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# Regeneration and Endocrinology in the Polychaete *Platynereis dumerilii*

## An Experimental and Structural Study*

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**Summary.** 1. In the polychaete *Platynereis dumerilii*, the hormone-elaborating portion of the prostomium was determined by means of prostomium transection and implantation experiments. The area in question lies between the two pairs of eyes, extending longitudinally from the posterior border of the anterior eyes to about the posterior border of the posterior eyes. This corresponds approximately with the brain area delimited by the anterior and posterior dorsoventral connective tissue tubes and which is covered ventrally by the infracerebral gland epithelium.

2. The infracerebral gland-complex and neurosecretory neurons within the brain were envisaged as possible sites of hormone synthesis.

3. The infracerebral gland-complex in *Pl. dumerilii* was investigated with light- and electron-microscopical techniques. A leaf-shaped area (measuring 120 by 95 µm at the most) of the pericapsular epithelium at the ventral side of the brain, adjacent to the main blood vessel and to its efferent branches, consists of specialized columnar epithelial cells. Numerous *a*-cells and scarce *b*-cells can be distinguished. Fibre tracts with glia fibres and axons (some being neurosecretory axons) descend from the neuropile and in part terminate with prominent end-structures at the inner face of the brain capsule in the gland region. Probably some axons penetrate the capsule and make contact with the gland cells. Neither structural nor experimental findings prove that the infracerebral gland synthesizes the brain hormone. Accessory functions are discussed.

4. Investigations in secretory brain cells of *Pl. dumerilii* are reported. In agreement with Müller (1973), a lack of correlation between the number of stainable neurosecretory neurons and the hormonal activity of the brain was found: in immature worms (to which high hormonal titers are ascribed) only few or even no neurosecretory brain cells at all were detectable. Prostomium transection and implantation experiments show further that not all regions of the brain which enclose neurosecretory neurons produce brain hormone. The results are discussed with reference to the hypotheses of Müller

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(1973) which suggest that the appearance of stainable neurosecretory brain cells indicates inactivation of neurons possibly previously involved with hormone synthesis.

**Key words:** Annelida – Nereidae – Endocrinology – Control of regeneration.

**Zusammenfassung.** 1. Beim Polychaeten *Platynereis dumerilii* wurde der hormonbildende Bereich des Prostomiums experimentell durch Prostomiumdurchschneidungs- und Implantationsversuche eingegrenzt. Das fragliche Areal liegt innerhalb der beiden Augenpaare und erstreckt sich vom Hinterrand der Vorderaugen bis etwa zum Hinterrand der Hinteraugen. Dies entspricht ungefähr dem Bereich des Gehirns der zwischen dem vorderen und dem hinteren, das Gehirn dorsoventral durchziehenden Bindegewebsstrang liegt und der ventral vom Epithel des Infracerebralkomplexes überzogen ist.

2. Als mögliche Bildungsorte für das Gehirnhormon werden in Betracht gezogen: (a) der Infracerebralkomplex, (b) neurosekretorische Neurone im Gehirn.

3. Der Infracerebralkomplex von *Pl. dumerilii* wurde licht- und elektronenoptisch untersucht. Ein im Umriß blattförmiges, maximal 120× 95 µm messendes Feld des Epithels, das die Gehirnkapsel an der Ventralseite des Gehirns außen bedeckt und das dem Hauptblutgefäß und seinen beiden abführenden Ästen benachbart ist, besteht aus höheren, spezialisierten Epithelzellen. Elektronenoptisch können zwei Zelltypen unterschieden werden: die überwiegenden *a*-Zellen und die selteneren *b*-Zellen. Aus dem Neuropilem nach ventral ziehenden Faserzüge mit Gliafasern sowie z.T. neurosekretorischen Axonen enden (teilweise mit ausgeprägten Endstrukturen) an der Innenseite der Gehirnkapsel. Einige Axone durchdringen diese möglicherweise, um mit den spezialisierten Epithelzellen in Kontakt zu treten. Weder schlüssige strukturelle noch experimentelle Befunde weisen die Infracerebraldrüse als eigenständige „Gehirnhormondrüse" aus. Verschiedene akzessorische Funktionen werden diskutiert.

4. Untersuchungen an neurosekretorischen Gehirnzellen bei *Pl. dumerilii* werden vorgelegt. Danach besteht Übereinstimmung mit Müller (1973) darin, daß eine bemerkenswerte Inkongruenz der Anzahl färberisch nachweisbarer, neurosekretorischer Neurone und der experimentell ermittelten hormonalen Aktivität der betreffenden Tiere besteht: so sind bei jungen, unreifen Individuen, denen hohe Hormontiter zugeschrieben werden, wenige, z.T. gar keine neurosekretorischen Neurone erkennbar. Unter Bezugnahme auf die Prostomiumdurchschneidungs- und Implantationsversuche ist weiter festzustellen, daß nicht in allen Gehirnbereichen, in denen neurosekretorische Zellen vorkommen, Fähigkeit zur Hormonbildung zukommt. Die Resultate werden im Hinblick auf die Thesen von Müller (1973) erörtert, denenzufolge das Auftreten färbbarer Neurosekrete als Indiz für die Inaktivierung möglicherweise zuvor hormonbildender Neurone gewertet werden kann.

**Schlüsselwörter:** Annelida – Nereidae – Endokrinologie – Regenerationssteuerung.

The almost innumerable investigations on the prominent developmental feature of polychaete and oligochaete annelids, i.e. their considerable potentiality to regenerate amputated posterior body parts and, in many cases, to restore anterior structures also, have mostly covered problems of morphogensis and histogenesis (for references see Berrill, 1952; Hofmann, 1966 and the exhaustive bibliography of Boilly, 1967c). Nevertheless, quite a number of authors were interested in the analysis of factors governing the regeneration processes. In nereids, some time ago Nusbaum (1908b, in *Nereis diversicolor*) and recently Boilly and Combaz (1970, also in *N. diversicolor*) have investigated the influence of the ventral nerve-cord on the emergence and symmetry of the regenerates. The nervous system also influences regeneration via the supraoesophageal ganglion, whose endocrine role was first suggested in a *Lycastis* species by Harms (1948). Reliable experimental evidence for this concept was provided by Casanova (1955) in *Platynereis massiliensis*, a hermaphrodite polychaete. The hormonal character of the prostomial factor promoting regeneration, found by Casanova, was proved by Hauenschild (1960a) in the gonochoric *Platynereis dumerilii* by his prostomium-replacement technique. It was also Harms (1948) who speculated on the function of neurosecretory cells, discovered by B. Scharrer (1936) in the brain of *Nereis virens*; he thought that similar cells in the supraoesophageal ganglion of *Lycastis* spec. might be involved in the control of regeneration. Initiated by the findings of these investigators, a large number of papers subsequently dealt with the problem of regeneration and endocrinology in annelids, particularly in nereids, which are considered in the present work (Durchon, 1956; Hauenschild and Fischer, 1962; Clark and Bonney, 1960; Herlant-Meewis and van Damme, 1962; Scully, 1964; Gabe, 1966; Hauenschild, 1966, 1974a; Hofmann, 1966, 1974a, c; Golding, 1967a–e, 1974a; Porchet, 1967; Porchet and Durchon, 1968; Porchet and Cardon, 1972; Müller, 1973; list not exhaustive).

In the present paper, data on the production of the regeneration-promoting hormone in *Platynereis dumerilii* are collected, and arguments are contributed to identify this hormonal factor with the hormone known to control maturation and epitoky (Hauenschild, 1974c, for a recent review). The particular aim of this work was an approach to the localization of the hormone-producing elements within the prostomium. This includes a first ultrastructural survey of the *infracerebral gland*, a complex probably with endocrine function in *Platynereis dumerilii*. It has already been described in several other nereid species and also in other polychaete families (Bobin and Durchon, 1952; Dhainaut-Courtois, 1966a, b, 1968b; Golding, Baskin and Bern, 1968; Baskin and Golding, 1970; Golding, 1974b, Müller, 1973; Whittle and Golding, 1974).

## Material and Methods

All experiments reported in this paper were performed on laboratory-bred *Platynereis dumerilii* (Audouin and Milne-Edwards). For the details of the anaesthesia, the surgical techniques and the post-treatment see Hofmann (1975, pp. 510–514).

*Test for Endocrine Activity.* The lack of physico-chemical assays to determine hormonal titers in animals either at different developmental stages or under particular experimental conditions, necessitates the use of biological assays. These have to consider morphogenetic events and their chronological order, and yield information only on the relative activity. In this paper, the degree of differentiation of posterior regenerates developed under various conditions, was used as a measure for hormonal activity. In addition, the time of survival and the emergence of symptoms of maturation and epitoky were taken into account. The assay partly utilizes the ability of isolated prostomia of *Platynereis dumerilii* (Fig. 1) to survive for weeks or even months and to exert endocrine control functions if implanted into the coelom of decerebrated host animals. If a typical pygidium was formed and complete atokous parapodial segments were proliferated, the prostomial fragment submitted to the test was considered "active" (Fig. 2a). If the pygidium was defective, only rudimentary or no parapodes at all were formed and if precocious maturation and heteronereid transformation led to death within two weeks ("epitokoid development", Hofmann, 1966), the prostomial fragment was considered "inactive" (Fig. 2b). An observation period of about two weeks was sufficient to decide whether the prostomial structure tested was active or not. Posterior amputation of the worms was performed between segments 20 and 21 or between segments 40 and 41. Prostomial fragments to be tested were either left *in situ* or transplanted into the host's body cavity. An incision was therefore made by ablation of one parapode of segment 12 or 13, or of segment 25 if more posterior portions of the worms were used as host-fragments.

Individuals dying within the first three days because of damage inflicted by the experimental procedure, and animals which rejected or resorbed the implanted prostomial tissue, are not considered.

*Histological Methods.* Anterior ends of worms deprived of their jaws were fixed usually in Bouin's fluid, occasionally in Helly's fluid, then dehydrated and embedded in Tissuemat special paraffin and cut into 5 µm serial sections. Staining: Azan-novum (Geidies) hematoxylin (Weigert)-light green-orange-G. Paraldehyde-fuchsin (according to Gabe, 1966) and a modified alcian blue technique were used for the detection of neurosecretory material. For comparative purposes, serial sections of anterior ends of *Nereis diversicolor* were investigated. All photomicrographs were taken by a Zeiss photomicroscope.

*Electron-microscopical methods.* Prostomia amputated from intact worms with about 50 parapodial segments, were fixed in phosphate-buffered 3.5% glutaraldehyde at pH 7.4 for 1 h at 20° C and postfixed in phosphate-buffered 1% $OsO_4$, to which 10% sucrose was added, for $1^1/_2$ h at 0° C. In some cases prefixation with glutaraldehyde was omitted. After dehydration the prostomia were embedded in araldite. Sections were cut with glass knives on a Reichert OMU 2 microtome. Thick sections (0.5–1 µm) were stained according to Richardson et al. or with toluidine blue. Ultrathin sections were stained with uranyl acetate, followed by lead citrate and examined by a Zeiss EM 9 microscope.

## Results

### *A. Experimental Results*

#### LOCALIZATION OF THE ENDOCRINE ACTIVE AREA OF THE PROSTOMIUM

In two series of experiments the hormonal activity of different portions of the prostomia with the corresponding brain-areas was tested. In the first series (experiments No. 1–13), the portions to be tested were left in situ, in the second series (experiments No. 14–22), the respective prostomial parts were excised and implanted into debrained worm fragments. The results are represented in Table 1. Regeneration at the prostomial rudiment which was observed in the first series has previously been described by Hofmann (1975).

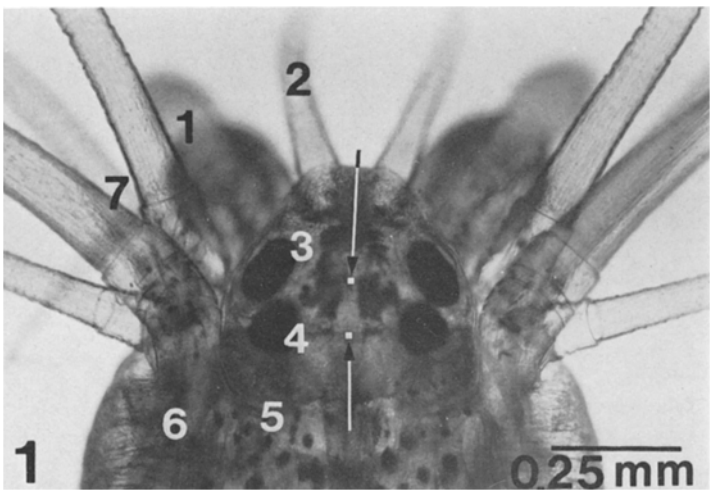



**Fig. 1.** Anterior end of *Platynereis dumerilii*, dorsal view, photomicrograph of live specimen. *1* palpus, *2* antenna, *3* anterior eye, *4* posterior eye, *5* nuchal organ, *6* peristomium, *7* peristomial cirri, *Arrows* point to the position at which the anterior and the posterior tubular strands of connective tissue emerge at the dorsal side of the brain (see also Fig. 3)

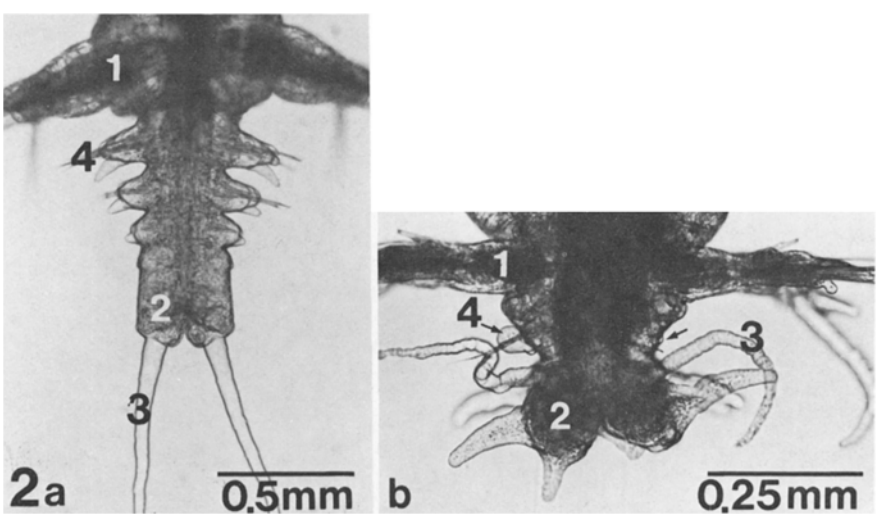
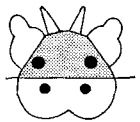
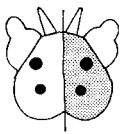
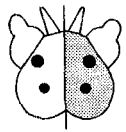
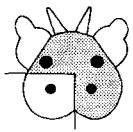
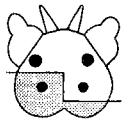
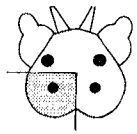
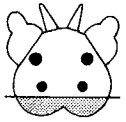
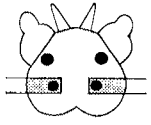
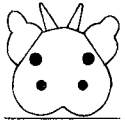
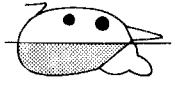
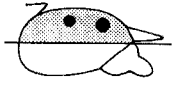
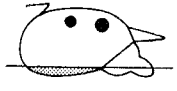
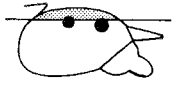



**Fig. 2a and b.** *Platynereis dumerilii*, posterior regenerates. Photomicrographs of live specimens. **a** typical caudal regenerate grown under the hormonal control of an intact prostomium. The endocrine status of prostomia of experimental animals which exhibited this type of regenerate was designated "*active*". **b** atypical posterior regenerate developed under anhormonal conditions. The endocrine status of prostomia of experimental animals exhibiting this type of regenerate and showing epitokoid development was designated "*inactive*". Note rudimentary parapodium (arrow) and the digitiform outgrowths of the pygidium, typical for the epitokous males, in which they serve for evacuation of sperm. *1* parapodium of former wound-segment, *2* regenerated pygidium, *3* urite (appendage of pygidium), *4* parapodium of regenerated segment. (From Hofmann, 1975)

**Table 1.** *Platynereis dumerilii*: Endocrine status of various prostomial fragments

The prostomial fragments which were subjected to the test are represented by stippled areas. In experiments Nos. 1–9 the prostomia were transected dorsoventrally; in experiments Nos. 10–13 horizontal transections were performed and in addition in Nos. 12 and 13, the remaining brain tissue was removed from the dermalmuscular tunic. In experiments Nos. 14–22 prostomial portions were excised by dorsoventral sections and were subsequently implanted into debrained worm fragments

| Number and type of experiments | Number of animals operated, surviving | Endocrine status of the "in situ" prostomial fragments as revealed by the morphology of the caudal regenerates | | Number and type of experiments | Number of animals operated, surviving | Endocrine status of the implanted prostomial fragments as revealed by the morphology of the caudal regenerates | |
|---|---|---|---|---|---|---|---|
| | | active | inactive | | | active | inactive |
| 1  | 24 | 24 | 0 | 14  | 28 | 0 | 28 |
| 2  | 20 | 15 | 5 | 15  | 27 | 9 | 18 |
| 3  | 21 | 20 | 1 | 16  | 47 | 31 | 16 |
| 4  | 31 | 30 | 1 | 17  | 38 | 21 | 17 |
| 5  | 20 | 19 | 1 | 18  | 14 | 11 | 3 |

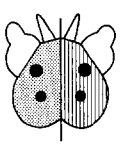
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 6 |  | 42 | 41 | 1 | 19 |  | 21 | 18 | 3 |
| 7 |  | 24 | 1 | 23 | 20 |  | 12 | 10 | 2 |
| 8 |  | 14 | 0 | 14 | 21 |  | 26 | 4 | 22 |
| 9 |  | 40 | 0 | 40 | 22 |  | 33 | 30 | 3 |
| 10 |  | 22 | 8 | 14 | | | | | |
| 11 |  | 25 | 23 | 2 | | | | | |
| 12 |  | 15 | 0 | 15 | | | | | |
| 13 |  | 14 | 1 | 13 | | | | | |

Following dorsoventral, transverse dissection of the prostomia between the anterior and the posterior pair of eyes (experiment Nr. 1), in 100% of the animals the formation of typical caudal regenerates, indicating the presence of hormone-producing structures in the prostomial remnant, was induced. The same applies to 75–97% of animals in which even smaller prostomial areas were left (experiments No. 2, 3, 4), also for 95% of those individuals with longitudinally halved prostomia, and for 95% of experimental animals in which only one posterior quadrant (including one posterior eye) was retained (experiment No. 6). If the section was performed further posterior in order to retain only the nuchal region and eventually the hindmost cells of the posterior brain-lobes (experiments No. 7 and 8), endocrine activity was detected only in one exceptional case. The ablation of the entire prostomium (experiment No. 9) led always to epitokoid development and death within two weeks.

Horizontal dissection of the prostomia and removal of their dorsal or ventral portion (experiments No. 10 and 11), brought about different results. When the ventral portion was retained, only 36% of the individuals developed typical caudal regenerates; all others underwent epitokoid development. However, when the dorsal portion was preserved, almost all individuals proved to have hormonally active structures. Additional excision of the brain rudiments following horizontal transection of the prostomia (experiments No. 12 and 13) obviously deprived the animals of their hormone-producing cells.

The results of the second series of experiments (No. 14–22) demonstrated that *prostomial parts of rather different size exhibited hormonal activity, but activity was not present in all regions.* If an anterior portion including the anterior pair of eyes and the foremost part of the brain was excised by a slightly angular section (experiment No. 14), none of those anterior fragments could induce typical caudal regeneration. This was in contrast to the potentialities of the posterior, complementary portions which were also subject to the implantation test (Table 2, experiment No. 23). If the section was carried out transversely between the anterior and the posterior pair of eyes, still the majority (67%) of the anterior portions proved to be inactive, but 87% of the complementary parts were active. The proportion of prostomial fragments proving to have hormonal activity increased with the inclusion of more posterior portions of the prostomium (experiments No. 16–20). However, lateral fragments which included the posterior eyes were inactive in 85% of cases. Considering the results of the experiments No. 1–21 (Table 1) and also the findings of the tests of complementary prostomial portions (Table 2, experiments No. 23–26), the general assumption that the hormone-producing elements in nereids are located within the brain is applicable to *Platynereis dumerilii*, as proposed for many years by Hauenschild. *As to their distribution, one might assume from the experiments described above that the brain-hormone-producing cells are situated in a more central, probably superior part of the supraoesophageal ganglion. The area appears to extend from about the posterior border of the anterior eyes to the posterior region of the posterior eyes, and to cover the space between both pairs of eyes.* This concept was examined by experiment No. 22 (Table 1) and proved to be valid: 91% of the "interocular" prostomial portions excised and transplanted into test fragments exhibited hormonal activity.

**Table 2.** *Platynereis dumerilii*: Endocrine status of implanted complementary prostomial fragments
The experiments were designed so that both prostomial fragments which were obtained from isolated, dorsoventrally transected prostomia, were transplanted into separate, debrained worm fragments. The prostomial portions subjected to the test are represented by stippled and hatched areas

| Number and type of experiment | | Number of fragments implanted | | Endocrine status of the implants as revealed by the morphology of the caudal regenerates | | Successful transplantation of both prostomial fragments of one donor | Endocrine status of the implants | |
|---|---|---|---|---|---|---|---|---|
| | | | | active | inactive | | both active | both inactive |
| 23 |  | anterior fragm.<br>posterior fragm. | 28<br>31 | 0<br>27 | 28<br>4 | 28 | 0 | 1 |
| 24 |  | anterior fragm.<br>posterior fragm. | 27<br>24 | 9<br>20 | 18<br>4 | 24 | 8 | 4 |
| 25 |  | anterior fragm.<br>posterior fragm. | 28<br>29 | 16<br>9 | 12<br>20 | 26 | 4 | 4 |
| 26 |  | left fragments<br>right fragments | 20<br>18 | 10<br>11 | 10<br>7 | 18 | 6 | 3 |

From the results of the dissection experiments, especially from those series in which complementary prostomial portions were tested, within the limits of precision imposed by the surgical methods applied, there is *no evidence of the existence of two separate areas of hormone producing cells.* The low proportion of both complementary fragments showing activity in experiments No. 23–26 represented in Table 2, pleads rather in favour of one homogenous endocrine area, subdivided by the arbitrary sectioning.

Furthermore, it should be emphasized that in all series strict alternative reactions were observed: either development of typical caudal regenerates, essentially linked with inhibition of maturation and epitoky and with prolonged survival; or formation of atypical, rudimentary regenerates, accompained by precocious maturation and metamorphic changes (called "epitokoid development") and death within two weeks. *There was no indication of qualitatively different functions in cells located at different positions within the endocrine area.*

These experimental results will be compared subsequently with the histological, cytochemical and ultrastructural data on neuroendocrine phenomena worked out hitherto in *Platynereis dumerilii.*

## *B. Structural Observations*

### 1. NEUROSECRETORY CELLS IN THE BRAIN OF PLATYNEREIS DUMERILII

Investigations were carried out in intact animals of both sexes with about 50 parapodial segments. Specimens of this size readily formed caudal regenerates upon posterior transection. A group of 6 animals was fixed every Tuesday during period of 5 weeks. This covered more than one illumination-cycle of 3 weeks LD 16/8 h alternating with one week of administered low intensity light instead of the normal dark-phase. Another group of 4 regenerating animals was fixed without regard to the illumination-cycle. Thereafter, the number of brain-cells staining with paraldehyde-fuchsin or alcian blue was determined in serial sections. Besides secretory ganglionic cells, fuchsinophilic aggregations (type 3-cells of Hauenschild and Fischer, 1962) were observed at the posterior border of the brain and also in other regions of the prostomium. Recently, these aggregations could be detected in electronmicroscopic preparations (personal observations). The number of neurosecretory neurons counted was unexpectedly small. In complete series of sections only 1 to 16 fuchsinophilic cells per prostomium were recorded, about 70% of prostomia investigated exhibited 2 to 6 cells. In accordance with Hauenschild and Fischer (1962) these cells were located in the antero-dorsal region and/or in the dorsal or ventral posterior region of the brain. No apparent differences in the number of neurosecretory cells within the experimental groups, or throughout the entire series could be seen. Regenerating specimens neither proved more suitable for the detection of neurosecretory cells nor contained a higher number of these cells than non-regenerating worms. *The endocrine activity exhibited by the prostomia of animals of that size and at comparable stages of sexual development rather contrasted with the insignificant number of neurosecretory neurons which were throught to elaborate the brain hormone.* These observations, however, are in agreement with Müller (1973) (see also discussion) who found that during ontogenesis the number of peptidergic, PAF-positive brain cells in *Pl. dumerilii*-♀♀ is reciprocal to the hormonal activity detected experimentally.

### 2. THE INFRACEREBRAL GLAND-COMPLEX

In their study on the brain of *Perinereis cultifera*, Bobin and Durchon (1952) described a "complexe cérébro-vasculaire". This structure was investigated by Dhainaut-Courtois (1966a, b; 1968b) in *Nereis pelagica*, also in *Nereis limnicola* and 3 other species of nereids by Golding et al. (1968) with light- and electron-microscopic techniques. As in the aforementioned species, in *Platynereis dumerilii* an infracerebral gland-complex is also present. *This complex consists 1. of an area of specialized epithelial cells at the ventral side of the brain capsule, adjacent to the dorsal blood vessel and to its efferent branches, 2. of fibre tracts descending from the neuropil to the ventral, inner side of the brain capsule, and 3. of sites at which these fibres make contact with the capsule or even penetrate the collage-*

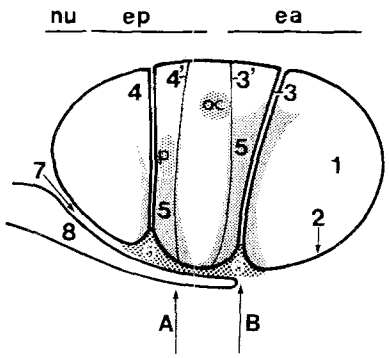



**Fig. 3.** *Platynereis dumerilli.* Schematic drawing of a median section of the brain in which the constituents of the infracerebral gland complex are represented. Arrows *A* and *B* indicate the positions at which the transverse sections used for Figures 4 and 5 were cut. Detailed explanations in the text. For abbreviations see legend of Figure 5

*nous layer.* Figure 3 gives a schematic representation of the constituents of the complex in *Pl. dumerilii.* Some more details are illustrated in Figures 4 and 5. In this species, particularly the epithelial portion is considerably less prominent than in *Nereis limnicola*; its development is even inferior to that of *Nereis diversicolor.* The extracapsular "gland" cells, which differ notably from the very flattened "ordinary" pericapsular cells, extend along the median groove formed by the ventrally protruding brain lobes. To evaluate the area covered by the "gland" cells in prostomia of immature animals with about 50 parapodial segments, the lateral extension of the "gland" was measured in 5 µm thick serial transverse sections and then the data were plotted as diagrams. *According to these, the glandular area has (in the horizontal projection) the shape of a leaf,* the tip of which lies slightly rostral to the anterior connective tissue tube, and its base caudal to the posterior tissue tube. The maximal width of the area in different animals varied between 80 and 95 µm, while the longitudinal extension was found to range from 90 to 120 µm. Besides the specialized "gland" cells, "ordinary" pericapsular cells are present marginally, and cells and fibres suspending the median blood vessel and its branches were also observed. Their nuclei are slender and elongated. The epithelial configuration of the "gland" results from taller cells with relatively large, roundish or oval nuclei and, in light-microscopic preparations, with very faintly staining cytoplasm (Figs. 4 and 5). The proximal portion of the "gland" cells is attached to the brain capsule or to its delaminated parts, while the distal portion of many of these cells is exposed to the coelomic sinus. But undoubtedly a considerable number of cells makes contact with endothelial cells of the blood vessels. *In contrast to other species, no discrimination between different types of "gland" cells was possible at the light-microscopic level.* Paraldehyde-fuchsin-positive cells could not be detected in the epithelium of animals of that developmental stage. However, it should be noticed that Müller (1973), without going into details, reported $^{3}$H-cystine-incorporation in the "gland" of *Pl. dumerilii,* but it was not possible to decide whether the labeling resulted from local synthesis or from products synthesized elsewhere and transferred to the "gland" cells, perhaps by axonal transport. In electron microscopic preparations the epithelial "gland" cells, *a*-cells according to Golding et al. (1968) ($C_1$-cells of Dhainaut-Courtois, 1968a)

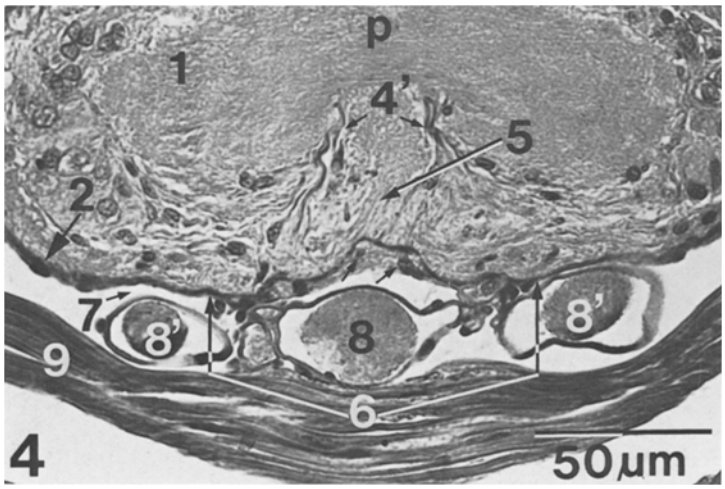



**Fig. 4.** *Platynereis dumerilii.* Infracerebral gland. Transverse section of the posterior ventral region of the prostomium (Plane *A* in Fig. 3). Tissuemat-paraffine section, 5 µm

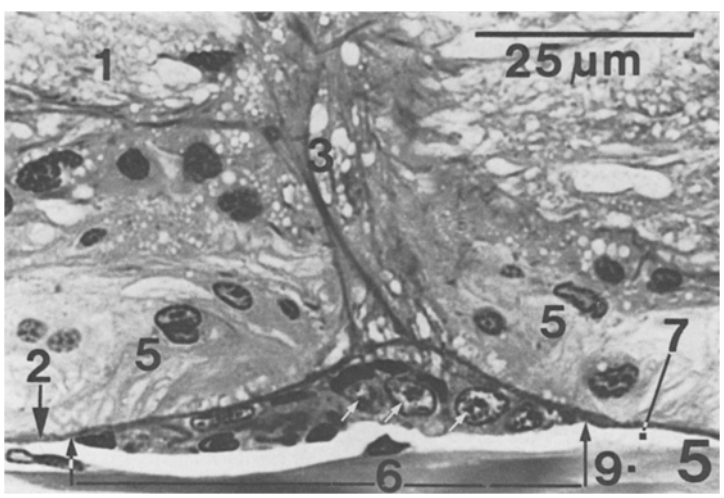



**Fig. 5.** *Platynereis dumerilii.* Infracerebral gland. Transverse section of the anterior ventral region of the prostomium (Plane *B* in Fig. 3). Semi-thin Araldite section. *ea* region of the anterior eyes, *ep* region of the posterior eyes, *nu* nuchal region, *oc* optic commissure, *p* posterior commissure of the brain, *1* brain with marginal layers of ganglionic cells and central neuropile, *2* brain capsule, covered by pericapsular epithelium, *3* anterior, impaired tubular strand of connective tissue, *3'* anterior minor strand, *4* posterior paired tubular strands of connective tissue, *4'* posterior minor strands, *5* fibre tract running towards the ventral, inner face of the brain capsule, *6* area of infracerebral gland cells (cells with largest nuclei, arrows) infested with pericapsular cells and cells suspending the blood vessels, *7* coelomic sinus, *8* median blood vessel, *8'* efferent branches of median blood vessel, *9* muscle fibres

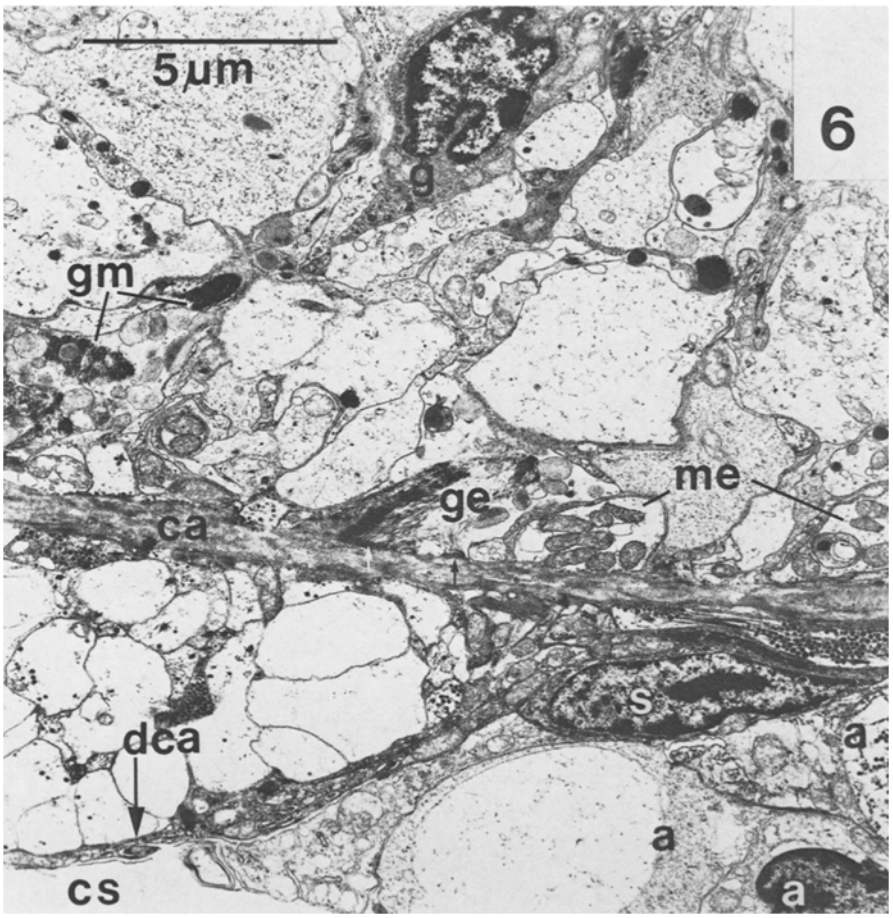



**Fig. 6.** *Platynereis dumerilii.* Infracerebral gland. Transverse section (region posterior to arrow *B* in Fig. 3). Magnification: ×6,800. Note mitochondrial endings and ending of glial fibre (with hemidesmosomes, arrows) at the inner face of the brain capsule. Processes of *a*-cells and blood vessel suspending cell at the other side of the capsule

represent the predominant cell type (Figs. 6–9). The *a*-cells show oval nuclei, their organelles such as mitochondria and golgi and fibrillar material are often concentrated in the basal part; vacuolization frequently appears in the apical portion. Granules resembling elementary secretory granules are exceptional, and enlarged, active golgi-complexes, as one would expect to find in glandular cells, were not observed. Exceptionally a second type of "gland" cells was present. It resembles the fuchsinophilic, stellate *b*-cells described by Golding et al. (1968) in *Nereis limnicola* and *N. diversicolor*, which corresponds to the type $C_2$-cells of Dhainaut-Courtois (1968a). These cells are infested by *a*-cells and contain some granules with the appearance of elementary neurosecretory granules. Occasionally free coelomocytes can also be detected in the "gland"

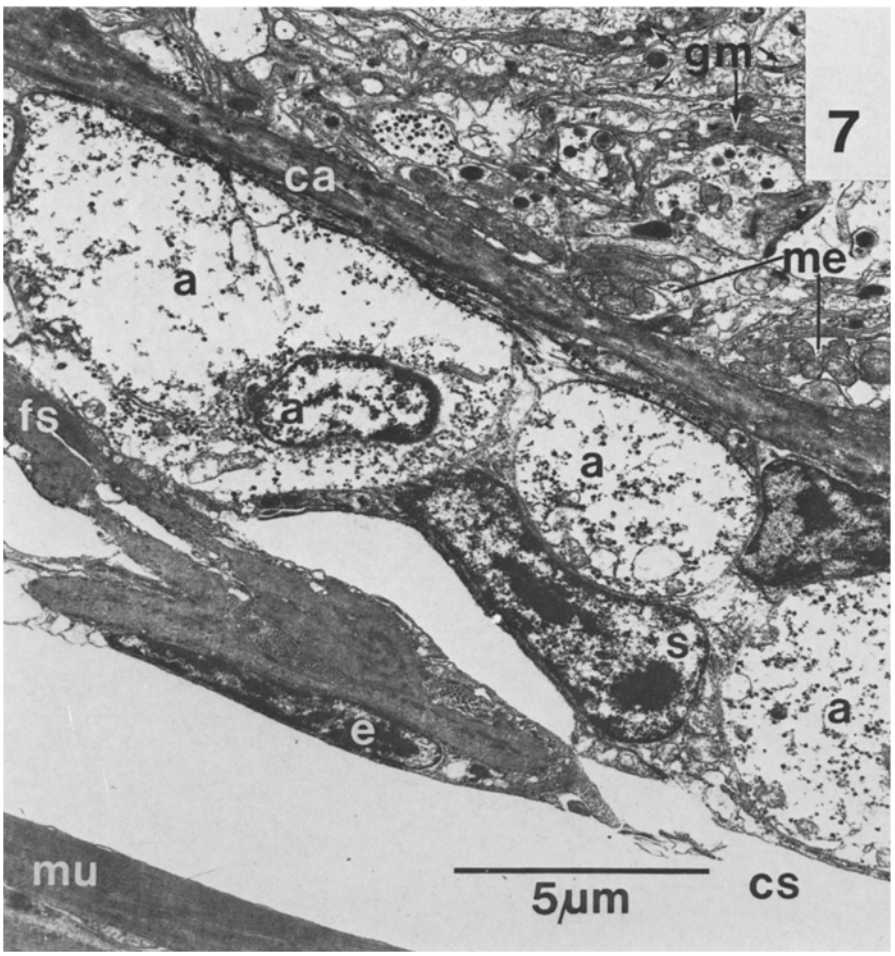



**Fig. 7.** *Platynereis dumerilii.* Infracerebral gland. Transverse section (region marked by arrow *B* in Fig. 3). Magnification: ×6,800. Note mitochondrial endings at the inner face of the brain capsule and the adjacent network of glial lamellae. The capsule is covered by *a*-cells

area, especially in the regions where the connective tissue tubes invaginate.

As described for other nereids, *fibre tracts within the brain descend from the neuropil to the ventral part of the brain capsule* (Figs. 3–5). These tracts contain glial fibres and also nerve fibres. Moreover, two prominent structures pass dorsoventrally through the supraoesophageal ganglion in the sagittal plane: *the anterior and the posterior connective tissue tubes.* It appears from serial sections that these tubes result from medioventral invaginations of the collagenous brain capsule. Upon reaching the dorsal side of the brain, the tubes meet the dorsal part of the capsule, which is continuous with the basal membrane of the epidermis in the dorsomedian portion of the prostomium. In sections stained with azan-novum, the capsule and the basal portions of the tubes stain an intense blue, but they gradually tinge yellow (like muscle fibres) in the

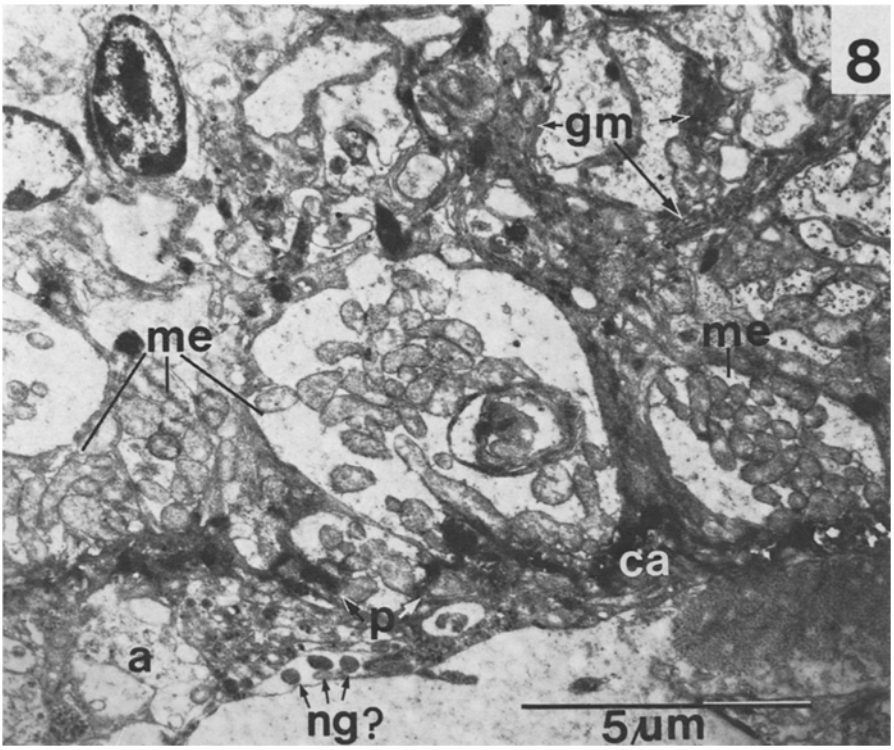



**Fig. 8.** *Platynereis dumerilii.* Infracerebral gland. Sagittal section (region posterior to arrow *B* in Fig. 3). Magnification: ×7,000. Note the large mitochondrial endings and perforation of the brain capsule. A cell process in the glandular region perhaps contains elementary secretory granules

superior portions. This observation was confirmed by Fischer, who found a muscular component within the tubes in electron-microscopic preparations (Fischer, personal communication). The tubular, collagenous sheath is surrounded with a remarkable number of glial fibres radiating into the neuropil. Frontal sections proved that the anterior strand consists of a single tube, whereas the posterior tube contains paired elements, as has already been documented by Hauenschild (1959) and Müller (1973). Between the tubular strands two groups with a variable number of minor strands extend dorsoventrally. Their staining properties are similar to those of the tubes. In Figure 3 each group is represented by one single strand.

*The anterior fibre tract* which has an approximately conical shape is grouped about the anterior connective tissue tube. The numerous glial elements are joined by nerve fibres which appear to originate from groups of dorsolateral ganglion cells located anteriorly and posteriorly to the tube. Unfortunately, it has not yet been possible to trace individual nerve fibres all along their length to establish the exact morphological relations between the cells of origin and their target.

*The posterior fibre tract* is located mainly anterior to the posterior tube. Few nerve fibres appear to descend from the posterior region of the brain,

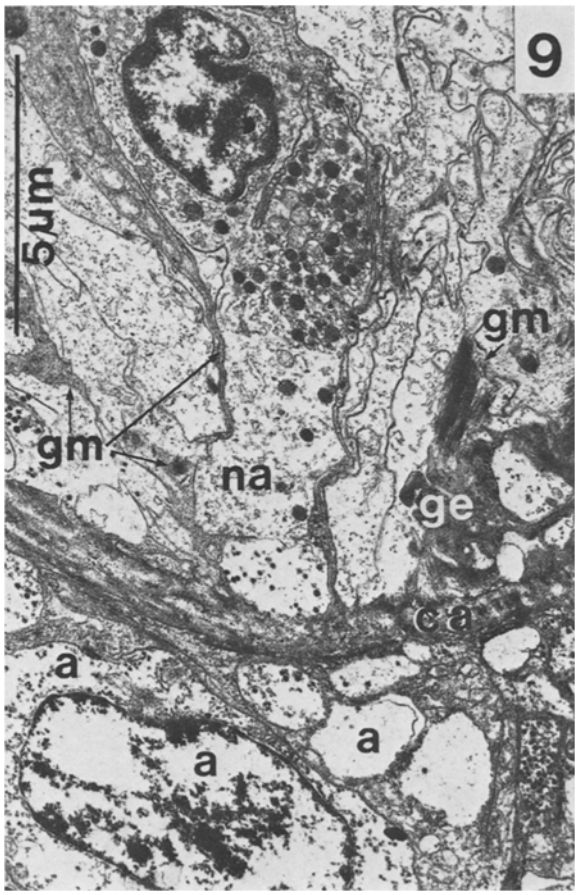



**Fig. 9.** *Platynereis dumerilii.* Infracerebral gland. Transverse section (region marked by arrow *B* in Fig. 3). Magnification: ×7,000. Neurosecretory axon surrounded by glial processes ending at the brain capsule. Note *a*-cells at the external face of the capsule. *a* *a*-cell, nucleus of *a*-cell, *ca* collagenous brain capsule, *dca* delamination of brain capsule, *cs* coelomic sinus, *e* nucleus of endothel cell, *g* glia cell within brain, *ge* ending of glial fibres, *gm* glial material and lamellae, *me* mitochondrial ending, *mu* muscle fibres, *na* neurosecretory axon, *ng* neurosecretory granule, *p* perforation of brain capsule, *s* nucleus of blood vessel-suspending cell, *fs* fibres suspending blood vessel

but the major portion of the tract, which is directed to the capsule, becomes visible below the posterior commissure of the brain (Figs. 3 and 4). *It appears from a great number of series of sections examined that, except for the tubes, the number and the configuration of the above-mentioned fibrous components is subject to considerable variations.*

Electron-microscopic investigations in *the sites of contact* of the different fibres at the brain capsule provided findings in *Platynereis dumerilii* which correspond with many facts known from other nereids. The numerous glial fibres form endings with hemidesmosomes at the capsule (Fig. 6), which shows the structural periodicity of collagen (Figs. 6–9). *The most frequent and rather*

*prominent structures on the internal surface of the capsule are enlarged endings of fibres filled with particularly large mitochondria* (Figs. 6–8). As in *Nereis limnicola,* mitochondria in these endings may contain inclusions and concentric lamellae. Among the axons directed to the capsule in the region of the anterior fibre tract, only few are neurosecretory axons (Fig. 9). Their endings appear less differentiated than in other species. Nevertheless, this observation might fit with the relatively few neurosecretory perikarya detected within the brain of animals of that stage. Further investigation is necessary to prove whether or not there are changes in the number and the ultrastructural features of the axons considered and in the respective perikarya as sexual development proceeds.

It appeared already from paraffin sections that the capsule is perforated at some places in the region of the infracerebral gland. Ultrastructural investigations revealed such perforations: an example is given in Figure 8. The picture even suggests that mitochondria from a mitochondrial ending penetrate into the glandular area where the capsule is discontinuous. Golding et al. (1968) demonstrated the passage of axons with elementary granules through the capsule and discovered axonal branching between the infracerebral gland cells. Some of our records suggest the presence of axons also in the "glandular" region of *Platynereis dumerilii.* However, convincing evidence as presented by the aforementioned authors for *Nereis limnicola* (loc. cit. pp. 213–215) was not available from this investigation.

## Discussion

In a previous paper (Hofmann, 1975), anterior regeneration in *Platynereis dumerilii* and its neuroendocrine implications have been studied in detail; the present investigations refer to experiments concerned with the hormonal control of caudal regeneration.

### *Requirement of Hormone in Different Phases of Regeneration*

It is an established fact that regeneration of posterior segments in nereids, in contrast to most of the other polychaetes (Hofmann, 1974a), requires the presence of a brain hormone. Furthermore, Hofmann (1966), in *Pl. dumerilii,* found a relationship between the rate of regeneration of segments to the position along the antero-posterior axis at which the amputation was performed. Considering one particular position, in immature worms one single prostomium produced enough hormone to ensure segment regeneration at the optimal rate. In accordance with Golding's (1967b) results in *Nereis diversicolor,* the regeneration-promoting hormone proved to be produced continuously and not to be released only upon injury of the animals by amputation of posterior segments. Findings in *Pl. dumerilii* (Hofmann, 1966, p. 418) do not support the recent assumption of Boilly (1974) and Golding (1974a) that in *Nereis diversicolor* wound healing and pygidium formation, in contrast to segment proliferation, escape the endocrine control of the brain. The present author proposes that

the threshold value of hormone stimulation for these initial processes is considerably lower than that required for the formation of complete parapodial segments; he further suggests that, after decerebration, the hormone concentration takes longer to fall short of the low threshold in *N. diversicolor* than in *Pl. dumerilii*, probably even longer than estimated by Golding (1974a).

### *Hormonal Activity at Different Ontogenetic Stages*

During ontogenesis, elaboration of the regeneration-promoting hormone was first detected in 6–7 day old individuals exhibiting the three deutometameres (Hofmann, unpublished observations): in 28% (=8 out of 28) of the 4-segmented fragments taken from immature donors, the implanted anterior ends induced regeneration of a pygidium and up to 5 segments within two weeks. Following transplantation of the anterior ends of young worms with 4–7 parapodial segments, 75% (=21 of 28) of the implants showed hormonal activity and provoked regeneration of 1 to 6 ($\bar{x}=3.1$) segments until the end of the second week p.o. This is in accordance with Hauenschild's (1966) statement that the prostomia of 10 to 20 days old, 3–4-segmented worms, apart from controlling sexual development, also induce regeneration of up to 6 parapodial segments within the period of survival when implanted into worm fragments. *These observations indicate that the production of the hormone starts in those individuals which terminate the nectochaetal stage and settle down to enter the substrate-bound growth-phase.* In animals approaching maturity and entering metamorphosis, the ability to respond to caudal amputation by pygidium formation and segment proliferation declines. In females, regeneration ceased when the oocyte diameter exceeded 150 µm. Prostomia of these worms did not exhibit hormonal activity when transplanted into debrained fragments of non-reproductive worms (see Hofmann, 1974c).

### *Is the Regeneration-promoting Hormone Identical with the Brain Hormone Controlling Gametogenesis and Epitoky?*

Hauenschild (1974c) reported that the brain hormone ("Juvenilhormon") produced by *Pl. dumerilii*-♀♀ at different developmental stages caused homologous effects. None of the experimental results indicated the elaboration of a specific maturation hormone apart from a hormone controlling gamete development in its earlier phases. Both functions appear to be exerted by one hormone.

Considering the regeneration-promoting hormone in *Pl. dumerilii*, strict correlation of two effects was apparent: the development of typical caudal regenerates, even if stimulated by the implanted anterior ends of very young animals, was coincident with the inhibition, at least for a notable period, of maturation and epitoky (Hauenschild, 1960a, 1966; and personal observations mentioned above). Correspondingly, in worms approaching maturity and entering metamorphosis, the ability to form posterior regenerates decreases (Hauenschild, 1966; Hofmann, 1974c). However, additional implantation of prostomia of immature

donors into animals of the afore-mentioned advanced stages led in a high proportion of cases again to caudal regeneration and to considerably delayed achievement of the reproductive form (Hofmann, 1974c). *Subject to future biochemical verification it is deduced, that the induction of regenerate formation and subsequent proliferation of parapodial segments as well as control of maturation and epitoky are differentiated functions of one single hormone.* The specificity of the hormone's action appears to be mediated by the different concentrations of hormone produced during ontogenesis; at high levels segment proliferation (normal and regenerative) is promoted, initial phases of gametogenesis are admitted, but rapid progress of sexual differentiation is prevented; at decreasing levels maturation can proceed, but regeneration becomes progressively inhibited; the development of the reproductive, non-regenerating heteronereis form is achieved when the hormone titer reaches zero (for reviews see Durchon, 1970; Hauenschild, 1966, 1974c).

### *Species Non-Specific Action of the Hormone*

Personal investigations (unpublished) in decapitated fragments of *Platynereis dumerilii* with 40 parapodial segments into which prostomia of relatively small individuals of *Nereis diversicolor* (species without heteronereis form) were implanted, proved that the species-non-specificity of the hormone not only applies to the maturation- and metamorphosis-controlling component (see Hauenschild, 1956b; Boilly-Marer, 1962), but also to the regeneration-promoting component of the hormone's action. From 21 individuals which retained the implant, 8 did not regenerate parapodial segments but performed maturation and developed host-specific heteronereis patterns before death within 13–15 days. The other individuals survived 13–81 days and developed typical regenerates with 1–9 ($\bar{x}=3.6$) segments. Except for two fragments, the individuals *with* regenerates finally also underwent heteronereid metamorphosis. In control animals with in situ prostomia the rate of segment formation was higher and maturation usually did not interfere within about two weeks time. The results obtained from the experimental group might, therefore, reflect interspecific differences in hormone production.

### *Origin of the Hormone*

The main problem of this work was the search for the origin of the hormone. Only preliminary information is available from experiments designed to isolate the active compound from prostomial homogenates (Cardon, 1970) and the chemical composition is still obscure. Therefore elaborate techniques to detect the sites of synthesis and the pathways of transport by methods of immunospecific recognition of the hormone are at present not at our disposal. We have to refer to dissection and implantation experiments and further to histological and ultrastructural attempts to localize the hormone-producing cells. In *Platynereis dumerilii* the experiments proved that the hormone is elaborated

in the interocular portion of the prostomium which extends from about the posterior border of the anterior eyes to the posterior region of the posterior eyes. According to the histological observations, this portion coincides approximately with the area delimited by the dorsal points of emergence of the anterior and the posterior connective tissue tubes (Figs. 1 and 3). Furthermore, this portion is congruous with the leaf-shaped area at the ventral side of the brain which is covered by the infracerebral gland epithelium.

In *Nereis diversicolor*, Durchon and Dhainaut-Courtois (1964) found spermatogenesis-inhibiting activity only in 15% of the anterior prostomial fragments, which were excised at the optic commissure and then associated with isolated parapodia. Golding (1967c), working with the same species, did not detect regeneration-promoting activity in prostomial areas anterior to the posterior eyes. Personal histological observations in *N. diversicolor* (unpublished) showed that the anterior fragments (quoted inactive by the afore-mentioned author) comprised the prostomial portion which is situated rostral to the position of the anterior tissue tube. The latter emerges in this species at the dorsal side exactly between the anterior and the posterior pair of eyes. As in *Platynereis dumerilii*, the infracerebral gland epithelium roughly covers the area at the ventral side of the brain outlined by the anterior and the posterior tissue tubes. These observations together suggested that in *Nereis diversicolor* the hormone is likewise produced within the particular portion of the brain associated with the infracerebral gland epithelium.

### *Structure and Significance of the Infracerebral Gland-complex*

In *Pl. dumerilii* glia and nerve fibres, some of them with neurosecretory granules, were found to end at the internal face of the brain capsule in the area covered by the gland epithelium. Endings with large mitochondria are frequent. Penetration of fibres into the glandular region is probable, but their relation to the numerous *a*-cells and scarce *b*-cells could not yet be elucidated. For a comprehensive discussion of structural and neurophysiological aspects of the infracerebral gland-complex in several polychaete families see Whittle and Golding (1974).

The disposition of the neuroendocrine elements in the vicinity of blood vessels suggested that the infracerebral gland was a neurohemal organ, involved with the release of neurosecretory material. However, most of the authors are sceptical as to its functions. Baskin and Golding (1970), working with the viviparous *Nereis limnicola*, found hormonal activity both in dorsal and ventral brain portions from which they obviously could not separate the infracerebral gland. The activity was inferior to that of intact brains, but synthetic activity of the gland could not be excluded. Baskin (1970) observed hypertrophy and enlarged nuclear volume in glandular *a*-cells of *N. limnicola* related to oogenesis and viviparity. Relations to hormone production were no longer proposed. In an ultrastructural study on the infracerebral gland in *Perinereis cultrifera*, Dhainaut-Courtois (1968b) observed asynchronous development of $C_1$-cells (*a*-cells) and $C_2$-cells (*b*-cells). $C_1$-cells, which were found to resemble insect *corpora allata* cells, show a high degree of differentiation later in ontogenesis but persist

until the heteronereis stage; $C_2$-cells differentiate earlier and partly disappear at maturity. Endocrine functions under the neurosecretory control of the brain were suggested.

*In Platynereis dumerilii, although the striking conformity of the general morphological data with the experimental findings was apparent, more detailed studies did not provide evidence that the "gland" is the source of the brain hormone.* This results partly from experiment No. 11 (Table 1) in which excision of the ventral brain portion with the adherent gland epithelium did not terminate the hormonal activity of the prostomial remnant in a substantial number of operated animals. It further appears that under the particular experimental conditions the "gland" is not even required as a release organ for the secretion synthesized elsewhere and perhaps delivered by axonal transport. Nevertheless, the observations do not justify the conclusion that the infracerebral gland-complex is not involved with the release of the brain hormone in the intact animal.

Though highly speculative, proprioceptive and regulative functions of the complex should also be considered, since negative feed-back effects from the coelomal contents of maturing females on brain hormone production had been demonstrated by Porchet (1967), Porchet and Durchon (1968), Porchet and Cardon (1972) in *Perinereis cultrifera* and by Hofmann (1974c) in *Platynereis dumerilii.* These speculations have some support from the findings of Whittle and Golding (1974), who considered the $C_2$-cells (*b*-cells) in phyllodocids and also in nereids to be bipolar neurons with proximal processes penetrating into the neuropile. These bipolar neurons could be sensory.

### *Neurosecretory Brain Cells and their Relation to Hormone Production*

Neurosecretory brain cells, detected in nereids by Scharrer (1936), were already thought to be involved with regenerative processes by Harms (1948) in *Lycastis* spec. Since the initial experimental work of Durchon (for references see Hauenschild, 1966), most investigators concluded that neurosecretory neurons of the brain are connected with the maturation-inhibiting and regeneration-promoting hormone. As noted in the articles of Gabe (1966), Golding (1967f) Dhainaut-Courtois (1968a) and Müller (1973), it is not possible to homologize the descriptions of neurosecretory cells and their alterations in different developmental stages and under various experimental conditions in different species of nereids. Further, it is obvious that histological, ultrastructural and cytochemical data do not permit appropriate correlations with experimental facts. Golding (1967d) noticed changes in neurosecretory cells in the ganglionic nuclei 4,5 and 6 in the anterior part of the brain of *Nereis diversicolor* following amputation of posterior segments. However this part of the ganglion did not exhibit hormonal activity when transplanted into a decerebrated host.

Autoradiographic studies of Müller (1973) in *Platynereis dumerilii* provided surprising results. In females the first ganglion cells were found to differentiate into neurosecretory cells when the oocytes had reached diameters of 30–50 µm.

The number of secretory neurons increased rapidly with the oocyte growth: at ∅ of 75–90 µm a mean number of 280 cells was reached, at ∅ of about 180 µm (mature ova) a maximal number of 450 was reached. Thereafter the number of cystine-incorporating neurons decreased rapidly in the reproductive worms. Prominent alterations were found in the anterior dorsal and the posterior ventral groups of neurosecretory cells whereas only minor changes occurred in other groups within the brain. In regenerating, young females (oocyte-∅ 50–60 µm) the number of neurosecretory neurons did not differ significantly from that of control animals (see also page 56), but in worms at advanced stages (oocyte-∅ 140–170 µm) the mean number of 360 secretory neurons was reduced to 98 in those individual which were allowed to regenerate for one week.

The findings that the number of discernible neurosecretory neurons is reciprocal to the experimentally detectable degree of hormone activity (see Durchon and Porchet, 1971) seriously challenged the concept according to which neurons which exhibit the tinctorial affinities of secretory cells concomitantly produce brain hormone. Müller (1973) proposed two alternative hypotheses for this phenomenon: 1. the neurosecretory neurons do produce the brain hormone, but only as long as they show the characters of "conventional" neurones. Thereafter, they adopt other, still unknown functions. The rate of $^{3}$H-cystine-incorporation is therefore reciprocal to the hormonal activity. 2. the peptidergic neurons produce a factor which is antagonistic to the brain hormone. This factor might either inhibit the synthesis of the hormone or paralyse its functions. The hypothetical factor could be produced in the hormone-synthesizing cells at later ontogenetic stages and could act intrinsically, or it could act extrinsically on special hormone cells. For an approach to the problem of the significance of the neurosecretory cells in *Platynereis dumerilii*, some of the cytological and experimental data must be considered.

In experiment No. 14 (Table 1) the excised anterior prostomial region enclosed the anterior part of the brain which comprises the *corpora pedunculata*, many of the prospective neurosecretory cells of the *partes laterales* and the *pars intercerebralis* (terminology of Müller, 1973) and probably part of the large neurons of the antero-dorsal group, found by Hauenschild and Fischer (1962) and Müller (1973). Surprisingly, this portion of the prostomium did not exhibit hormonal activity. In a subsequent series (experiment No. 15, congruent with section *B* of Hauenschild, 1966), the prostomia were sectioned transversely between both pairs of eyes. In these cases the entire antero-dorsal group of future neurosecretory brain cells was included in the anterior prostomial fragment. Nevertheless, only one third of the number of transplants proved to be hormonally active. It should be added that from other sets of the same experiment different results were obtained: Hauenschild (1966) noted 3 active anterior fragments in a series of 4, but in another of our own series (not included in Table 1), only one of 18 implanted anterior fragments showed brain hormone activity. As to the posterior region of the prostomium, in experiment *D* of Hauenschild (1966) a transverse section of the prostomium across both posterior eyes was performed. The posterior remnant certainly enclosed both posterior brain lobes which contain prospective neurosecretory neurons, but in none of the three cases was hormonal activity detectable.

*The facts reported permit the conclusion that in certain regions of the brain of Pl. dumerilii-♀♀ numerous neurons are present which, at least at advanced stages of sexual development, have the morphological and tinctorial properties of neurosecretory neurons, but do not produce brain hormone, even at earlier stages of ontogenesis when most of the cells still have the appearance of conventional neurons.*

However, in the more central region of the supraoesophageal ganglion which was found to produce brain hormone, prospective neurosecretory cells are also localized. They can be classified among different topographed groups and, according to Müller's (1973) data, increase in number when maturation proceeds. It is actually impossible to decide whether the hormone is produced by future neurosecretory cells in this area of the brain or is synthesized by cells which never aquire the cytological affinities of neurosecretory cells, but are controlled by some of these secretory neurons. In any case, the latter do not appear to be homogeneous in function.

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