BOL. SOC. BIOL. DE CONCEPCION, TOMO XLV, pp. 175-188, 1972

STRUCTURE AND REGENERATION OF THE BRAIN AND EYE IN DUGESIA DOROTOCEPHALA (PLATYHELMINTES, TURBELLARIA)

POR

IVONNE C. HERMOSILLA

Depto. Biología Celular
Instituto Central de Biología

INTRODUCTION.

Fresh water planarians have been widely used for experiments owing to two facts: their remarkable regenerative power and their special place in the evolution of the nervous system. The regenerative capacity has been attributed, by most authors, to totipotent parenchyma cells dispersed through the body, which are able to differentiate histologically into the excised tissues (Dubois, 1949; Wolff, 1962; Lender, 1965; Stéphan-Dubois, 1965). Moreover, it is in these animals that, concurrently with the formation of a head, the intensive centralization of the nerve cells into the first primitive head ganglion begins.

Regarding the origin of the nervous system during regeneration, two hypothesis are advanced at the present time. One idea is that the nervous structure are formed from the cells of the old nervous system (Bardeen, 1902; Bondi, 1959); a second hypothesis is that the regenerative cells arise in the blastema, determine the new nervous system which later joins the old nervous tracts (Flexner, 1897).

Recent studies in planarian regeneration, tend to demonstrate that the new nerve cells result from the neoblast differentiation (Lender, 1952, 1962; Torok, 1958, Bacsy and Torok, 1962). However, the question arises here as to the manner in which the neoblasts differentiate into nerve cells. According to Lender (1951), the cerebral ganglia of *Polycelis* develops in the head blastema by means of self-differentiation, independently of the old nerve elements. On the other

hand, Torok (1958) and Bacsy and Torok (1962) found that the head ganglia in Dendrocoelum does not regenerate by self-differentiation, but as a result of a continuous process of regeneration induced by the old nerve trunks.

In connection with the differentiation of the planarian nervous system, the eye regeneration has been studied. Most authors emphasize the absence of nerve communication between the regenerating eyes and the cerebral ganglia concluding that the eyes appear by a process of self-differentiation. In the last decade, Wolff and Lender (1950), Lender (1956, 1962) demonstrated a so-called "organisine" produced by the brain which is able to induce the neoblasts of the blastema to differentiate in the direction of eye formation. Torok's studies (1958) have shown that the morphogenetic activity of the nervous system is not limited to the head ganglia, since the eye is induced by the nerve cords in the absence of the cerebral ganglia.

In the present state of investigation there is no doubt that these problems need further study. The present paper is an attempt to clarify the structure and regeneration of the brain and eyes in *Dugesia dorotocephala.*

MATERIALS AND METHODS.

The experiments were carried out on fully develop individuals of *Dugesia dorotocephala* that had been starved during 3 or 4 pre-operative days. The operations were performed without narcosis by means of a razor blade. The level of decapitation was always just behind the eyes and auricles. The operated animals were kept in darkness, in Petri dishes containing pond-water at room temperature of 22-24°C. The water was changed every day. The regenerating animals were not fed during the experiment, since it has been observed that starvation decreases the rate of asexual fission and stabilizes the internal milieu (Padersen, 1958).

In order to study the course of regeneration, some animals were fixed ½ hr, 6 hr, 24-48-60-72-84-96-108-120 hrs after decapitation. The fixatives used were Zenker and formalin-ethanol-acetic. To prevent contraction the animals were stretched between two slides and then inmersed in the fixative. After embedding in paraplast 5u transverse and sagittal sections were cut. The staining methods tested were Mallory, Giemsa, copper sulfate silver nitrate, Bodian, Heidenhain's iron hematoxylin and eosin.

RESULTS.

I.— Normal Planarians.

In *Dugesia dorotocephala* the head ganglion consists of two cerebral masses united anteriorly. Posteriorly the two masses diverge, each projecting posteroventrally until they are separated by a distance equal to their width (Fig. 1, Fig. 2). The diverging nerve masses are connected by from four to seven commissures (Fig. 3). Numerous cerebral nerves run from the outer surfaces of the head ganglion to the periphery of the head.

The level of divergence of the cerebral masses marks the anterior edge of the eyes. Slightly posterior to this level, the head ganglion reaches its maximum dorsal elevation and closely approaches the ventral surfaces of the eyes. Each cerebrae sends an optic nerve to the inner surface of the nearer eye (Fig. 4).

The posterior ends of the cerebral ganglia form a transition zone between the brain and the cords. This is rather gradual, although a decrease of the number of the cells is evident. The nerve cords continue ventrally in close relationship with the ventral muscles and epidermis. Cyanophilic glands are abundant prepharyngeally, most of them located ventrally to the head ganglia but taking a dorsal position along the nerve cords. This change in position takes place approximately at the level of the anterior gastric diverticulum. The nerve cords run to the posterior extremity of the animal. They are connected at frequent intervals by commissural fibers, and also give off fibers to the margins of the body.

The general histology of the central nervous system in *D. dorotocephala* consists principally of unipolar and bipolar neurons.

The unipolar neurons have usually a round chromatin-poor nucleus; the nucleolus is very often absent. This type of nerve cell exists abundantly distributed in the outermost zone of the cerebral ganglia; within the ganglia they are few in number, appearing scattered between the central fibers. They decrease in number along the nerve cords (Fig. 5-a).

The bipolar neurons appear to be of at least three types. The largest one shows an elongated nucleus in which small granules appear to be arranged in circles as viewed in a tissue section; no nucleolus is present. They are few in number and tend to be distributed along the cerebral nerves and ventral nerve cords (Fig. 5-b). Another type of bipolar neuron of regular size and marked bipolarity shows an ovoid or round large nucleus often containing a conspicuous nucleolus. This type is common along the cerebral nerves, commisures and nerve trunks (Fig. 5-c). A third type of bipolar neuron is seen in close relationship with the vertical muscles in the cerebral region. Here the nucleus is slightly ovoid and granular, the cytoplasm, usually well-

stained, is distributed along two prolongations, one leading into the muscles, the other one into the fiber bundles of the ganglion (Fig. 5-d).

On two occasions only a type of round, large nucleus slightly granular was found inside the cerebral nervous mass. Although its cytoplasm does not stain with basic dyes, it appears to be abundant. The entire cell is bounded by a membrane rather irregular in shape. The general appearance of these cells resembles some kind of supporting cells (Turner, 1946), (Fig. 5-e).

In the regions of head parenchyma close to and along the anterior cerebral nerves are examples of two types of non-nervous cells whose cytoplasmic boundaries are difficult to detect though their nuclei stain easily. In one type the nucleus stains moderately and shows numerous and conspicuous chromatin granules. By their appearance and by the fact that they are common in regions where some process of active differentiation is going on, they have been designated as "activated nuclei". The other type hereafter called "dense nuclei" apparently less abundant is smaller in size, stains heavily and the chromatin material is abundant. No nucleolus was observed in either of these two type of nuclei. Along the anterior cerebral nerves both types increase in number towards the margins of the body (Fig. 6).

All types of the nerve cells and the "activated" and "dense nuclei" are evident with Giemsa stain. They are stained with hematoxylin also, however with this stain one cannot distinguish the "activated nuclei" from most of the neuron nuclei.

Both Giemsa and hematoxylin show are presumably nerve fibers extending from the unipolar and bipolar cells into the fiber bundles. Such extensions in association with the "activated" or "dense nuclei" were found in very few cases.

Another important type of cell for this study is the neoblast. These appear to be most abundant prepharyngeally where they are found between the gastric diverticula and the ventral nerve trunks and also clumped together and scattered throughout the cephalic region. In addition they are widely and sparsely distributed throughout the parenchyma of the whole body. The shape of these cells is rather variable. The large nucleus may be round or ovoid; it usually shows one or two nucleoli densely stained with the basic dyes (Figs. 8-9). The scanty and basophilic cytoplasm is usually spindle-shaped, although round neoblasts are also common in intact planarians.

According to all descriptions of the turbellarian eye, it contains two regions — an optic cup formed by the pigment or accessary cells and a sensory region composed of visual cells or retinulae.

Dugesia normally has one pair of eyes placed very near together on the antero-dorsal surface. Each optic cup is formed by a single

layer of pigment cells whose granules are concentrated at the inner surface of these cells facing the distal portions of the retinulae. There is a dorso-lateral opening by which the retinula cells penetrate into the ocular cavity. A corneal membrane, formed by cytoplasmic expansions of the pigment cells, closes the ocular opening (Fig. 7).

The cell bodies of the retinulae, containing the nuclei are located outside of the cup. Their nuclei are large and granular. The cytoplasm has two long processes. One of these is directed through the cornea into the ocular cavity forming the slender distal portion of a retinula. Within the cup this slender process enlarges into a cone-shape "middle region" and still more distally into the rhabdomes (Fig. 7). These rhabdomes are distributed at different levels within the ocular cavity. The "middle region" is slightly stained a lilac color by Mallory's and intensely by the hematoxylin. It is possible to observe the longitudinal fibrils which continue from the "middle piece" into the rhabdome where in electron micrographs they seem to branch and form longitudinal lamellae (Press, 1959).

The other prolongation of the nerve cells body of the retinula circles outside of the optic cup to a ventral position, joining others to form the optic nerve which then establishes contact with the optic region of the brain. At this point conspicuous commissures connect the two optic regions of the brain.

II.— *Regenerating Planarians.*

Just after decapitation a strong contraction of the wounded areas occurs. Healing proceeds from the lateral surfaces. Internal tissues such as nervous system and digestive tract remain exposed at the cut surface during at least the first 10 hours. Sections cut 24 hours after decapitation show the wound covered with neoblasts.

Increase of the neoblasts at the anterior extremity begins at about 6 hours. As the growth of the blastema continues a few deeply staining nuclei are found in it. These "dense nuclei" decrease in number from the lateral regions of the blastema towards its center. Together with the neoblasts they accumulate in front of the cut ends of the nerve cords.

Approximately 24 hours after decapitation a considerable increase of the "dense nuclei" is evident at the anterior ends of the nerve cords, distributed especially at the dorsal and ventro-lateral regions close to the regenerating cords (Fig. 9). No such nuclei were seen around the regenerating gastric diverticula. By this time not only the neoblasts but most of the cells throughout the entire parenchyma appear with processes. Numerous neoblasts continue to accumulate during the next two days in the anterior region.

By the second day the old nerve cords have develop a three-lobed expansion of new fibers completely surrounded by a mixture of

neoblasts, "dense nuclei" and some "activated nuclei" (Fig. 9). By the second day the proliferation in the central zone of the blastema has increased rapidly. At this time the cells close to the nerve fibers are of three types: "dense nuclei", "activated nuclei" and neoblasts. The "dense nuclei" have become larger and now stain slightly lighter, allowing one to observe clumps of chromosomes. The "activated nuclei" show conspicuous chromatin granules. The neoblasts gradualy become less basophilic, their nuclei assuming the fine granularity of nerve cell nuclei and their nucleolo disappearing by lost of stainability. The transition towards the nerve cell bodies is already evident by the 2nd day. Finally, on the 3rd and 4th day, definite cytoplasmic extensions of both unipolar and bipolar neurons are visible.

By the end of the 2nd day, the primordium of the cerebral ganglia is determined although at this time the head has not attained its maximum size. The entire ganglionic primordium is richly surrounded by cells in active proliferation and differentiation. The first ventral commissures of the ganglia have appeared as outgrowths which have not as yet met in the midline. Cerebral nerve outgrowths from the posterior regions of the primordium is almost established by the 3rd day (Fig. 10).

The largest lobes are those associated with the eyes; here in the optic lobes the fibers and surrounding cells assume a vertical orientation directed towards the developing eyes.

The eye differentiation is iniciated during the 2nd day after decapitation (Fig. 11), simultaneously with the clear differentiation of the vertical muscles in the same region. In the dorso-lateral regions of the blastema and just in front of the optic lobes of the regenerating brain, a small group of differentiating cells assumes a spheric shape in the center of which there are scattered a few light brown pigment granules. On the 3rd day this sphere of cells forms an oval sac, flattened dorso-ventrally and elongated antero-posteriorly. Its cells are differentiating into the pigment cells of the eyes, gradually increase in number and in pigment content. Inside the cup is a lightly stained, noncellular material. This material shows no definite structure and has been assumed by Lender (1952) to be a jelly-like substance. Vertical muscles which traverse the head reach the optic cup and become distributed around the cup. Across the opening of the cup the lightly staining cornea is formed probably by cytoplasmic extension from the surrounding pigment cells.

Among the numerous differentiating cells, 2 or 3 elongated cells begin to assume the appearance of the retinula cells. By the 4th day most of the differentiating retinula cells are seen in front of the cup opening, although a few are located median, lateral and ventral to the cup. Some of these cells which lie close to the cup have a distal process directed into the optic cavity. Inside the cavity the slender process is continuous with the "middle region" and the rhabdome,

which have differentiated between the 3rd and 4th days. In the next day each retinula cell shows a proximal process which appears to be growing around the outside of the cup towards its postero-ventral surface where it will later become a part of the optic nerve (Fig. 12).

In spite of the proximity observed between the optic lobes and the regenerating eyes no nerve fiber connections were obvious through the 7th day. It is possible that the vertical muscles which traverse the ganglion and reach the optic cup obscure the pathway of the first few fibers of the optic nerve. At any rate the optic nerve is clearly visible on the 8th day.

In summary the nervous tissue appears to be well differentiated as to brain shape and cell types present by the 5th day after decapitation. Further increase in head size is mainly due to increase and further differentiation of the parenchyma cells. While the optic cup and its visual cells are well differentiated by the 5th day, the proximal processes of the retinula cells have not formed a well-defined optic nerve until the 8th day.

DISCUSSION.

Data concerning techniques for the demonstration of invertebrate nerve cells and nerve fibers are very poor; probably due to the difficulty of stainning methods. Staining with hematoxylin, Mallory and Giemsa good results were attained specially after a foregoing steep in Zenker fixation. These results may be due, probably to the affinity of the neuro fibrils to the molybdate-ions and to the Methylen-blue. The metal ions of fixative may also being significative in the results (Garzó, Torok and Rappay, 1960). However the Betchaku's and Bodians's techniques gave foor results; in these cases the fixative used, it was not Zenker.

The morphological features of the central nervous system in *D. dorotocephala* seen in this study agree with those of Hyman (1925). Furthermore the general review of the histology of the ganglia and nerve cords, although it does not pretend to be conclusive, is in agreement with the data reported by Monti (1897). Regarding the eye structure in this species, coincides in all respects with the results described by Taliaferro (1920), Lender (1952) and Press (1959).

The observations of the regenerative processes can be explained in either of two ways: (1) Regeneration of the nervous tissue is a result due entirely to the active participation of the neoblasts; or (2) Regeneration of the nervous tissue involves the participation of neoblasts and "dense nuclei". This latter suggests that the allegedly totipotent neoblasts do not act alone in the nervous system regeneration.

The assumption that the brain forms by the proliferation of nerve trunks without the intervention of neoblasts (Bondi, 1959) appears not to be the case. On the contrary, the neoblasts take an active role in the process (Lender, 1952, 1965; Stéphan-Dubois, 1965) in *D. dorotocephala.* However, their role from the beginning of the blastema formation in the nervous tissue regeneration appears not to be independent, but rather to be carried out in conjunction with the "dense" and "activated nuclei".

Since the appearance of the "dense nuclei" around the ends of the nerve cords in already evident at 24 hours after decapitation and by the 4th day has assumed the same distribution observed along the anterior nerves of the normal planarians, it is possible that these "dense nuclei" are necessary as in the beginning of the nervous regeneration as in the maintenance of the normal state of nervous tissue. Although the "activated nuclei" are seen in close relationship with the "dense nuclei" and show that quite likely they are derived from the "dense nuclei", no definitive conclusion as to their origin can be drawn from this study. The fate of these "activated nuclei" in nervous differentiation was not clearly established. However, cells in tiers or clumps many times observed in between the differentiation nerve cells, suggest by their spacial relation to the "activated nuclei" that they have just derived by a celular process from the cells containing the large "activated nuclei". To resolve this uncertainty, marking experiments need to be performed to find the origin, and follow the history of the "dense nuclei".

The histological studies in *D. dorotocephala* regeneration support Torok's hypothesis by demonstrating that a morphogenetic activity during regeneration is exerted by the two main nerve cords. The new ganglia are not initiated by a process of self-differentiation (Lender, 1951) independently of the old nerve cords, but by a continuous process from the old nerve trunks which appear to induce the neoblasts in a gradual postero-anterior differentiation.

According to Lender (1952) some neoblasts in the head are stimulated by the cerebral inductive factor called by him "organisine" to differentiate into the eye structure. This "organisine" has been thought to act before the optic nerve has reestablished the connection between the brain and the eye. The histological observations of the regenerating *D. dorotocephala* confirms this hypothesis, to the extent that no connections are visible between the regenerating brain and eyes until the optic cup and the retinula cells are already differentiated. Since in eye regeneration there was again observed the appearance of "dense and activated nuclei" among the differentiating neoblasts, a possibility of interactions between them and the neoblasts is suggested.

SUMMARY

Histological studies were performed in normal and regenerationg planarians in order to clarify the structure and the regeneration of the central nervous system and eyes in *Dugesia dorotocephala.* The brain is formed by two cerebral masses united anteriorly. Both cerebral ganglia continue posteriorly throughout the ventral nerve cords. As the cerebral ganglia as the ventral nerve cords are connected by commissural fibers. Most of the cells in the central nervous system are unipolar and bipolar neurons. In addition "dense" and "activated" nuclei which are in close relationship with the anterior cerebral nerves are detected in normal planarians. Neoblasts are scattered along the nervous structures. The eye is composed of the pigment cup and of retinula cells. The retinula cells are bipolar neurons whose distal portion penetrates into the ocular cup; the proximal portion of optic nerve enters into the ocular region of the brain.

Regeneration of the brain is due to the neoblast participation. However the evident eppearance of the "dense" and "activated" nuclei from the beginning of the process, suggest that this process is not determined exclusively by the neoblast but also by these two "nucleus" types. The head ganglia do not regenerate by self-differentiation alone, but as a result of a continuous process induced by the olf nerve cords. The 2nd day marks the appearance of the ganglionic primordium and the beginning of the eye differentiation. Nerve connections between eye and brain were found on the 8th day after decapitation.

RESUMEN

Se realizan estudios histológicos en planarias normales y en regeneración para clarificar la estructura y la regeneración del sistema nervioso central y de los ojos en *Dugesia dorotocephala.*

El cerebro está constituído por dos masas cerebrales unidas anteriormente. Ambos ganglios se continúan posteriormente a través de los cordones nerviosos ventrales. Tanto los ganglios cerebrales como los ventrales se conectan por fibras comisurales. La mayoría de los neurones en el sistema nervioso central son de tipo unipolar y bipolar.

En estrecha relación con los nervios cerebrales anteriores se encuentran núcleos "densos" y "activados".

Los neoblastos se encuentran esparcidos a lo largo de las estructuras nerviosas.

El ojo está compuesto de la copa pigmentada y de células retinulares. Estas últimas son neurones bipolares cuya porción distal penetra a la copa óptica; la porción proximal del nervio óptico entra a la región ocular del cerebro.

La regeneración del cerebro es debido a la participación de neoblastos. Sin embargo no se descarta la participación de los núcleos densos y activados desde el comienzo del proceso regenerativo. Los ganglios cefálicos no regeneran por autodiferenciación, sino como resultado de una inducción contínua, ejercida por las cuerdas nerviosas antiguas. Durante el segundo día aparece el primordio ganglionar y el comienzo de la diferenciación ocular. Conexiones nerviosas entre el ojo y el cerebro fueron establecidas en el octavo día después de la decapitación.

REFERENCES

BACSY, E. and TOROK, L. J.
1962 Contributions to the regenerative capacity of *Dendrocoelum lacteum*. Acta Biol. Hung. *12* : 313-327.

BARDEEN, CH. R.
1902 Embryonic and regenerative development in planarians. Biol. 3 : 262-288.

BETCHAKU, T.
1960 A copper sulfate-silver nitrate method for nerve fibers of planarians. Stain Technology *35* : 215-218.

BONDI, C.
1959 Osservazioni sui rapporti tra rigenerazione degli occhi e sistema nervoso in *Dugesia lugubris*. Arch. Zool. Ital. *44* : 141-150.

BULLOCK, T. H., and HORRIDGE, G. A.
1965 Structure and Function in the Nervous System of Invertebrates. Vol. *I*. Freeman, W. H., San Francisco.

CASTLE, W. A.
1928 An experimental and histological study of the life-cicle of *Planaria velata*. J. Expt. Zoo. *51* : 417-477.

DUBOIS, F.
1949 Contribution a l'étude de la migration des cellules de regeneration chez les planaires dulcicoles. Bull. Biol. *83* : 213-283.

FLEXNER, S.
1897 The regeneration of the nervous system of *Planaria torva* and the anatomy of the nervous system of double headed forms. J. of Morph. 14 : 337-346.

HYMAN, H.
1925 The reproductive system and other characters of *Planaria dorotocephala Woodworth*. Trans. Am. Micros. Soc. XLIV : (2) 51-89.

LENDER, TH.
1951 Sur les propriétés et l'étendu du chapm d'organization du cerveau dans la régénération des yeux de la planaire *Polycelis nigra*. C. R. Soc. Biol. *145* : 1211-1214.

LENDER, TH.
1952 Le role inducteur du cerveau dans la régénération de yeux d'une planaire d'eau douce. Bull. Biol. *86* : 140-215.

LENDER, TH.
1956 Recherches experimentales sur la nature et les propriétés de l'inducteur de la régénération des yeux de la planaire *Polycelis nigra*. J. Embryol. exp. Morph. *4* : 196-216.

LENDER, TH.
1962 Factors in morphogenesis of regenerating fresh-water planaria. Advances in Morphogenesis *2* : 305-331.

LENDER, TH.
1965 La régénération des planaires. Proc. Regeneration in Animals. NorthHoll. Publ. Co., Amsterdam : 95-111.

GAZSO, R. L., TOROK, L. J. and RAPPAY, G.
1961 Contribution to the histochemistry of the nervous system of planarian. Acta Biol. Hung. *11* : 411-428.

MONTI, R.
1897 Sur le system nerveux des dendroceles d'eau douce. Ital. Biol. *27* : 15-26.

PEDERSEN, K. J.
1958 Morphogenetic activities during planarian regeneration as influenced by triethylene melamine. J. Embryol. Exp. Morph. *6* : 308-334.

PEDERSEN, K. J.
1959 Some features of the fine structure and histochemistry of planarian subepidermal gland cells. Z. Zellforsch. *50* : 121-142.

PEDERSEN, K. J.
1959 Cytological studies on the planarian neoblast. Z. Zellforsch. *50* : 799-817.

PEDERSEN, K. J.
1961 Studies on the nature of planarian connective tissue. Z. Zellforsch. *53* : 569-608.

PEDERSEN, K. J.
1963 Slime-secreting cells of planarians. Ann. N. York. Acad. Sc. *99* : 424-443.

PRESS, N.
1959 Electron microscope study of the distal portion of a planarian retinular cell. Biol. Bull. *117* : 511-517.

STEPHAN-DUBOIS, F.
1965 Les neoblasts dans la régénération chez les planaires. Proc. Regeneration in Animals. North-Holl. Publ. Co., Amsterdam :212-229.

TALIAFERRO, W. H.
1920 Reactions to light in *Planaria maculata* with special reference to the function and structure of the eyes. J. Exp. Zool. *31* : 69-116.

TOROK, L. J.
1958 Experimental contributions to the regenerative capacity of *Dugesia lugubris*. Acta Biol. Hung. *9* : 79-98.

TURNER, R. S.
1946 Observations on the central nervous system of *Leptoplana acticola*. J. Comp. Neurol. *85* : 53-65.

WOLFF, E.

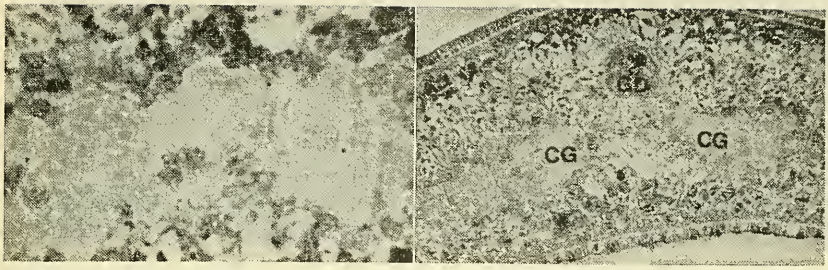
1962 Recent researchs on the regeneration of planarian Regeneration, *20th Growth Symposium* : 53-89.

WOLFF, E. and DUBOIS, F.

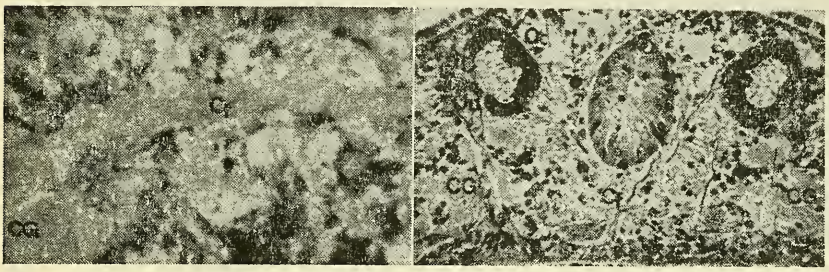
1947 La migration des cellules de régénération et les facteurs qui la provoquent chez les planaires. C. R. Acad. Sci. *241* : 1387-1388.

WOLFF, E. and LENDER, Th.

1950 Sur le role organisateur du cerveau dans la régénération des yeux chez une planaire d'eau douce. C. R. Acad. Sci. *230* : 2238-2239.



1 2



3 4

5 6

Fig. 1.— Traverse section of the anterior cerebral region in the normal specimen of *D. dorotocephala* (Giemsa stain, x 450).

Fig. 2.— Transverse section showing the divergence of the brain at the level of the gastric diverticulum (Giemsa stain, x 280). GD = Gastric Diverticulum; CG = Cerebral Ganglia.

Fig. 3.— Commisular fibers at the cerebral level (Giemsa stain, x 450). Cf = Commisural fibers.

Fig. 4.— Transverse section of a normal planarian at the eye level (Heidenhain's iron hematoxylin and eosin, x 280). Oc = Optic cup; On = Optic nerve; C = Cornea; CG = Cerebral Ganglia; Cf = Commisural fibers.

Fig. 5.— Different types of nerve cells in the Central Nervous System of D. *dorotocephala*.

Fig. 6.— "Dense" and "activated nuclei" along the anterior region of the brain (Giemsa stain, x 300). Dn = "Dense nucleus; Ac = "Activated nucleus".

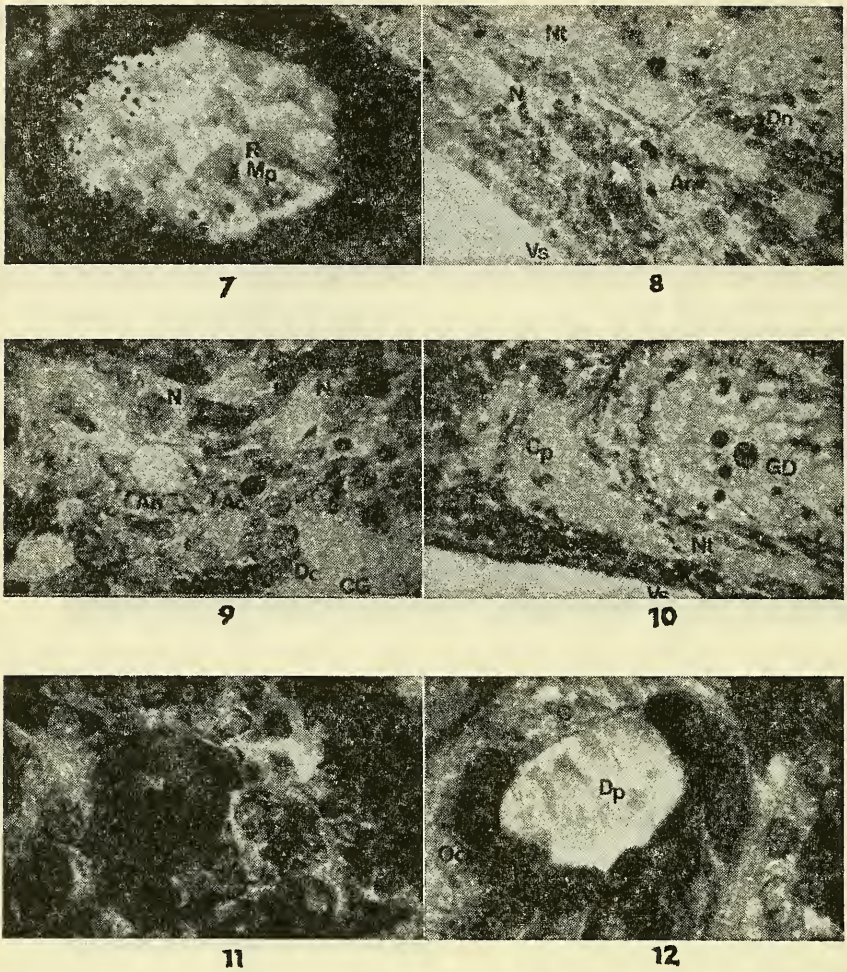


Fig. 7.— Transverse section of the eye in a normal planarian (Mallory stain, x 615). Mp = "Middle piece"; R = Rhabdome.

Fig. 8.— "Dense and activated nuclei" around the old nerve trunks of 1 - day regenerating planarian (Giemsa stain, x 300). Nt = Nerve trunk; N = Neoblast.

Fig. 9.— Nerve cell differentiation of 4 - day regenerating planarian (Giemsa stain, x 615). N = Neoblast; Dc = Differentiating cells.

Fig. 10.— Longitudinal section of the cephalia primordium of 3 - day regenerating planarian (Mallory stain, x 300). Cp = Cerebral primordium; Nt = Nerve trunk; GD = Gastric Diverticulum; Vs = Ventral surface.

Fig. 11.— Transverse section of 2 - day regenerating planarian showing the begining of eye differentiation (Mallory stain, x 615).

Fig. 12.— Transverse section of 6 - day regenerating planarian showing a differentiated eye (Mallory stain, x 615). Oc = Optic cup, C = Cornea. Dp = Distal portion of a retinula cell into the ocular cavity.