PLATE I

Fig. 1. – *Dendrocoelum lacteum* cross-section. Note the faint silver impregnation (arrow) around a testis (*te*). ×530.

Fig. 2. – *Dugesia lugubris* cross-section. Note the clear silver impregnation (arrows) around a testis (*te*). ×530.

PLATE II

Fig. 3. – Cephalic ganglia neurosecretory cells showing large nucleus (*n*), extensive rough endoplasmic reticulum (*rer*), several free ribosomes (*r*), glycogen particles (*g*) and mitochondria (*m*). Note: neighbouring nerve fibres (*nf*). ×20,000.

Fig. 4. – Neurosecretory cell showing a well-developed Golgi apparatus (*ga*) near its nucleus (*n*). Note: swollen cisterna end (arrow) very similar to free dense-cored vesicles (*dc*). ×30,000.
Inset shows in detail dense-cored vesicles.
Note: limiting membrane (arrows). ×40,000.

Fig. 5. – Ventral cord nerve fibres showing glycogen particles (*g*), mitochondria (*m*), microtubules (*mt*) and dense-cored vesicles (*dc*). ×47,000.