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Systematic Characters of Some Polychaetes (Annelida) at the Level of the Chemical Composition of the Jaws

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Abstract—The mandibles and maxillae of the buccal ventral organ of 2 species of Eunicidae (*Marphysa sanguinea* and *Eunice torquata*) are highly calcified, in contrast to the jaws of 4 species of other families of 'errant' predacious Polychaetes (Nereidae, Nephthyidae, Aphroditidae and Glyceridae) with axial proboscis. The amino acid composition of the structural proteins of these buccal pieces is also different in the two groups. The structural proteins of the jaws of *Glycera convoluta* (Glyceridae) are essentially made up of glycine and histidine (up to 86 residues per 100 residues). These chemical characters confirm the phyletic relationships proposed by Dales.[1]

Introduction

The stomodeal proboscis of predacious polychaetes appears either as a ventral buccal organ (Eunicidae, Amphinomidae) or as an axial muscular pharynx with a more or less elongated buccal tube before it (for instance Phyllodocidae, Glyceridae, Nephthyidae, Aphroditidae and Nereidae). These two types of proboscis differ in their mode of formation[1] and in their morphological features.[1,2]

In many families of predacious polychaetes, the axial proboscis is armed with one or two jaws, while the ventral buccal organ is equipped with two series of jaws, the dorsal ones (called maxillae) and the ventral ones (called mandibles).

There are very few data about the chemical composition of these structures. Hyman[3] demonstrated that the jaws of *Nereis virens* do not contain chitin. Jeuniaux,[4] and Desière and Jeuniaux[5] confirm the lack of this substance in the buccal tube, the jaws and the paragnaths of *Perinereis cultrifera* and in the jaws of *Sthenelais boa*. On the contrary, the analysis of a sample containing the jaws of the ventral organ of four species of Eunicidae (*Marphysa bellii, M. sanguinea, Arabella iricolor* and *Lumbriconereis impatiens*) revealed the presence of chitin in very low amounts (0·13%, w/w) in addition to protein-like substances (46·9%, w/w) and unidentified mineral components. Using histochemical methods, Michel[6] recently demonstrated the lack of chitin in the jaws of *Nephtys hombergii*. She also revealed that protein components with phenolic residues are present in these structures and that the proteins are hardened by quinonoid links. Protein components with phenolic residues are also present in the jaws of *Glycera convoluta* but quinonoid links have not been detected.[7]

The present paper deals with the chemical analysis of the jaws of different species of annelid Polychaetes with either type of proboscis, especially the qualitative and quantitative analysis of the mineral components,

[1]Dales, R. P. (1962) *Proc. Zool. Soc. (London)* **139,** 389.
[2]Desiere, M. (1967) *Ann. Soc. R. Zool. Belg.* **97,** 65.
[3]Hyman, L. H. (1966) *Biol Bull.* **130,** 94.
[4]Jeuniaux, Ch. (1963) *Chitine et Chitinolyse,* Masson, Paris.
[5]Desiere, M. and Jeuniaux, Ch. (1968) *Ann. Soc. R. Zool. Belg.* **98,** 43.
[6]Michel, C. (1971) *Ann. Histochim.* **16,** 273.
[7]Michel, C. personal communication.

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TABLE 1. LOSS OF WEIGHT OF BUCCAL SKELETAL PIECES OF 4 SPECIES OF POLYCHAETES IN 0·5 N HCl

Material studied	Initial dry wt (mg)	Weights after treatment with 0·5 N HCl (mg)	Loss of wt (mg)	Acid soluble components (%)
Marphysa sanguinea (5 specimens)				
mandibles	25·60	3·00	22·60	88·28
maxillae	42·80	5·20	37·60	87·85
Eunice torquata (7 specimens)				
mandibles	11·20	1·20	10·00	89·20
maxillae	11·10	1·85	9·25	83·30
Perinereis cultrifera (7 specimens)				
jaws	9·90	9·90	0	0
Sthenelais boa (7 specimens)				
jaws	1·90	1·90	0	0

the amount of chitin and a comparison of the composition of the protein fraction.

Results

Mineral Components

The results of the quantitative determinations of mineral components in the jaws of the different species are listed in Tables 1 and 2. Table 1 shows the weights of the samples before and after treatment with 0·5 N HCl, the differences corresponding to the amounts of acid-soluble mineral components. Table 2 lists the percentages of acid-soluble Na, Ca, Mg and K found in the jaws, and the percentages of these mineral components calculated as carbonates. A comparison of Tables 1 and 2 shows that the loss of weight after treatment with 0·5 N HCl and the amount of carbonates calculated from the values of the cations found are in good agreement.

The data presented in Table 2 reveal that the jaws of the 2 species of Eunicidae sharply differ in their mineral composition from the jaws of polychaetes with axial proboscis: the former are strongly mineralized, the latter contain mineral components in only very low amounts. In the case of Eunicidae, maxillae and mandibles have a similar mineral composition both within the same species and between species.

Organic Components

The application of the specific test of Jeuniaux[9] for chitin to maxillae and mandibles of *Marphysa sanguinea* did not reveal any trace of chitin in these structures.

Table 3 summarizes the results of the amino acid analysis of the protein components of the jaws of *Glycera convoluta, Nephthys hombergii, Sthenelais boa* and *Perinereis cultrifera* and of the maxillae and mandibles of *Eunice torquata.* The values of each amino acid (when present in measurable amounts) are expressed in µg/mg of dried material and as number of residues per 100 total amino acid residues ('molar fraction'). The sum of the different amino acids analysed gives an estimate of the total amount of protein, with respect to the dry weight of each material. Quantitatively, the proteins of the jaws of polychaetes with axial proboscis represent between 45 and 62% (w/w) of the initial material, while those of *Eunice* maxillae and mandibles respectively are only 11·8 and 7·9% (w/w). This difference originates from the relative importance of mineralisation in the latter structures. Qualitatively the structural protein components of the jaws of all the species so far studied are characterized by high percentages of glycine (40% in *Eunice torquata,* 43–62% in the 4 species with axial proboscis) and by very low amounts of the sulphur containing amino acids.

Nephthys hombergii, Sthenelais boa and *Perinereis cultrifera* have a somewhat similar amino acid pattern, which is in favour of a certain degree of isology between the protein components of the jaws of these 3 species. *Perinereis cultrifera* has however a higher proportion of tyrosine and lower amounts of serine. *Nephthys* has lower amounts of alanine and histidine. The jaws of *Nephthys hombergii, Sthenelais boa* and particularly those of *Perinereis cultrifera,* all contain aromatic amino acids. This observation confirms the presence of aromatic tanned proteins as being responsible for the hardness of the jaws.

[8]Dales, R. P. (1963) *Annelids,* Hutchinson, London.
[9]Jeuniaux, Ch. (1966) *Bull. Soc. Chim. Biol.* **47,** 2267.

TABLE 2. Ca, Mg, Na AND K, AND MINERAL COMPONENTS CALCULATED AS CARBONATES IN THE BUCCAL SKELETAL PIECES OF 4 SPECIES OF POLYCHAETES

Material studied	Ca	Mg	Na	K	$CaCO_3$	$MgCO_3$	Na_2CO_3	K_2CO_3	Total carbonates (calc. % (w/w))
				(% (w/w) dry wt)					
Marphysa sanguinea									
mandibles	31·84	0·95	0·33	0·036	79·60	3·32	0·63	0·05	83·60
maxillae	32·18	0·72	0·35	0·045	80·36	2·50	0·65	0·06	83·57
Eunice torquata									
mandibles	33·64	1·08	0·23	0·033	84·10	3·80	0·52	0·05	88·47
maxillae	30·08	1·09	0·26	0·034	75·17	3·83	0·59	0·05	79·64
Perinereis cultrifera									
jaws	0·074	0·073	0·055	0·011	0·185	0·25	0·09	0·002	0·527
Sthenelais boa									
jaws	0·243	—	0·022	0·030	0·610	—	0·05	0·042	0·702

On the contrary, in the jaws of *Eunice torquata* and *Glycera convoluta,* the percentage of aromatic amino compounds is very low.

The amino acid pattern of the protide components of the jaws of *Glycera convoluta* is very exceptional in that glycine and histidine amount to 85·6% of the total amino acid residues. The protide components of the maxillae and those of the mandibles of *Eunice torquata* are very similar in their amino acid composition: the only noticeable difference lies in their aspartic acid content. Their amino acid pattern differ from those of the protide components of the jaws of the species with axial proboscis in their higher percentage of threonine, glumatic acid and arginine and in their lower amounts of glycine and histidine.

Conclusions

The chemical composition of the jaws of the species of predacious polychaetes with axial proboscis so far studied (belonging to the families Nereidae, Nephthyidae, Aphroditidae and Glyceridae) strongly differs from that of the maxillae and mandibles of 2 species of Eunicidae, a family which is morphologically characterized by the presence of a ventral buccal organ.

These differences lie mainly in the relative amount of mineral substances: maxillae and jaws of Eunicidae are highly calcified while the jaws of the other families contain only minute amounts of inorganic cations. Such a difference is as sharp as that which distinguishes the tegument of most Crustacea to the cuticle of all Insects.

The amino acid composition of the protide fraction of the maxillae and mandibles of Eunicidae also exhibit some differences when compared to that of the jaws of the other families of Polychaetes so far studied. In the

TABLE 3. AMINO ACID ANALYSIS OF THE PROTIDE COMPONENTS OF THE JAWS, MAXILLAE OR MANDIBLES OF 5 SPECIES OF POLYCHAETES

	Glycera convoluta (jaws)		*Nephthys hombergii* (jaws)		*Sthenelais boa* (jaws)		*Perinereis cultrifera* (jaws)		*Eunice torquata* mandibles		*Eunice torquata* maxillae	
Amino acid	M.F.	µg/mg	M.F.	µg/mg	M.F.	µg/mg	M.F.	µg/mg	M.F.	µg/mg	M.F.	µg/mg
Asp	1·93	12·00	4·90	39·36	6·26	40·92	12·90	98·81	9·20	9·45	4·74	7·20
Thr	1·13	6·16	1·54	10·88	2·55	14·69	1·24	8·37	4·11	3·71	4·32	5·76
Ser	1·98	9·31	7·84	47·68	6·83	33·78	2·22	12·91	9·63	7·49	9·23	10·60
Glu	2·32	16·16	5·90	53·20	5·24	38·44	4·90	42·10	11·62	13·39	13·38	22·75
Pro	+	+	1·19	8·07	+	+	2·89	18·71	3·32	2·88	4·96	6·36
Gly	62·07	190·58	56·18	223·62	50·10	162·30	43·26	164·26	40·92	20·84	39·16	29·46
Ala	3·28	12·55	4·95	24·58	9·91	40·02	9·10	43·04	7·94	5·04	9·20	8·62
Cys	—	—	—	—	—	—	—	—	TR	TR	+	+
Val	0·56	3·03	1·04	7·25	0·86	4·85	0·98	6·49	1·06	0·94	1·27	1·66
Met	—	—	0·73	6·74	—	—	—	—	+ +	+ +	0·28	0·49
Ile	0·41	2·50	0·57	4·56	0·38	2·46	0·43	3·29	0·70	0·71	0·89	1·34
Leu	0·64	3·94	0·43	3·47	2·66	17·15	1·24	9·37	1·15	1·17	1·71	2·56
Tyr	0·17	1·53	3·27	37·25	1·33	12·33	7·79	84·58	0·46	0·68	0·44	0·95
Phe	0·21	1·69	3·05	31·41	3·66	30·61	1·90	18·61	0·58	0·76	0·72	1·40
Lys	0·61	4·23	1·60	14·32	+	+	0·42	3·62	0·71	0·81	0·89	1·51
His	23·59	174·17	2·95	28·28	8·16	63·57	7·79	71·15	1·19	1·46	0·65	1·19
Arg	1·03	8·66	3·77	41·09	2·00	17·77	2·87	29·91	7·34	10·24	8·11	16·72
Total	99·93	446·51	99·91	581·76	99·94	478·89	99·93	615·22	99·93	79·57	99·95	118·57

The results are expressed in µg/mg of dried material, and as the number of residues for every amino acid per 100 amino acid residues: 'molar faction' = M.F.

former structures the proportions of threonine, glutamic acid (or glutamine) and arginine are twice those observed in the latter, while the proportion of histidine is considerably lower.

On the other hand, the chemical compositions of the jaws of the 3 species belonging to the Nephthyidae, Aphroditidae and Nereidae are obviously similar: the jaws, devoid of chitin, have indeed identical mineral composition and contain similar amounts of proteins which present a rather high degree of isology. On the contrary the jaws of *Glycera convoluta* (Glyceridae) are highly different: they contain very peculiar protide components with glycine and histidine amounting to 86% of the total amino acids so far identified, contrasting with the proteins of the jaws of the three other predacious Polychaetes with axial proboscis.

The characters of the chemical composition of the buccal skeletal pieces thus appear in good agreement with the taxonomical and phylogenetical relations within Polychaetes recently proposed by Dales.[8] The Eunicidae obviously belong to a phylogenetic line distinct from that of the other 'errant' predacious Polychaetes. Within the latter group the Glyceridae probably represent a divergent branch as suggested not only by morphological and histochemical arguments, but also by the very peculiar proteic fraction of their jaws.

Experimental

The animals were collected at Roscoff (France) except for *Eunice torquata* (Banyuls, France). They were preserved in 70% EtOH.

Mineral components. The following samples were analysed for mineral composition: jaws of 7 specimens of *Perinereis cultrifera* (Nereidae), of 7 specimens of *Sthenelais boa* (Aphroditidae) and jaws of 2 species of Eunicidae: *Marphysa sanguinea* (5 specimens) and *Eunice torquata* (7 specimens). The dorsal and ventral jaws of each of these 2 last species were analysed in two separate samples. After isolation, the jaws were carefully cleaned to be free of any trace of muscular fibers, washed several times with H_2O and dried on filter paper. The dry wt (95°) was then determined. The dried jaws were treated with 0·5 N HCl, the residues were separated and the loss of weight determined. Na, K and Ca in the HCl were determined by flame spectrophotometry (Eppendorf), Mg by Atomic Absorption (Perkin Elmer).

Organic constituents. Test for chitin: The jaws of 8 specimens of *Marphysa sanguinea* were isolated, cleaned from muscular attachments and washed several times. They were treated with 0·5 N HCl to eliminate mineral components. The residues, after treatment with 0·5 N NaOH at 100° to eliminate the protide components without destroying chitin, were tested for 'total' chitin[9] with the enzymic method using highly purified chitinases[4,9] (E.C. 3.2.1.14).

Protide amino acids were analysed in acid hydrolysates of jaws or of maxillae and mandibles of 5 species (*Glycera convoluta, Nephthys hombergii, Sthenelais boa, Perinereis cultrifera* and *Eunice torquata*). After extraction, cleaning and several washings the dried pieces were weighed. The maxillae and mandibles of *Eunice torquata* were previously decalcified. The material was then hydrolysed with 6 N HCl at 110° in sealed tube. After evaporation, citrate buffer was added to the sample and the insoluble residue discarded. A Beckman model 120 C amino acid analyzer equipped with a single column system was used for amino acid determinations.