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Halogenation and quinone-taning of the organic tube components of some Sabellidae (Annelida Polychaeta)

J. Vovelle*, M. Rusaouen-Innocent*, M. Grasset* and M. Truchet**
* Laboratoire d'Histologie et Cytologie des Invertébrés Marins,
** Laboratoire d'Histophysiologie fondamentale et appliquée,
Université P. et M. Curie, 12, rue Cuvier 75005 Paris, France.

Abstract : Histochemistry, histoenzymology, and microanalysis have been applied to the study of tube-making in two Sabellidae (*Sabella pavonina* and *Spirographis spallanzanii*). The investigation examined the tube constituents and the glands which supply them in two distinct secretory sequences. The organic tube adds to the wrapping of a roll which includes exogenous material gathered by the building organs and whose spiral deposit on the upper rim ensures the growth of the outer layer, with an inner sheath elaborated by the ventral thoracic shield. This sheath is mostly composed of sulphated glycosaminoglycans, rich in sulphur and calcium. The wrapping of the initial roll built at the level of the parallel folds separating the ventral lobes of the collar, comes from the tyrosine-rich superficial secretions of the collar and mainly from gland clusters previously described. The most anterior cluster secretes a sulphur-poor proteoglycan. The roll strengthening is correlated to exogenous halogenation, thus according with the long-noticed high level of iodine in sabellid tubes. We link this halogenation with the presence of a catecholase in "Meyer's anterior gland" secretion, suggesting quinone-tanning hardening, as well as the presence of DOPA oxidase in the shield.

Résumé : L'édification du tube de deux Sabellidae (*Sabella pavonina* et *Spirographis spallanzanii*) a été étudiée par des méthodes histochimiques, histoenzymologiques et microanalytiques. Appliquées aux composants endogènes du tube et aux formations glandulaires qui les produisent, elles ont montré qu'il y a deux séquences sécrétoires. Le tube organique est en effet formé de deux enveloppes : l'une, externe, est initialement un cordon déposé en spirale au bord de l'ouverture supérieure, assurant la croissance du tube, l'autre est élaborée par le bouclier thoracique ventral. L'enveloppe interne est surtout formée de glycosaminoglycanes sulfatés, riches en soufre et en calcium. Le cordon qui forme l'enveloppe externe s'élabore au niveau des replis séparant les lobes ventraux de la collerette et enrobe, dans des sécrétions, les matériaux exogènes rassemblés par les organes collecteurs. Ces sécrétions sont d'une part celles d'un massif glandulaire, jadis figuré par Meyer, dont le plus antérieur élabore un protéoglycane pauvre en soufre, d'autre part celle, superficielle et riche en tyrosine, de la collerette. La consolidation de ce cordon s'accompagne d'une halogénation à partir d'éléments du milieu extérieur, probablement responsable de la richesse en iode des tubes de ces Sabellidae. Une catécholase, dans la sécrétion des glandes de Meyer antérieures, et une DOPA-oxydase, dans le bouclier, seraient responsables du durcissement par tannage quinonique des deux composantes organiques du tube.

INTRODUCTION

This study was undertaken to re-examine some old results using more modern techniques. Cameron's work (1914-1915) showed that the tubes of some Sabellidae contained much iodine. In North American species *Sabella columbiana* and *Bispira polymorpha* the proportion of iodine reached 0,74 % of the total dry weight of the tubes, and even 0,80 % in *Eudistylia vancouveri* (according to O'Donoghue, 1924, in Oglesby, 1969). More recently the importance of these concentrations has been stressed in reviews on the subject

(i.e. Vinogradov, 1953). The concentration of this halogen is not uncommon in polychaetes or other marine invertebrates. In polychaetes, following Swan's (1950) initial data, Gorbman *et al.* (1954) and Fletcher (1970) used radioactive tracers to appreciate the selective fixation of iodine in polychaete chaetae (Nereidians). The former suggests the formation of mono- and diiodotyrosine while Fletcher favours a correlation with a process of quinone tanning. These facts and hypotheses can be linked with several recent works emphasizing the part of halogenous scleroproteins in "skeletal parts", among others : Roche (1952), Makarieva *et al.* (1981) on sponges and Coelenterata, Barrington & Thorpe (1968) on tunicates and Hunt & Breuer (1973) on molluscs. In a previous study, we used microanalysis and labelling to assess the concentration and distribution of iodine and bromine linked with a recognized quinone tanning of chaetae in several species of polychaetes (Vovelle *et al.* 1983).

The original work of Cameron (1914-1915) remained neglected for a long time. In a recent comprehensive paper on polychaete tubes, Gaill and Hunt (1988) do not refer to it, and only use Defretin's (1971) survey as a reference about the Sabellidae. The latter used biochemical and histochemical methods to identify the secreted organic material of the tube in several French species ; he determined the amino-acid composition of the protein fraction, the polysaccharide composition of the mucous fraction (in which he gives some importance to hyaluronic acid), but he overlooked the possible halogenation of secretions.

In spite of their diversity, the means of hardening of polychaete tubes have a common feature. These processes successively involve several endogenous and/or exogenous materials associated with different glandular sites. This applies to Sabellariidae (Vovelle, 1965) and Amphictenidae (Truchet & Vovelle, 1977), with the alternative occurrence of quinone-tanning or biomineralization of their tube cement. Although people still refer to the secretion of Sabellidae as "mucus", which is unsatisfactory, at least the dynamic process of tube making and the organs involved in it are well-known (Nicol 1930) and the work is mentioned in all the books dealing with the physiology of invertebrates. She showed that exogenous material is first collected and sorted by the branchial crown, and carried along from the mouth to the ciliated ventral sacs, before being associated with secretions and moulded into a "string" (or roll) by the parallel folds which force it between the collar, which is ventrally hypertrophied into two lobes. The roll is then laid on the edge of the tube by the action of the collar folds and thus the cylinder builds up in a way sometimes compared to Mexican pottery. The ventral gland shield is in its turn involved in the thickening of the inner organic tube. Defretin (1971) mentions the contribution of supposed gland formations in the ventral sacs and Meyer's (1887) anatomical drawings represent "ventral glands" (Bauchdrüsen) located under the collar lobes, at the root of the ventral sacs before the shield plates.

In the present study microanalytic techniques have been associated with histochemistry and histoenzymology ; it has been focussed on the tube of two Sabellidae abundant on French shores, and on the part played by the various successive gland sites which build it up and that we needed to clarify morphologically and histologically.

MATERIALS AND METHODS

Specimens of *Sabella pavonina* Savigny and *Spirographis spallanzanii* Viviani were collected on Penpoul strand near Saint-Pol-de-Léon (Brittany) and supplied by the Marine Station of Roscoff. They were kept alive in a closed circuit sea water aquarium for a period varying from 2 days to 2 months.

Fixation, microscopic anatomy and cytology

Fixatives used for the histological, histochemical and microanalytic studies were Bouin's fluid, saline formaldehyde, and McGee Russell's Alcohol-Formaldehyde for the preservation of mineral elements. Material from the basal part of the crown and the fifteen first metameres was embedded in paraffin and cut into sagittal or transverse sections for examination.

Material was also fixed in 2 % buffered glutaraldehyde (pH 7.2), then embedded in Araldite and provided semi-thin sections for cytological study (after buffered toluidine blue staining) and microanalysis.

Sections of the tubes were obtained using a cryostat or fixed in alcohol-formaldhyde and embedded in paraffin or dehydrated and embedded in Araldite and cut into semi-thin sections (1 to 2 µm).

Histochemistry, histoenzymology

The protocols of the following methods were applied according to the treatise of Gabe (1968) and Ganter & Jollès (1969-1970).

Proteins

* Thiol groups, DDD method (Barrnett and Seligmann, 1954) ;
* Reducing groups, Adam's method (1956) ;
* Reducing aromatic radicals, Argentaffin reaction (Masson, 1926) ;
* Diphenols, Extemporaneous diazotation in toto at 4 °C ;
* Tyrosine, Glenner and Lillie's method (1959) ;
* "Proteins or phenolic compounds", Malachite green (Smyth, 1951).

Polysaccharides

* PAS reaction (Hotchkiss-Mac Manus) ;
* Alcian blue reaction (pH 1 and 2.6) ;
* Metachromatic reaction with buffered toluidine blue (pH 1.3 and 4.5).

Mineral elements

* Calcium, GBHA method on cryostat sections (Kashiwa and Atkinson, 1966) ;
* Ferric iron, Prussian blue method (Perls).

Enzymes

Tests were carried out on *in toto* pieces of material prefixed in 80 ° alcohol for 5 min and on cryostat sections of fresh material. Controls were obtained by incubation in different media of the two symmetrical halves of a sagitally sectioned animal, or by preinhibition (5 min) and simultaneous inhibition by 1 % sodium diethyldithiocarbamate solution.

* Polyphenoloxidases

Catecholase : Smyth's method (1954) ;

DOPA reaction : Gomori's method (1954) ;

* Peroxidases

Benzidine method (Prenant, 1924).

Microanalysis

Wavelength dispersive X ray microprobe.

Sections (6 µm) of material embedded in paraffin were set on terphane slides. The same techniques were used for cryostat or paraffin sections of tube fragments. For microanalysis we used a MS46 Cameca microprobe fitted with crystal wavelength dispersive spectrometers : KAP or TAP for P and S elements, PET for Ca, Fe, I and P, LIF for Br under following conditions : 15 or 25 kV acceleration tension, 40 nA sample current, probe diameter approximately 1 µm. The mean and maximal figures retained for each type of structure are expressed in counts per second ($c.s^{-1}$)

Secondary ion mass spectrometry (SIMS).

Sections (6 µm) of the tube, collar and shield embedded in paraffin, and semi-thin sections (2 µm) of Araldite-embedded material were spread on gold supports.

Analyses were performed with a Cameca SMI 300 ion microanalyser as follows : primary ion beam Oxygen, $0+_2$, 7.5 µA, defocused ; diameter of the diaphragm of the emission lens, 200 µm ; energy band pass, 13eV ; magnification, 115 ; lateral resolution on ion images, 0.7 µm ; mass resolution (M/dM), 300. Spectral range, 2 to 265, both positive and negative secondary ions ; images were obtained separately (intensities and exposition time given for each image).

RESULTS

Tube structure

Direct examination under a dissection microscope confirms the existence of two complementary layers - an outer "detritic" part which has incorporated the exogenous material and an inner "organic" part. The latter is obviously composed of homogeneous elastic translucid material, which appears to throw superficial blades or filaments into the thicker blackish outer detritic material. From this outer part, flakes come off, leaving beneath more or less subdivided transverse striations (average spacing of 80 µm in *Sabella*).

The organic inner part is composed of successive stratified layers less than 100 µm thick in cryostat or semi-thin sections. These layers are deposited by a periodical shield secretion on the whole inner surface. The outer part is made of pelitic silt, but the material also includes remnants of hard pieces of protists or algae, spines or cylidrical segments from sponge spicules or from the chaetae of the animal itself. The interface in-between the two parts consists of a specific material, denser than the inner layer, protuding to build slantwise partitions every 80 µm or so, which correspond to the previously mentioned blades. They

divide the exogenous material into regular recesses which can be identified as the wrappings of the roll laid and flattened at the anterior growth zone of the tube (Figs 4 a & b, 6 a).

Tube building : morpho-functional marks.

Our observations of *Sabella* and *Spirographis* confirm the course of the particular elements at the level of the ventral sacs and the collar. A whitish fine-grained packed material gathers and actively moves between the two rims of the ventral sacs which fit together like lips. But an undescribed process needs to be mentioned : the particles, starting from the anterothoracic zone, brush past the twin lobes of the collar whose superficial secretions are thus able to contribute to the blend near the basal dimples of the ventral sacs (F).

From observation of paraffin and semi-thin sections the following remarks can be drawn :

1. no glands are present in the ventral sacs, which are lined with a ciliated epithelium.

2. the parallel folds anteriorly display a highly ciliated epithelium which is posteriorly replaced by an evacuating zone of deep-rooted gland cells (Fig. 2e). Histochemical methods reveal two areas within the gland evacuating zone. We suggest naming them "Meyer's anterior gland", and "Meyer's posterior gland". This latter expands to the lateral parts of the collar ventral lobes. In the external epithelium of those lateral lobes, one can observe thickening zones which produce a superficial secretion. "Meyer's anterior gland" is characterized by its secretion and structure. It can be divided into medioventral symmetrical masses, each subdivided into two parts. The outer one grows into the collar lobes, the inner one is highly vascularized and bounded by nervous and muscular zones. Its dead-end capillaries may, by turgescence, help as much the dynamics of the building organ, as the supply of the gland cells. The posterior gland can be considered as a whole because its opening join the end of the parallel folds under the collar lobes. However histochemistry shows that its secretions are less different from those elaborated by the shield and discharging at its surface, than from those issuing from "Meyer's anterior gland". The shield itself is not absolutely regular, since the first two plates after the edge of the collar are wider and thicker than those following.

HISTOCHEMISTRY

Apart from a few variations mentioned later, *Sabella* and *Spirographis* sections have provided similar results to the histochemical tests (Table I). Histochemistry has improved the knowledge of the limits and evacuation processes of the gland involved in tube building. They have been classified from forward to backward in their order of intervention, but it should be kept in mind that other sites may provide complementary secretions : the epithelium of the crown and of the mouth collecting gutters, of the parapodial gland folds, and of the abdominal extension of the shield.

The following sites have beewn examined :

- The anterior medioventral Meyer's gland (Ma) under the parallel folds
- The cell clusters (T) which form thickened bands of pigmented cells in the external epithelium of the collar lobes.

TABLE I

HISTOCHEMISTRY	Meyer's Gland		Collar	Shield				Tube	
	anterior	posterior	external	anterior	posterior	mucocytes	inter-segment	internal	superficial partitions
Proteins									
DDD (thiols)	0	0-ϵ		0-ϵ	0-ϵ				
Adams (reducing G.)	0	0		+	+			+	+
Argentaffin (aromatic)		(+)		+					
Diazotation (diphenols)	0	0	0	0	0				
Malachite green (tanning)	0	+		+	+				
Glenner (tyrosine)			+	(+)	(+)			(+)	+
Polysaccharides									
Haemalum (mucoïds)	+	0	0	0	0	0			
PAS	+	0	0	0	0	0			+
Alcian blue pH 1 & 2.6	0	++	0	++	+	+		+	+
Toluidine blue pH 1-4.5	0	+	0	$++^{m}$	$+^{m}$	$+^{m}$		$+^{m}$	$+^{o}$
Mineral elements									
GBHA (Ca)						+		+	+
Perls (Fe^{+++})							+		
Enzymes									
Smyth (catecholase)	+	0	0	0	0				
DOPA reaction	0	0	0	+	+				
Benzidine (peroxidase)	0	0	0	0	0				

(m : metachromatic, o : orthochromatic).

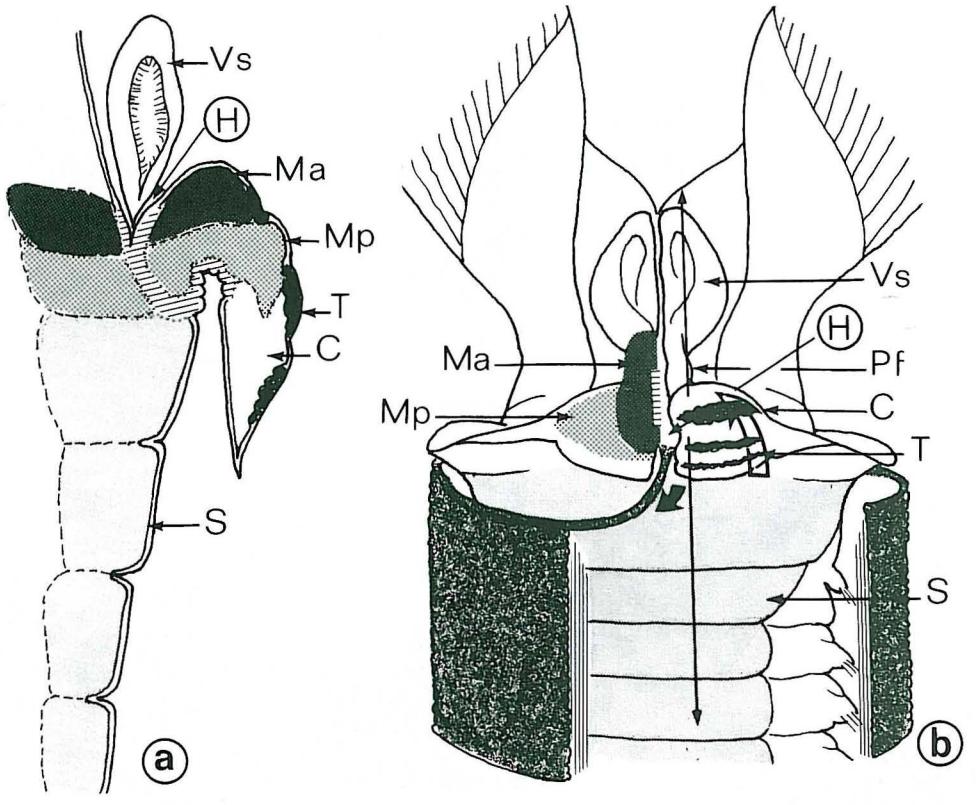

Fig. 1 : Schematic views of anterior part of the thorax of *Sabella*.
a) parasagittal section, b) ventral view (C : collar, H : supposed halogenation site, Ma : anterior Meyer's gland, Mp : posterior Meyer's gland, Pf : parallel folds, S : ventral shield, T : tyrosine-rich collar epithelium, Vs : ventral sacs) ; hachure lines for the discharge of Meyer's glands.

- The anterior ventral thoracic shield which fills the two metameres posterior to the collar with a maximum width.
- The posterior thoracic ventral shield corresponding to the six groove parted following segments.

Protein secretions

Tyrosine residue secretions are found in the three transverse glandular thickenings of the outer epithelium of the collar ventral lobes, and to a lesser extent upward in the collar and mouth collecting gutters. Granular secretions are evacuated on the surface (Fig. 3 a & b).

Ortho- and paradiphenols are not detectable by extemporanous diazotation on "in toto" parts. However the argentaffin reaction shows the presence of reducing groups on some melanised sites in the collar outer epithelium (possibly premelanin sites). It also gives a faint result on some secretions of the "Meyer's posterior gland" (Mp) and the anterior shield. On those two sites, Malachite green reaction gives a significant indication to identify the aromatic compounds of quinone-tanning.

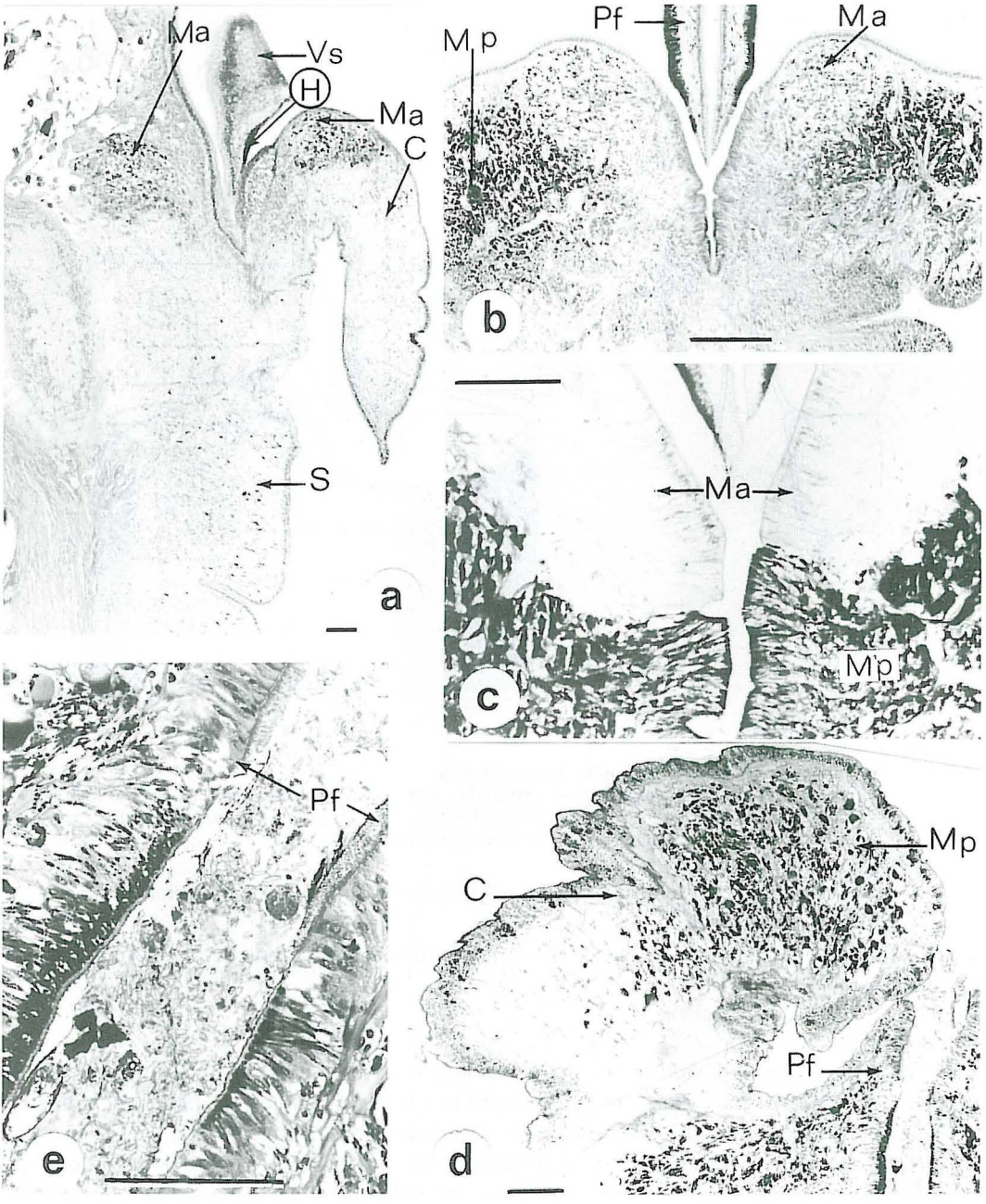

Fig. 2 : Histochemical reactions of polysaccharides in Meyer's glands. Legends as in Fig. 1.
a) parasagittal section of *Sabella* (anterior part of the thorax), Haemalum-picroindigo carmine. b) frontal section of parallel folds and collar lobes, Haemalum-Malachite green. c) Same place, Toluidine blue pH 1. d) Semi-thin transverse section of the ventral groove and collar folds of *Spirographis*, Toluidine blue pH 1. e) Detail of the same : secretory and ciliated parts of the groove, initiation of the sheet ; (bars : 100 µm).

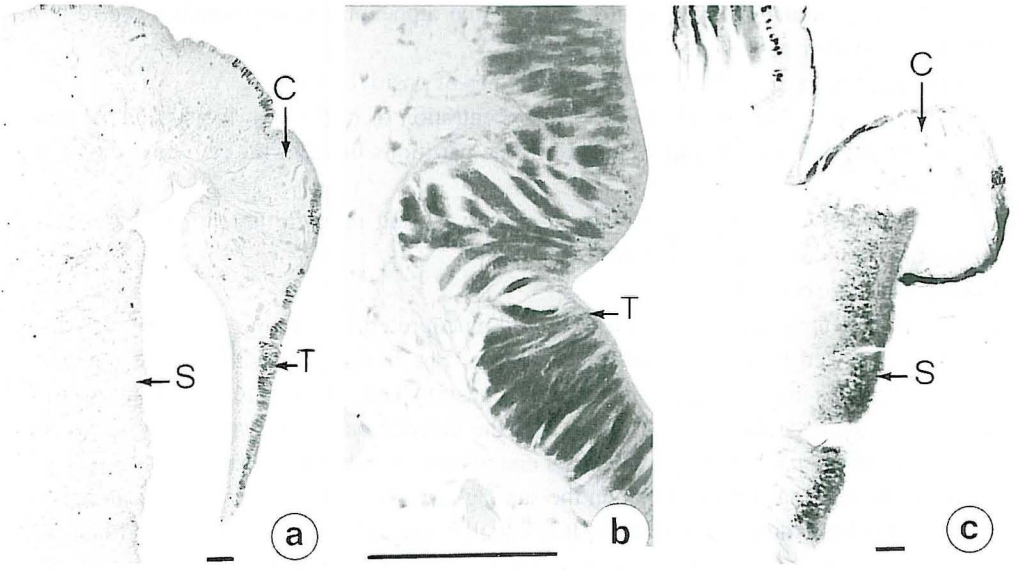

Fig. 3 : Partners for quinone-tanning. Legends as in Fig. 1.
a) parasagittal section of *Spirographis*, Glenner and Lillie reaction for tyrosine. b) Detail of the same, collar tyrosine-rich secretions of the external epithelial cells. c) Cryocut parasagittal section of *Sabella* after "in toto" DOPA reaction (bars : 100 µm).

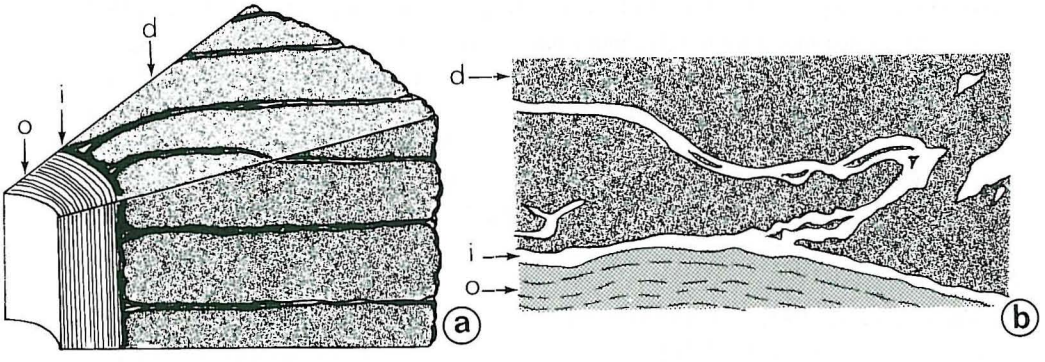

Fig. 4 : Schematic representation of the tube structure.
a) Three-dimentional interpretation of the connections of the different components analyzed in Fig. 5. (o : inner organic tube, i : intermediate organic sheath, d : detritic outer tube).
b) Interpretation of the field analyzed in Fig. 6.

Sulphydryl radical proteins are absent from the gland sites when they appear on the blood vessels. A part of the shield gland reacts positively to Adam's method, thus suggesting the presence of -SH reducing groups. These secretory cells are situated in the medium part of the tissue. But this information cannot be regarded as proving the presence of

cystein. Adam's reaction may equally reveal some diphenols, among which those responding to argentaffin on the same sites.

Tube paraffin sections have provided two types of positive results :

. Glenner and Lillie's method shows a concentration of tyrosine in the superficial outer film of the organic sheath, and continued in the partitions linked with the wrapping of the detritic roll.

. Adam's method gives a homogeneous positive result through the entire organic tube. DDD only provides a faint colouration.

Polysaccharide secretions

"Mucoïd" neutral mucopolysaccharides and mucoproteins as well as proteoglycans have been characterized by Gabe's haemalum method (Fig 2a & b) ; in both species the gland cells of "Meyer's anterior gland" have given a positive and selective result. Their secretion appears as small regular grains evacuated in the anterior part of the gutter of the parallel folds. The deeper part of the gland shows concretions which react intensely. APS reaction confirms the previous observations on the same gland. It provides a moderate staining surrounding the haemalum reacting sites. It is equally noticeable in the filament cartilage, the cuticle, and some cells of the collar inner face. The results obtained by the toluidine blue and alcian blue methods fit together : the reactive sites are the "Meyer's posterior gland" and its extensions within the lateral part of the collar, and the gland clusters of the posterior and anterior shield (Figs 2 c & d). The even metachromatic staining, obtained at all pH values used, suggests the presence of sulphated mucosubstances. Several types of cells give different results. The most metachromatic are dilated goblet-shaped mucocytes situated beneath and close to the shield epithelium. Most other reacting cells might be regarded as "principal", since they provide the major part of the gland tissue. They are intensely coloured from blue to purple essentially visible on the well graded small spherules of the secretion. The doubt between ortho- and metachromatic results may be due to a significant presence of basic proteins. Those observations are roughly the same on both species. "Meyer's anterior gland" gives a negative result ; it is also worth mentioning that the principal cells evacuating zones of the "Meyer's posterior gland" are situated in the bottom part of the parallel folds gutter ; the evacuating zones of the identical "principal" cells of the shield are present all over its epithelial coating.

The histochemical tests on the tube sections show that the superficial layer of the organic tube and its partitions are PAS positive, with the deeper part of the tube giving a weaker result. The whole organic tube is alcian blue positive and metachromatic with toluidine blue. Its external layer is rather orthochromatic (Fig. 6a).

Mineral constituents

GBHA has been used to detect calcium in cryostat sections. The shield gland tissue gives a positive result, within mucocyte cells, as well as the homogeneous material of the inner organic tube.

Prussian Blue only shows the presence of Fe in thin granulations between the gland clusters in the shield intersegment epithelium. The reaction is somewhat variable from one specimen to another, but it plays no part in secretions and is totally absent from the organic tube.

HISTOENZYMOLOGY (Table I)

Two parallel techniques show the existence of a catecholase and a DOPA oxidase at different levels. Positive results have been obtained on twenty samples of *Sabella* and confirmed on the same sites in two *Spirographis*.

Catechol reaction appears positive in "Meyer's anterior gland", located ventrally on both sides of the parallel folds, extending to the crown ventral lobes. This reaction is inhibited by diethyldithiocarbamate, thus proving the presence of an enzyme. However all animals do not react positively. 23 have been tested in seven attempts ; 10 of them did not respond and 3 gave a weak result. Such a ratio of negative results can be due either to experimental parameters (fixation time, correct proportioning of substrate, or temperature) or individual and variable secretory cycles. However, in more than half the cases a local blackish-brown colour indicates an important enzyme activity. Cryostat sections of "in toto" parts provide little further information, since the blurred result makes a characterisation of cellular types impossible. But the map of the reacting sites is precise enough and corresponds to "Meyer's anterior gland" and to haemalum-coloured secretion cells. The catechol reacting ventral gland zone of *Sabellaria* (Vovelle, 1965) shows a similar connection. It seems that the abundant vascularisation of the concerned site is strengthened as if blood was locally involved in the positive result (whether enzymatic or not).

DOPA reaction does not affect the "Meyer's anterior gland", but a blackened hue spreads over all the shield ventral gland zone. All the specimens (21) incubated in DOPA answer positively even if the reactivity varies from one worm to another ; at any rate DOPA affords a more contrasted and conspicuous response than catechol (Fig. 3 c). No reaction appears on control half worms incubated in water. The reaction is inhibited by diethyldithiocarbamate, thus proving the presence of an enzyme. The two largest plates, situated immediately after the collar, are particularly reactive. Less marked results concern the crown pinnules and the ventral sacs, and the symmetrical gland clusters of the thoracic neuropods. On those sites, we note some variations between specimens and the common positive action of the inhibitor. DOPA cryostat sections of "in toto" specimen provide better images than the catechol ones. Many shield secretory cells show a granular black pigmentation ; the cellular bodies of these cells are located half way within the shield and their extensions can be followed through the epidermis. There is a clearcut boundary between the anterior part of the collar and Meyer's gland which do not react and the shield pigmented sites. In spite of similitudes of structures and sites, there is no evidence that the DOPA reacting shield cells are the same as those reacting to tyrosine detection, no evidence either, that they correspond to argentaffine reaction on the same sites of the first two thoracic metameres.

WAVELENGTH DISPERSIVE MICROANALYSIS (Table II, Fig. 5 a & b and Table III)

I, S and Ca have been systematically traced ; we have also occasionally looked for P. Mg, Fe because of their presence in other polychaete tubes, as well as Al and Si, which cha-

TABLE II

	Chaetae		Animal Glandular shield		Inter-segment	Inner organical		Tube Superficial -partitions		External exogenous		Chaetae?
c.s^{-1}	Sa	Sp	Sa	Sp	Sa	Sa	Sp	Sa	Sp	Sa	Sp	Sp
Mg			10		5							
Al										354		
P			20		35	9		20		14		
S			**35**	**38**	10	**615**	**627**	**575→210**	**470→125**	45	45	
Ca			**118**		40	**95**	**136**			↗2276		
Fe			0-ϵ		**145↗250**	14	11			↗780		
I	**↗150**	**↗145**	0-ϵ	0-ϵ		11	12	**25→45**	**50→110**	8	8-30	**110**
Br	**↗75**											
Si										↗2180	917	

Sa : *Sabella*, Sp : *Spirographis* ; → evolution depending on topography ; ↗ indicating higher values ; bold type : mean of 10 different points.

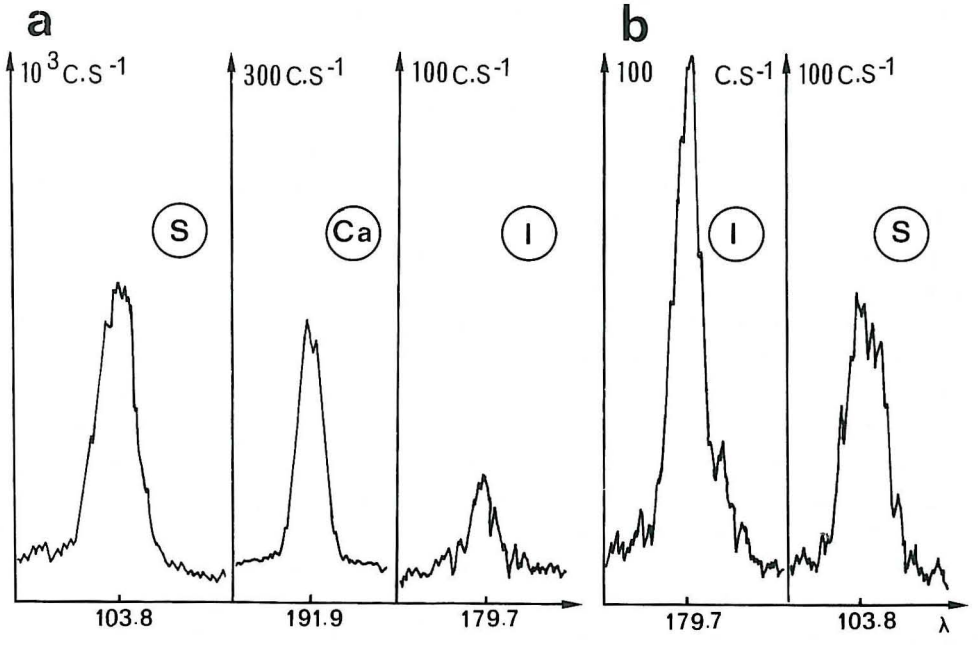

Fig. 5 : Recordings with the wavelength dispersive X-ray microprobe, concerning two characteristic points of the tube.
a) Organic inner layer.
b) Partitions of the organic intermediate tube (PET crystal for Ca and I, TAP crystal for S).

racterize the exogenous part of the tube. In *Sabella* and *Spirographis*, assays have been performed on paraffin sections of fixed animals, and on tubes either in paraffin or cut by cryostat. Some slight discrepancies between the results may be due to the use of these different preparations.

In both species no **iodine** is found in the shield glandular tissues or in the outer edge of the collar. Several tests showed that iodine never stands out against the background noise. However, confirming our previous results in *Sabella* (Vovelle & *al.*, 1984), iodine is present in the chaetal cortex, associated with bromine. Significant results had been previously obtained on "in toto" chaetae (up to 500 c.s^{-1}) and on paraffin sections (iodine : 150 c.s^{-1}, Br 75 c.s^{-1}). The values vary along the shaft of the chaetae, according to their type, and reach 270 c.s^{-1} in the *Spirographis* uncini. The significant halogenation of chaetal scleroprotein is confirmed by our present results.

Polychaetes are known to regularly lose their oldest chaetae : this material carried along by upward currents inside the tube, could be incorporated in the exogenous coating and partially account for iodine. When the outermost part of the tube is examined by microanalysis, in both species it is possible to locate iodine emissive spots (110 c.s^{-1} in *Spirographis*) which could be related to chaetal fragments easily identifiable by microscopy. Iodine can also be detected at the level of the partitions rooted in the superficial layer of the organic tube corresponding to the wrapping of the roll building the primitive tube. Iodine concentration can be followed step by step along the course of the wrapping ; present at the interface between the inner organic tube and the outer detritic tube (average 25 c.s^{-1} in *Sabella*, 50 c.s^{-1} in *Spirographis*) it increases outward (peripheral values, *Sabella* 45 c.s^{-1}, *Spirographis* : 110 c.s^{-1}). On some particular spots iodine values may be as high as those found in chaetal cortex. Besides a part of the inner organic tube also includes some iodine (from 10 to 12 c.s^{-1} according to species) (Fig. 5a and b). The halogenation of the secreted material is more conspicuous on the initial roll wrapping, but in spite of the absence of the reaction of gland sites, it may occur at the level of the parallel folds where Meyer's gland secretions are evacuated and possibly in the ventral sacs where the building material of the roll is being mixed. We were able to puncture the heterogeneous contents of an active *Spirographis* ventral sac and make a smear on carbonated terphan. After drying out, the evidence of a slight increase in iodine (less than 5 c.s^{-1}) could be found on 10 different emissive spots. In our control samples (the animal tissues and a dried brinedrop) no iodine was present.

In both species **sulphur** is present in the shield gland tissues. Its concentration in *Spirographis* changes from one level to another. In the main mass and evacuating zone of the Meyer's anterior gland it is faintly present (15 c.s^{-1}, TAP), in the underneath gland zone before the shield it reaches its maximum (from 90 to 100 c.s^{-1} in the evacuating zone under the collar). In the actual shield, S average values between 30 and 50 c.s^{-1} have been found, similar to those of the superficial "tyrosine" gland cells of the collar. The presence of sulphur in the shield and the posterior Meyer's gland is obviously related to their sulphated glycosaminoglycans reacting to toluidine blue. There is more sulphur present yet through the organic tube (with TAP crystal *Sabella* 615 c.s^{-1} and *Spirographis* 627 c.s^{-1}). Its outward concentration gradient in the organic partitions which dive into the detritic tube is the opposite of the iodine concentration gradient : *Sabella* from 575 c.s^{-1} to 210 c.s^{-1}, *Spirographis* from 470 c.s^{-1} to 125 c.s^{-1} in the bulk of the detritic tube much weaker results are obtained.

In *Sabella* shield, **calcium** values reach 115 c.s^{-1}, and are roughly the same in the organic tube : *Sabella* 93 c.s^{-1}, *Spirographis* 135 c.s^{-1}. This endogenous element is obviously linked with the organic secretion.

Typical concentrations of mineral calcium (1000 to 2275 c.s^{-1}) are found in some detritic particles of the heterogenous outer tube. Traces of **magnesium** associated with calcium have also been recognized.

We have looked for **phosphorus**, because of the significant presence of calcium, and of its importance in some other polychaete tubes. The results obtained either in the shield or the organic tube, between 10 and 20 c.s^{-1}, are fifty times weaker than those found in *Onuphidae* for instance. Therefore, it seems that phosphorus plays no significant part in Sabellid tube composition.

Iron appears only in the external tube detritic part, and fitting our histochemical results, in the shield intersegment epithelium (*Sabella* 145 c.s^{-1} 250 c.s^{-1}) where its presence is linked with an increase in phosphorus content (35 c.s^{-1})

An important mineral fraction of the external tube is rich in **aluminium** (355 c.s^{-1}) and **silica** (2200 c.s^{-1}).

ION MICROANALYSIS

An estimation of the elements involved in the building of the inner tube and of the detritic outer tube as well as the evaluation of their possible presence in the secretory tissues was obtained by examination of the peaks of the spectra. In decreasing order of importance, Ca, Cl, S are present in secretory tissues, Ca, S, Cl, Mg, I and Br, in the organic tube, and Al, Si, Ca, Fe, and traces of exogeneous iodine in the outer detritic tube. More detailed information is provided by the different images of repartition, element by element.

Secretory tissues

Sections of *Sabella* ventral shield fixed in glutaraldehyde show an even distribution of sites of S and Cl within the tissue cellular bodies. Only traces of other halogens F, I and Br are noticeable on the same sites after a long exposure (the spectrum of negative elements hardly shows an I peak with a mass 127). On sections of parts fixed in alcohol-formaldehyde, Ca is conspicuously present, along with some Mg and traces of Sr and Ba all through the ventral shield.

Spirographis collar ventral part shows reacting sites at the level of the protein-secreting external epithelium, and internal epithelium. In decreasing order, Ca, Cl and some S are revealed. The epithelium surface can be outlined by a tenuous edging of Br and a touch of I after a long exposure.

In *Spirographis*, we have examined the evacuating zone of the anterior glands, at the level of the parallel folds, and specifically where it is close to the collar ventral lobes, more caudally than the enzymatic part of Meyer's glands. The epithelium, crossed by the evacuating extensions of the secretory cells underneath, provides excellent ion emission images. In decreasing order Ca, Cl, and S are found. The surface is outlined by a tiny edging of Br, and hardly noticeable I.

The material gathered between the two parallel folds is mainly rich in Si (diatoms), particles of Al and Ca are also present on randomly distributed sites.

Tube (Fig. 6 b, c, d, e)

In *Sabella* outer exogenous layer, no I peak is visible on spectra, but Ca Al, Si, Fe, Na, K are conspicuously present ; also detectable to a lesser extent are Mg, Sr, Cu, and a few detritic I spots. The fairly good concentration of Ca evenly distributed all through the organic tube, is similar to the one found in the shields.

In *Spirographis*, the outer detritic tube is rich in Al, Si (Fig. 6 e), Ca, Fe (with a mass 56+ where it competes with CaO). The inner organic tube includes in decreasing order : Ca, S, Cl, Mg, I and Br. Ca, Cl and S are evenly distributed through the tube. A significant observation must be now mentioned : the outer zone and the external partitions issued from the organic tube show an increase of I (Fig. 6b) and even of Br, which does not affect the main part of the organic tube. Therefore this increasing halogenation is linked with the detritic roll wrapping.

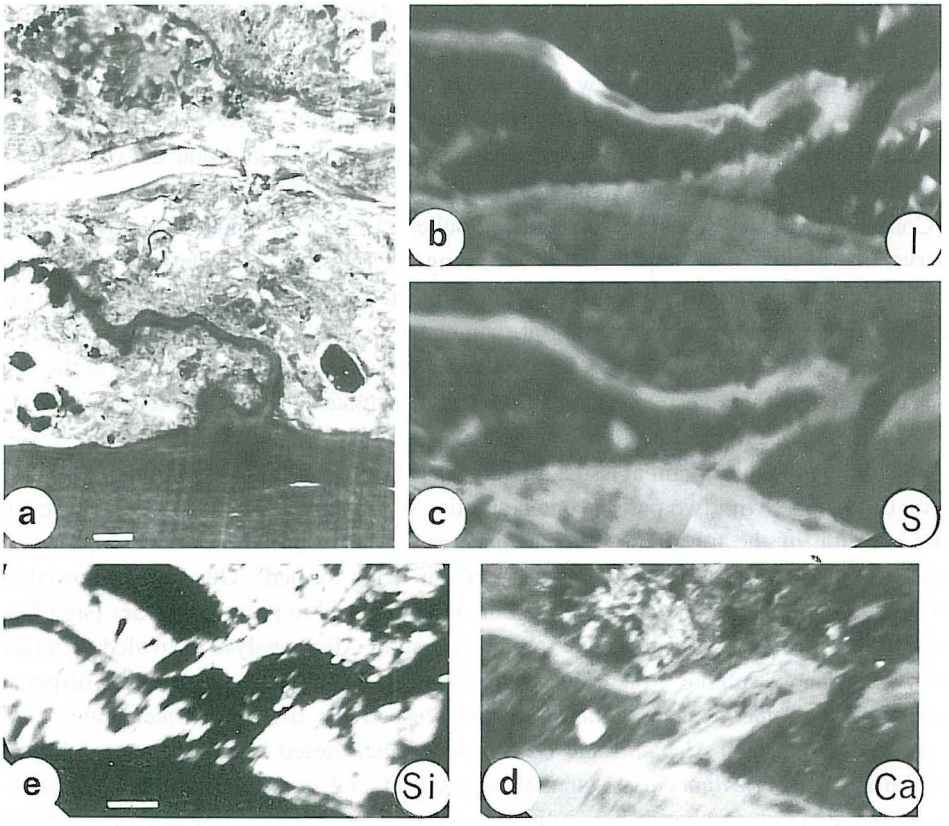

Fig. 6 : Ion microanalyser image of *Spirographis* tubes sections.
a) Semi-thin section of the tube, buffered Toluidine blue ; b, c, d, e, microanalysis of a neighbouring section concerning the following elements, with mention of their intensity and time of exposure : b) I (127^-) 6.10^{-15} A, 50s ; c) S (32^-) 4.10^{-15} A, 50s ; d) Ca (40^+) 1.10^{-13} A, 10s ; e) Si (28^+) 2.10^{-15} A, 3 min ; bars : 25 µm).

DISCUSSION

Our results demonstrate the existence of two sequences in the tube-building of the two studied Sabellidae. Some new data need to be discussed :

. the importance and duality of an anterior glandular formation, which we call "Meyer's gland" ;

. the participation of the external epithelium secretions (tyrosine) of the collar to the first sequence, when the ventral sacs are not concerned in this process ;

. the composition of the inner organic tube which appears rich in calcium and sulphur, possibly linked to polysaccharides ;

. the likelihood of two different quinone-tanning processes corresponding to the two sequences ;

. the confirmation of an halogenation process which involves exogenous iodine and may be correlated to the first quinone-tanning process.

Concerning the first point, we need to credit Meyer (1887) of the description of the glandular clusters associated with the parallel folds and opening into the folds groove : even if he missed their connection, he drew them on one of his plates exactly where we have identified them, at the basal part of the ventral sacs. Our results revealed that Meyer's gland is composed of two quite distinct parts. Histochemistry demonstrated that the only anterior gland elaborates mucoïd compounds likely to vector a catecholase activity.

Concerning other glandular secretions, Soulier (1891) had underlined the exclusive intervention of the ventral shield and its wide range of secretory cells, Defretin (1971) mentioning Nicol as a reference suggested that the collected particles were coated in the ventral sacs "by secretions of glands opening at their inner surface". Actually, the description by Nicol (1930) of the mixing motion in the ventral sacs was more discerning : "at the same time the parallel folds secrete mucus so that a continuous string is formed in which are embedded particles of mud from the ventral sacs".

Concerning the third point at issue, Defretin (1971) recognized the heterogeneous constitution of the tube, with two parts : "one consisting of sulphur-rich scleroproteins, forms the solid skeleton of the tube. The other, which consists of glycoproteins, is trapped in the meshes of the scleroprotein structure as the tube is being formed". Our results showed that the sulphur component from the inner organic tube is linked to polysaccharides (significant metachromatic stains, weak response to thiol reagents). Microanalysis provided significant information about sulphur ; the values of sulphur given by the X wavelength dispersion microprobe on *Sabella* inner organic tube are respectively fifty and fifteen times higher than those obtained in *Alvinella* and *Hyalinoecia*, when tested in a similar way (Vovelle, unpublished). In *Hyalinoecia* the specific organo-mineral constituent, onuphic acid, is a phosphorylated polysaccharide which fixes calcium and magnesium (Table III). The cement of Amphictenidae (Truchet & Vovelle, 1977) and of Sabellariidae (Gruet *et al.*, 1987) shows similar chemical features. Phosphorus being absent, this similarity cannot apply to the Sabellidae studied. Yet, the part played by calcium linked with an organic secretion (ori-

TABLE III

$c.s^{-1}$	TAP Crystal P	TAP Crystal S	PET Crystal Ca	PET Crystal Fe
• *Sabella* Internal tube	9	695	93	14
• *Alvinella*	230	13	40	93
• *Hyalinoecia*	907	41	512	9

ginally the mucocyte secretion of the shield) is noteworthy, though five times less than with *Hyalinoecia*. It cannot be regarded, however, as a true mineralisation. The cation here can be compared to that found in the terebellid worm *Lanice* (Bielakoff *et al.*, 1975).

Another point at issue concerns the duality of nature and localisation between two O-diphenoloxidases (catecholase *s.s.* for the first sequence, DOPA oxidase for the second one). This occurrence is not unusual, it has already been mentioned in the range of gland sites involved in the quinone-tanning of the *Sabellaria* tube (Vovelle, 1965) and on the different sites of the dog-fish nidamental gland secreting the egg-capsule (Rusaouen-Innocent, 1990).

The last problem deals with the correlation of quinone-tanning and halogenation. Two independent and successive tanning processes may be involved in the reinforcement of the organic materials which form the roll wrapping of the outer tube and the thickening of the inner tube : only one is correlated to iodine fixation. We have excluded any endogenous halogenation of the secreted material as well as iodine concentration taking place within the tissue. We have assumed that halogenation occurs on the surface of the organ of mixing and shaping. Using labelled iodine we demonstrated (Vovelle *et al.*, 1984) that halogen fixation on the tyrosine-rich tanned cortex of *Pectinaria* chaetae operates from their extrusion into the outside medium. However halogen concentration (according to Fowden's definition, 1968) of iodine, bromine, even chlorine (whelk opercula after Hunt, 1972) on tyrosine-rich materials seems clearly linked to quinone-tanned sites. Hunt was the first to suggest alternative explanations for the observation : "It seems possible... that halogenation is a side effect of the tanning process" and "the presence of (phenol) oxidases would also be effective in oxidizing chloride and bromide in sea water to chlorine and bromine, which would then readily substitute on the tyrosine ring". If we accept Waite's view of scleroprotein halogenation (private correspondence) : "abiotic, following their secretion into iodide and bromide rich water", we nevertheless observe a selective concentration process which varies with species and structures. In *Sabella* and *Spirographis* tubes, as well as in chaetae, bromine is much less than iodine. It can be observed that in contrast with chaetae, the material of the tube involved in the halogen fixation includes a significant polysaccharide fraction and its hardening keeps it flexible and, for a time, adhesive. However, iodine concentration values obtained by X wavelength dispersion microanalysis are significant on some sites of the primitive roll wrapping where they may reach the values obtained in the chaetal cortex.

Our conclusions need to be confirmed by radioactive labelling. Even if our observations are not based on pure quantitative analysis they reinforce the results formerly obtained by Cameron (1914, 1915), in connection with a likely process of the strengthening of the tube.

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