Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

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Observations on the cells partaking in the regeneration of Dugesia lugubris

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **63** (1977), n.1-2, p. 86–89.

Accademia Nazionale dei Lincei

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Zoologia. — *Observations on the cells partaking in the regeneration of* Dugesia lugubris. Nota (*) di MARIO GRASSO (**), GIUSEPPE GARDENGHI (***) e MARIA VITTORIA DI GIOVANNI (**), presentata dal Corrisp. E. VANNINI.

RIASSUNTO. — Con l'ausilio di due diverse tecniche è stato possibile recidere nel tratto medio-anteriore del corpo ambedue i cordoni nervosi ventrali posteriori della planaria *Dugesia lugubris*. L'esame istologico, eseguito dopo 10 giorni di continua interruzione di tali parti del sistema nervoso, ha permesso di rilevare che i follicoli testicolari retrostanti la recisione, invece di scomparire del tutto come fanno quando una planaria venga decapitata dietro gli ovari, rimangono quasi inalterati. Solo quelli più vicini alla ferita perdono gli spermatogoni dello strato periferico, che si trasferiscono a formare una parte del blastema rigenerativo. Ciò induce a supporre, in accordo con analoghi reperti di altri ricercatori, che la scomparsa dei testicoli nei casi di ablazione di estesi territori del corpo possa dipendere in larga misura dalla partecipazione alla genesi dei blastemi rigenerativi, oltre che dei « neoblasti », anche di altri elementi cellulari in fase di sdifferenziamento, fra cui i suddetti spermatogoni.

Previous experiments carried out by one of our group (1) revealed that in sexually mature specimens of *Dugesia lugubris* ablation of the cephalic region by transverse cutting in front of the base of the pharynx and behind the ovaries resulted in the total disappearance of the testes in the posterior stump of the animal body, deprived of the brain and the first tract of the posterior ventral nerve cords. Ovaries and testes then reappeared as soon as the extirpated territory had completely regenerated. These results were later confirmed by other research workers (2).

At the time, the results were interpreted by hypothesizing an inducing morphogenetic influence emanating from the cephalic nervous system and affecting both the development and the preservation of the hermaphrodite genital apparatus. This hypothesis agreed well with the one put forward

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(1) M. GRASSO, *Fenomeni rigenerativi e apparato genitale in* Dugesia lugubris, « Boll. Zool. », *26*, 523 (1959); M. GRASSO, *L'organizzazione dell'apparato genitale ermafrodita in esemplari rigenerati e in esemplari bicefali di* Dugesia lugubris, « Rend. Accad. Naz. Lincei », *35*, 101 (1963).

(2) L. BRANDI and E. GHIRARDELLI, *L'azione del cervello sulla rigenerazione delle gonadi di* Dugesia lugubris, « Rend. Accad. Naz. Lincei », *35*, 120 (1963); W. TESHIROGI and H. FUJIWARA, *Some experiments on regression and differentiation of genital organs in a freshwater planarian*, Bdellocephala brunnea, « Sc. Rep. Hirosaki Univ. », *17*, 38 (1964).

by Wolff *et al.* [3] who reached the same conclusion studying other planarian organic apparatus.

The present research continues to deal with the regenerative histogenesis of the male genital apparatus, but this time the incision in sexually mature adult *Dugesia lugubris* was performed on the posterior ventral nerve cords in the mid-anterior tract of the animal body. To ensure a permanent break in these nerve cords, two different techniques were used.

a) In one group of 5 animals, the operation consisted in an incision on both sides of the mid-anterior tract of the animal body; small bistouries were used and the incision was renewed twice daily for a total of 10 days. This prevented the margins from mending (fig. 1 A).

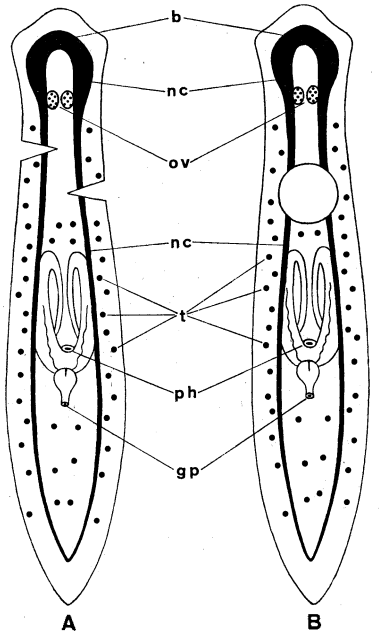

Fig. 1. – Bilateral incision of the posterior ventral nerve cords in sexually mature specimens of *Dugesia lugubris*. *A*) by cutting on both sides of the mid-anterior tract of the animal body; *B*) by removing a central disc from the mid-anterior tract of the animal body. *b*) brain; *nc*) posterior ventral nerve cords; *ov*) ovaries; *t*) testes; *ph*) pharynx; *gp*) genital pore.

b) In a second group of 5 animals the operation was carried out in the same body tract by removing a central disc of 1.5 mm diameter with a modified paracentesis needle, thereby producing a hole sufficiently wide to isolate the ventral nerve cords of the cephalic zone from those of the caudal part. Care was taken to renew the isolation twice daily for 10 days (fig. 1 B). All operated animals, including controls, were kept in a thermostatic cell at 20 °C for the entire experimental period. They were then fixed

(3) E. WOLFF and TH. LENDER, *Les néoblastes et les phénomènes d'induction et d'inhibition dans la régénération des Planaires*, «Ann. Biol.», *1*, 499 (1962); E. WOLFF, TH. LENDER and C. ZILLER-SENGEL, *Le rôle de facteurs auto-inhibiteurs dans la régénération des Planaires* (*Une interprétation nouvelle de la théorie des gradients physiologiques de Child*), «Rev. Suisse Zool.», *71*, 75 (1964).

in Serra liquid (16 ml ethyl alcohol at 95°,8 ml formalin and 1 ml glacial acetic acid), embedded in paraffin, cut transversally or frontally into 8 μ thick sections and stained with haematoxylin-eosin.

Histological examination of the animals operated by both techniques showed, 10 days after the recision of nerve cords, the following morphological characteristics: the testes located far from the wound were found to be almost unaltered both in their peripheral and central territories (Plate I, fig. 1); in the testicular follicles nearer the wound some peripheral spermatogonia seemed to migrate (Plate I, fig. 2), while in other testicular follicles the usual peripheral stratum of spermatogonia was missing (Plate I, fig. 3, fig. 4).

These findings, although at first seeming to be scarcely consistent with our idea of the importance of the nervous system in the preservation of the male gonads [4], may suggest a new view of the whole problem.

As previously demonstrated [1], the regression and therefore the disappearance of all testes can only be obtained by ablation of a very consistent anterior body region of planarians. The posterior stump requires a time lag of about 10 days for total regeneration of the lost piece and about 16 for *ex novo* regeneration of the gonads. However, in the anterior stump the hermaphrodite genital apparatus remains almost unaltered and the animal reacquires its earlier structural integrity within about 4 days.

In our present experiments, on the contrary, the mere recision of the posterior ventral nerve cords by either of the techniques described above evidently does not disturb the normal neurosecretory activity of the nervous plexus existing around the testes, which is notoriously necessary for germ–cell maturation [5]. This may explain the preservation of the testes further from the wound.

On the other hand, our observations on the particular regression of the testes nearest to the wound strongly suggest that after our operations the regenerative blastema may derive not exclusively from the usual "neoblasts", but also from the already differentiated cells derived from neoblasts which surround these testes as spermatogonia. This interpretation is in full agreement with previous findings [6], definitively confirmed in more recent research [7], on the participation of spermatogonia in regenerative pheno-

(4) E. VANNINI, *Introduction to the symposium on some aspects of sex differentiation in pluricellular animals at a lower order of organization: Porifera, fresh-water hydras and planarians*, « Boll. Zool. », *41*, 291 (1974).

(5) M. GRASSO and M. BENAZZI, *Genetic and physiologic control of fissioning and sexuality in Planarians*, « J. Embryol. exp. Morph. », *30*, 317 (1973); M. GRASSO, L. MONTANARO and A. QUAGLIA, *Studies on the role of neurosecretion in the induction of sexuality in a planarian agamic strain*, « J. Ultrastruct. Res. », *52*, 404 (1975).

(6) H. MANELLI and L. CONTOLI-AMANTE, *Aspetti dell'istogenesi rigenerativa in* Procerodes dohrni, « R. C. Accad. Naz. XL », *16*, 1 (1966).

(7) V. GREMIGNI and I. PUCCINELLI, *A contribution to the problem of origin of the blastema cells in planarians: a karyological and ultrastructural investigation*, « J. exp. Zool. », *199*, 57 (1977).

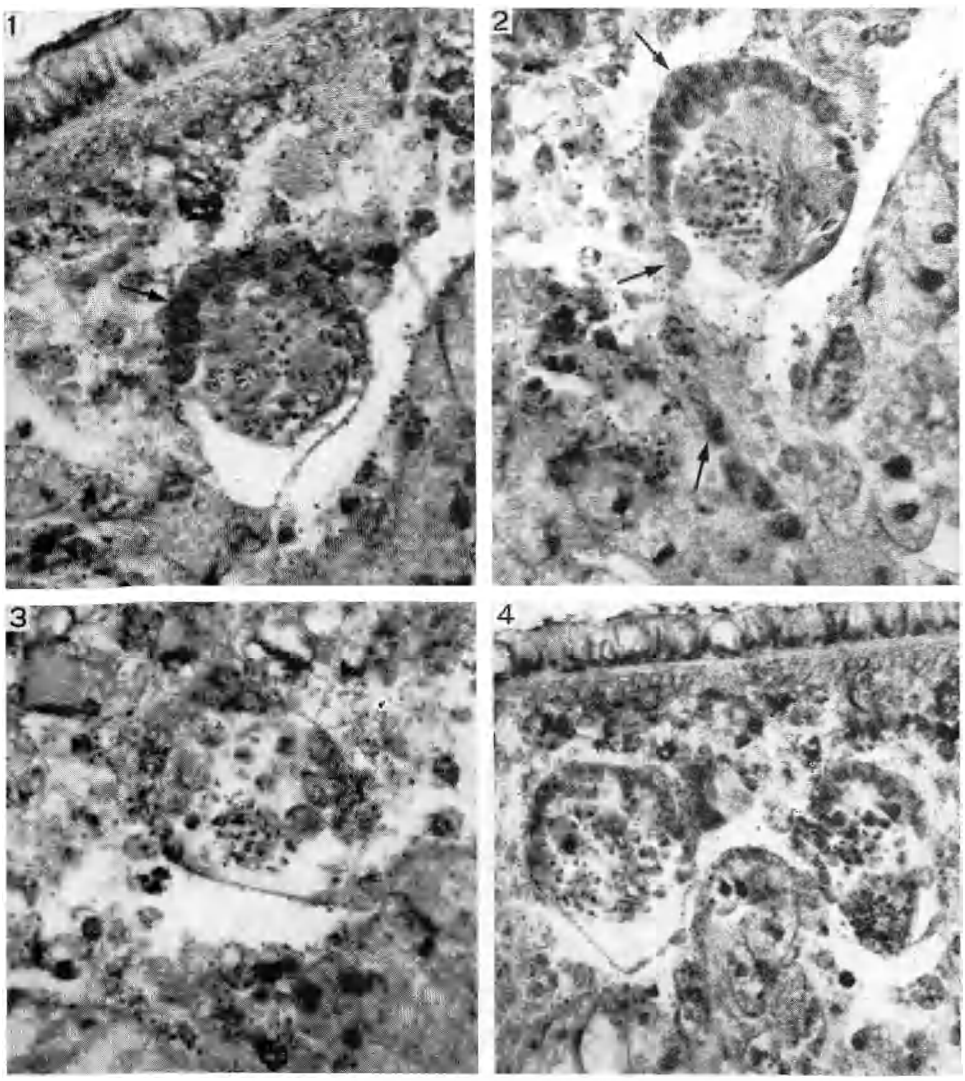

Fig. 1. – Unaltered testicular follicle. Fig. 2. – Testicular follicle from which peripheral spermatogonia (arrows) are migrating towards the regenerative blastema. Fig. 3 and fig. 4. – Testicular follicles in which the peripheral stratum of spermatogonia is already missing. 600×.

mena in planarians. Probably the previously quoted results [1] showing complete disappearance of testes after a complete decapitation of planarians largely depend on these facts.

It would therefore seem that our present investigation gives further support to the hypothesis that during regenerative phenomena dedifferentiated cells may redifferentiate into cells different from the original ones. Also Fedecka-Bruner's demonstration [8] of cellular cords extending from testicular follicles to the regenerative blastema in regenerating planarians seems to support our interpretation which could be considered an attempt at reconciling two apparently opposite theories on the origin of the planarian blastema: from totipotent undifferentiated "neoblasts" according to the French school of Wolff and Lender [3] [9], or at the expense of already differentiated cells capable of dedifferentiation according to other researchers, mainly American [10].

(8) B. FEDECKA-BRUNER, *Régénération des testicules des planaires après destruction par les rayons X*, in « Regeneration in animals and related problems », Kiortsis and Trampusch edit., North-Holland Publ. Co., Amsterdam, 185, (1965); B. FEDECKA-BRUNER, *Sur quelques conditions du maintien, de la régénération et de la maturation des testicules chez la planaire* Dugesia lugubris, « Bull. Soc. Zool. France », *92*, 287 (1967); B. FEDECKA-BRUNER, *Études sur la régénération des organes génitaux chez la planaire* Dugesia lugubris. I. *Régénération des testicules après destruction*, « Bull. Biol. France Belg. », *101*, 255 (1967).

(9) E. WOLFF and F. DUBOIS, *Sur une méthode d'irradiation localisée permettant de mettre en évidence la migration des cellules de régénération chez les planaires*, « C. R. Soc. Biol. », *141*, 903 (1947); TH. LENDER, *Factors in morphogenesis of regenerating fresh-water Planaria*, « Advanc. Morphogenesis », *2*, 305 (1962); M. J. SAUZIN-MONNOT, *Étude ultrastructurale des néoblastes de* Dendrocoelum lacteum *au cours de la régénération*, « J. Ultrastruct. Res. », *45*, 20 (1973); R. FRANQUINET, *Étude comparative de l'évolution des cellules de planaires d'eau douce* Polycelis tenuis (*Jijima*) *dans des fragments dissociés en culture* in vitro; *aspects ultrastructuraux, incorporations de leucine et d'uridine tritiée*, « J. Embryol. exp. Morph. », *36*, 41 (1976).

(10) P. STEINMANN, *Das Verhalten der Zellen und Gewebe im regenerierenden Tricladenkörper*, « Verh. Naturf. Ges. Basel », *36*, 133 (1925); P. STEINMANN, *Prospektive Analyse von Restitutionsvorgängen. I. Die Vorgänge in den Zellen, Geweben und Organen während der Restitution von Planarienfragmenten*, « Arch. Entw. », *108*, 646 (1926); L. WOODRUFF and L. BURNETT, *The origin of the blastema cells in* Dugesia tigrina, « Exp. Cell Res. », *38*, 295 (1965); E. D. HAY, *Dedifferentiation and metaplasia in Vertebrate and invertebrate regeneration*. In: *The Stability of Differentiate State*. H. URSPRUNG ed., New York, 85 (1968); E. D. HAY and S. J. COWARD, *Fine structure studies on the Planarian*, Dugesia. I. *Nature of the « neoblasts » and other cell types in noninjured worms*, « J. Ultrastruct. Res. », *50*, 1 (1975); S. J. COWARD, *Regeneration in Planarians: some unresolved problems and questions*, « J. Biol. Psych. », *11*, 15 (1969).