Marine Biology 49, 203-210 (1978)

MARINE BIOLOGY
© by Springer-Verlag 1978

Chemical Changes Accompanying Maturation of the Connective Tissue Skeletons of Gorgonian and Antipatharian Corals

W.M. Goldberg

Department of Biological Sciences, Florida International University; Miami, Florida, USA

Abstract

The skeletons of 3 gorgonian and 2 antipatharian corals were subjected to quantitative chemical analysis. Protein values ranged from 70.4 to 93.6%; ash from 3.0 to 19.4%; lipid from 0 to 8.4%; carbohydrate from 1.24 to 3.94% and halogen from 4.2 to 24.9% of the dry skeletal weight. Hydroxyproline and phenolic values were 0 to 3.0% and 11.6 to 25.0% of the protein, respectively. Lipid, present in 2 gorgonian species and 1 antipatharian, significantly decreased with age in all three cases. Glucose and galactose accounted for over 90%, and sialic acids for an additional 1 to 10%, of the carbohydrate in the gorgonian skeletons studied; the glucose content of the gorgonian skeleton decreased with age. The antipatharian skeletons possessed no glucose or galactose, but contained significant levels of amino sugars; the presence of chitin is confirmed. In the gorgonians, bromine and iodine, the predominant halogens, increased with skeletal age and were present in nearly equal amounts. Small amounts of bromine were found in the antipatharian skeletons, but very large amounts of iodine were found in older parts of the skeleton. The basal regions of both antipatharian species contained >23% by weight of iodine, the highest content of iodine yet recorded for any organism.

Introduction

Gorgonian and antipatharian coral skeletons are among the most resilient and chemically resistant proteinaceous materials known. In a previous paper (Goldberg, 1976), histochemical data and amino acid composition of several species were presented in an attempt to characterize these materials. This paper quantifies the components of "gorgonin" and "antipathin", with particular emphasis on the chemical changes which accompany the process of skeletal maturation.

Materials and Methods

Skeletal tips (yellowish, distal 2 to 4 cm) and basal regions (excluding the attachment disc) were taken from 3 species of gorgonian corals representing medium-sized colonies from different families [*Muricea muricata* (Pallas), *Swiftia exserta* (Ellis and Solander) and *Gorgonia ventalina* (Linnaeus)], from the Florida Keys, and 2 species of antipatharian corals [*Cirrhipathes lütkeni* (Brook) and *Antipathes rhipidion* Pax] from Jamaican waters. All analyses were done on these samples, except that halogen analyses of *G. ventalina* and *C. lütkeni* employed samples from La Parguera, Puerto Rico. Skeletons of at least three separate colonies of each species were cleaned of adherent material by allowing bacterial decomposition to occur in warm seawater for 2 to 3 days. Skeletons were rinsed in distilled water, checked microscopically for adherent material, and ground to a powder in liquid nitrogen. The powder was repeatedly extracted in triple-distilled, deionized water, dried to constant weight at 100°C, and stored in polyethylene vials until analyzed for protein, halogens, carbohydrate, lipid and total ash weight.

Protein. 100 mg samples were hydrolyzed for 6 h in sealed tubes at 105°C in 6N HCl; the hydrolysates were Millipore-filtered (0.2 µm) to remove insoluble humins. Protein was measured by biuret (Harleco). Standards employed 3X crystallized bovine serum albumin (BSA)

0025-3162/78/0049/0203/$01.60

(Miles Research Laboratories, Elkhart, Indiana, USA) to which 2% glucose (w/w) had been added. The same hydrolytic conditions described above were applied to the BSA material. The hydroxyproline content was measured by the benzene extraction technique of Woessner (1961). Tyrosine and other phenols were determined with Folin's reagent (Fisher) without the addition of copper (cf. Lowry *et al.*, 1951), using 6 h hydrolyzed tyrosine as the standard.

Halogens. Iodine, bromine and chlorine were measured by non-destructive neutron activation analysis. Powdered samples weighing 10.00 mg were placed in heat-sealed polyethylene vials, and delivered by means of a pneumatic system to the reactor core at the Puerto Rico Nuclear Center (Mayaguez, Puerto Rico). The irradiation time was 1.0 min at an average flux value of 1.4 X 10^{13} neutrons cm^{-2} sec^{-1} calculated from 10 µg standards of $MnSO_4$ dried on ashless filter paper. A standard was placed in each vial subjected to irradiation. A cooling period of approximately 5 min was allowed during transfer of the sample to the detection system, which consisted of a Ge(Li) spectrometer with a 10.6 cm^2 detector area facing the window. The detector was housed in a lead shield and was operated at a gain setting of 1 kev per channel. Data reduction employed an analog/digital converter which was programmed to calculate halogen mass when cooling time, sample geometry, detection efficiency, background and reactor flux were known. Program accuracy was checked by irradiation of standards containing 10 µg KI and NaBr on ashless filter paper together with a $MnSO_4$ flux standard. Peak energies, half-lives and cross-section values were taken from Lederer *et al.* (1967) and Adams and Dams (1969).

Carbohydrates. Samples were hydrolyzed for 4 h in either N-NaOH or 2N HCl at 80°C. The unhydrolyzed antipathin residue was removed and saved for separate analysis. Hydrolysates were adjusted to pH 6.8 and desalted through a column of mixed bed ion exchange resin (Biorad, Ag-501-X8, Richmond, California). The eluates were lyophilized and redissolved in glass-distilled, deionized water. Total carbohydrate was measured by the phenol-sulfuric acid method of DuBois *et al.* (1956), both before and after column chromatography, to detect losses (negligible in all cases). Samples were analyzed for sialic acids by the thiobarbiturate method of Warren (1959), and for total amino sugars by the method of Rondle and Morgan (1955). Standard curves were prepared with N-acetylneuraminic acid and equal mixtures of glucosamine and galactosamine. Quantitative analysis of glucose and galactose was performed colorimetrically using an enzyme-coupled chromogen technique (Glucostat and Galactostat Reagent, Worthington Biochemical Corp., Freehold, New Jersey, USA).

Qualitative identification of carbohydrate was performed by thin-layer chromatography. Plates of silica gel G (Analtech, Newark, Delaware, USA) were soaked in 0.08M borate buffer, pH 8.0, for 10 min, and activated by heating for 30 min at 110°C. A mixture of standards consisting of glucose, galactose, xylose, fucose, rhamnose, arabinose, glucosamine and galactosamine (Sigma) were spotted in parallel to skeletal preparations. The solvent system consisted of chloroform-acetic acid-water (3:3.5:0.5, v/v) (DeStefanis and Ponte, 1968). Plates were developed by running three times in the same dimension, followed by drying at 110°C. Spots were visualized using p-anisidine-phthalic acid reagent (Kirchner, 1967; p. 153).

Ash. Skeletal ash content was measured gravimetrically by combustion (12 h) in a muffle furnace at 550°C. It is assumed that halogen weight is not reflected here due to the relatively high temperature employed.

Lipids. Skeletal lipid content was estimated by difference before and after extraction of the dried, powdered skeletal material with chloroform-methanol (2:1, v/v) (Folch *et al.*, 1951). The extraction was carried out in a shaking water bath at 30°C for 72 h, after which the powder was repeatedly washed with suction in fresh solvent and dried to constant weight.

Results

The overall skeletal analyses are summarized in Tables 1 and 2. The general trend is toward increasing protein content with skeletal age (assuming proximal regions of the skeleton are the oldest). However, this trend is reversed in *Muricea muricata* and is not clear in *Cirrhipathes lütkeni*. No distinction is seen between the protein content of antipathins and gorgonins. Both skeletal types are composed of about 81 ± 10% protein (Fig. 1). No interspecific trends can be seen in the relationship of hydroxyproline and phenols to the protein content of the skeleton, and with the exception of *M. muricata*, no ob-

Table 1. Proximate analysis of gorgonian and antipatharian coral skeletons. Data expressed as percentage of dry skeletal weight ± standard deviation. Phenol and hydroxyproline values are expressed as a percent of the protein content and are not included in the totals (last column)

Species	Biuret protein (N=6)	Phenol	Hypro	Ash	Carbohydrate	Halogens	Lipid	Total
Muricea muricata								
tip	78.6±2.9	13.8±1.5	2.08±0.16	19.4±3.2	2.64±0.2	6.06±0.5	8.4±2.0	115.1
base	73.2±2.8	19.6±1.8	3.00±0.18	9.7±0.7	1.64±0.5	11.26±1.6	1.0±0.1	96.8
Swiftia exserta								
tip	70.1±1.5	9.0±0.2	2.60±0.07	18.1±2.3	3.48±0.4	5.53±0.7	8.2±1.8	105.4
base	80.1±2.4	13.2±0.2	2.37±0.75	9.6±1.1	2.76±0.2	12.04±1.1	0.1±0.1	106.6
Gorgonia ventalina								
tip	76.4±4.5	25.0±2.4	1.85±0.21	12.0±4.8	2.64±0.2	4.20±0.5	0	95.2
base	91.4±2.8	17.6±2.3	1.41±0.10	16.2±1.4	1.24±0.1	6.16±0.4	0	115.0
Cirrhipathes lütkeni								
tip	77.8±6.9	21.6±2.2	0	4.2±0.1	3.94±1.2	20.13±7.7	0	106.1
base	82.8±4.1	15.7±1.2	0	3.0±0.1	3.05±1.0	24.76±4.0	0	113.6
Antipathes rhipidion								
tip	84.8±4.7	11.6±0.3	0.17±0.03	8.4±2.1	2.83±0.8	13.78±3.7	2.2±1.2	112.0
base	93.8±4.1	15.1±1.3	0	6.7±3.3	3.58±0.9	24.93±2.1	0.5±0.2	129.2

Table 2. Summary of maturational differences in skeletons of Antipatharia and Gorgonacea

Property	Change with age in Gorgonacea	Change with age in Antipatharia
Total protein	70.1-91.4% of skeletal weight; increases in 2 cases, decreases in *Muricea muricata*	77.8-93.8% of skeletal weight; slight or insignificant increase
Hydroxyproline	1.4-3.0% of protein; slight decrease in 2 cases, distinct increase in *M. muricata*	Not consistently present
Phenol	9-25% of protein; increases distinctly in 2 cases, decreases distinctly in *Gorgonia ventalina*	11.6-21.6% of protein; decreases in 1 species, increases in another
Total carbohydrate	Decreases by 18-47%	Variable
Glucose	Decreases by 44-57%	Not present
Galactose	Increases by 27-74%	Not present
Sialic acids	Increases by 44-64%	Decreases by 50-53%
Amino sugars	Not present	Slight increase to no change
Total halogen	Increases by 36-54%	Increases by 19-45%
Iodine	Increases by 44-47%	Increases by 28-54%
Bromine	Increases by 14-54%	Decreases by 41-69%
Chlorine	Increases by 49-90%	Irregular
Lipid	Decreases when present	Decreases when present

vious difference between tip and base is apparent. Phenols (including tyrosine and its derivatives) account for a considerable proportion of the skeleton. However, it is not certain that only phenolic amino acids are the reacting species. Comparison with tyrosine values reported previously (Goldberg, 1976) indicate that this amino acid accounts for only about one-third of the gorgonian phenol values, and for about one-half of the antipatharian values reported here.

The ash content tends to decrease with age, although not in *Gorgonia ventalina*. Many species occasionally trap calcareous spicules in the growing skeleton (Neumann, 1911) which may account for some of the ash in gorgonians.

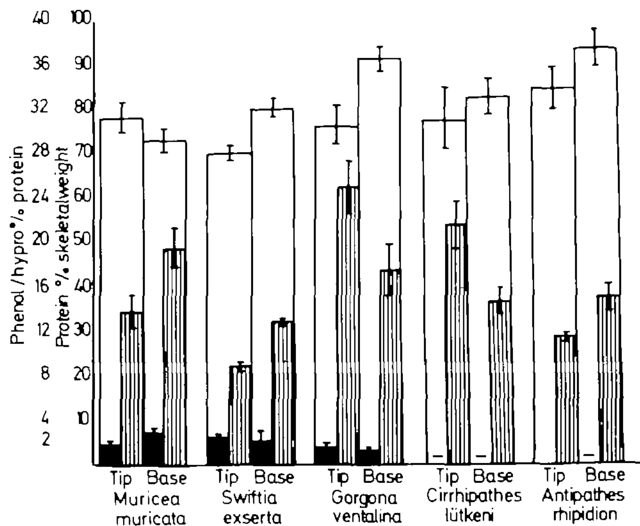

Fig. 1. Relationship of hydroxyproline (hypro, black bars) and phenol content (hatched bars) to total protein (open bars) of gorgonian and antipatharian coral skeletons ± standard deviations. Total protein is presented as a function of dry skeletal weight on separate ordinal scale

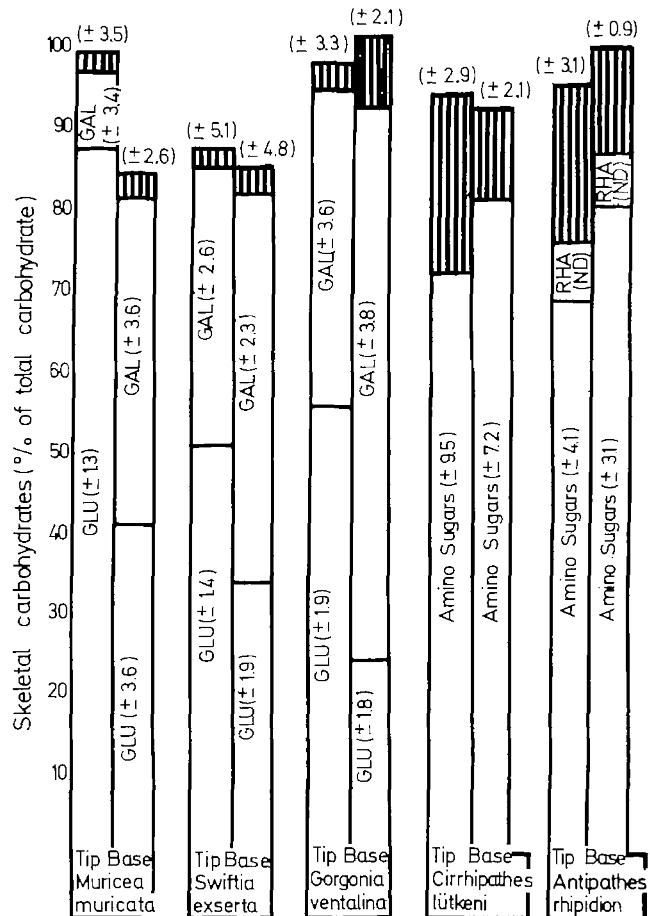

Fig. 2. Components of skeletal carbohydrates in tip and base of gorgonian and antipatharian corals; standard deviations in parentheses. GLU: glucose; GAL: galactose; sialic acid content is indicated by hatched portions of bars. For rhamnose (RHA) in *Antipathes rhipidion*, standard deviation was not determined (ND)

The carbohydrates account for <4% by weight of both the gorgonins and the antipathins. Total carbohydrate decreases with age in the gorgonians, primarily due to a net loss of glucose (Table 2; Fig. 2). Age variation in antipathin carbohydrate appears to be irregular in the two species studied.

Distinct differences in carbohydrate composition were found when comparing gorgonins to antipathins (Fig. 2). The former was composed almost entirely of glucose and galactose, while the antipathins contained principally amino sugars. Sialic acids were a minor but constant feature of both skeletal types. These results were qualitatively confirmed by thin-layer chromatography, and allowed additional identification by cochromatography of trace amounts of fucose and rhamnose in *Muricea muricata* tips. Rhamnose was also detected in *Antipathes rhipidion* tips and base.

Iodine, bromine and chlorine were present in all species examined, and in the gorgonins increased toward the base (Figs. 3 and 4). Gorgonin bromine and iodine were present in nearly equal amounts, with smaller quantities of chlorine. Individual colonies did not always conform to the pattern of the mean. The paired *t*-statistic compares tip and base of each colony analyzed and shows that increases in bromine and chlorine are significant only in *Swiftia exserta* and *Muricea muricata*, respectively (Table 3).

Iodine increases with skeletal age in all colonies of all gorgonian species. It is not certain that iodine increases distally in the antipatharian *Cirrhipathes lütkeni*, although it apparently does so in *Antipathes rhipidion*. The amount of iodine present in this group accounts for over 23% of the skeletal weight and >94% of all halogens in the basal portion. Bromine decreases with skeletal maturity in both antipatharians, and is present in relatively small amounts. Chlorine is distributed irregularly in small amounts.

Extractable lipid was found in two gorgonian species and one antipatharian (Table 1). This material was associated with protein since the extracts were ninhydrin-positive. Where it occurred, there was a distinct decrease in lipid toward the base of the colony.

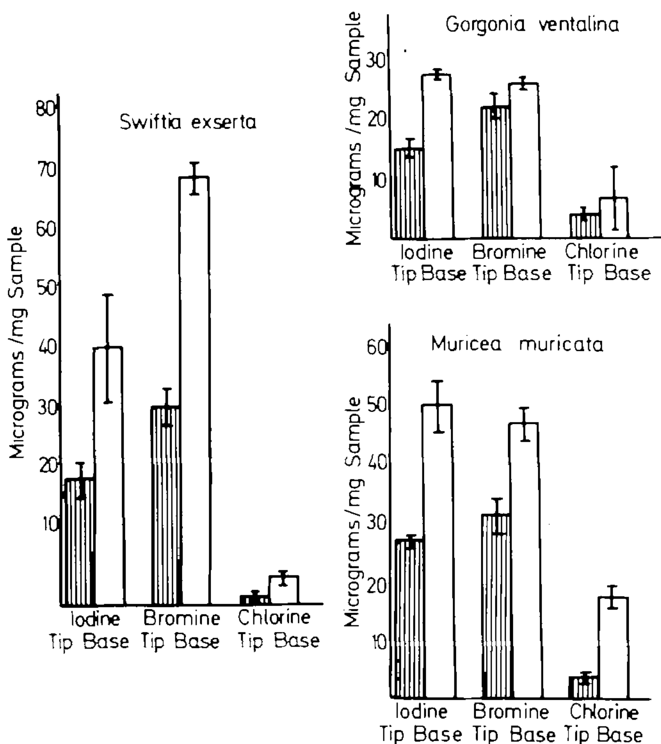

Fig. 3. Halogen content of gorgonian coral skeletons ± standard deviation. Hatched bars are skeletal tips; open bars are skeletal bases

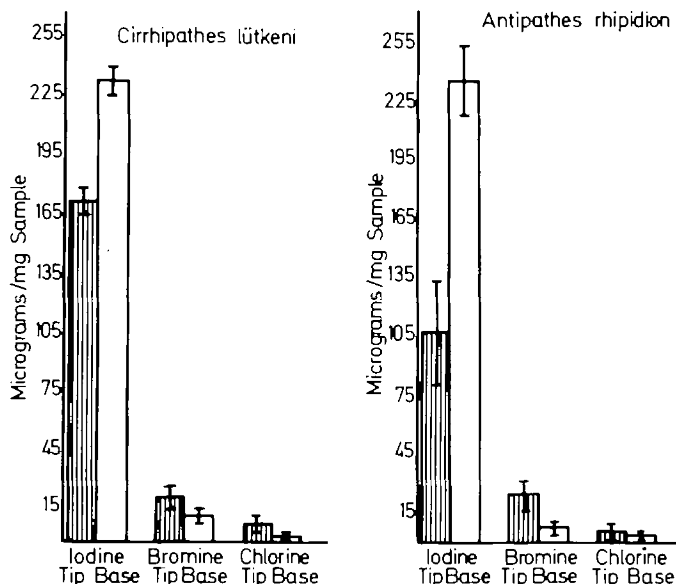

Fig. 4. Halogen content of antipatharian coral skeletons ± standard deviation. Hatched bars are skeletal tips; open bars are skeletal bases

Table 3. Significance of differences in halogen concentrations of tip and base pairs of gorgonian and antipatharian skeletons (paired t-statistic, N=3). I: iodine; B: bromine; C: chlorine

Species	t-values			Mean ratio base:tip			P		
	I	B	C	I	B	C	I	B	C
Muricea muricata	9.98	2.16	44.39	1.8	1.5	4.8	<0.01	<0.20	<0.001
Swiftia exserta	5.35	26.27	2.85	1.2	2.2	2.1	<0.05	<0.002	<0.2
Gorgonia ventalina	30.23	1.70	3.57	1.8	1.2	1.9	<0.002	<0.30	<0.1
Cirrhipathes lütkeni	1.25	1.52	1.02	1.4	0.5	1.0	<0.4	<0.30	<0.5
Antipathes rhipidion	4.89	3.02	0.49	2.2	0.3	1.0	<0.05	<0.10	<0.7

Discussion

Neither gorgonin (Szmant-Froelich, 1974) nor antipathin are homogeneous substances. The major component in both types is protein, but the presence of other materials complicates its analysis. When used with alkaline copper sulfate, Folin's reagent reacts poorly with both antipathins and gorgonins, apparently due to interference by high glycine levels which substantially depress the Folin-copper color (Lowry *et al.*, 1951). Kjeldahl analysis gives spuriously high results with certain species, particularly *Muricea muricata* and *Cirrhipathes lütkeni*. The use of ninhydrin suffers from losses of amino acids in the formation of humins, whether or not a reducing agent ($SnCl_2$ or thioglycollate) is added. Autoclaving before analysis is not an effective means of removing carbohydrate. Basic hydrolysis would overcome such difficulties, except that antipathins are incompletely degraded by 5N NaOH at 105°C for 24 h. The biuret method was used as a compromise, since a 6 h acid hydrolysis minimizes humin formation, solubilizes all of the skeletons studied, and gives the most consistent results. It should be stressed, however, that some of the more labile proteins may become abiuret during hydrolysis, resulting in an underestimate of the total

Table 4. Comparative halogen analysis of gorgonian and antipatharian coral skeletons. ND: not determined

Species	Halogens (as percent dry weight of skeletons; regions undefined or averaged)			
	Iodine	Bromine	Chlorine	Source
Gorgonians				
Muricea muricata	3.94	ND	ND	Cook (1904)
	0.30	0	0	Mörner (1907)
	3.8	3.8	1.0	This paper
Gorgonia (*Rhipidogorgia*) *flabellum*[a]	1.91	ND	ND	Cook (1904)
	0.45	0.37	0.05	Mörner (1907)
	1.15	ND	1.24	Mendel (1900)
	0.80	ND	ND	Sugimoto (1928)
	0.62	0.73	ND	Roche (1952); Roche *et al.* (1963)
Gorgonia ventalina	0.22	0	ND	Roche (1952); Roche *et al.* (1963)
	2.2	2.5	0.5	This paper
Antipatharians				
Antipathes spp.	0.02–1.79	0.38–1.53	0.42–0.73	Mörner (1908)
	1.29–2.18	0	ND	Roche (1952); Roche *et al.* (1963)
Antipathes abies	4.34	0	0.37	Allen (1930)
Antipathes rhipidion	17.1	1.6	0.57	This paper
Cirrhipathes anguina	2.90	ND	ND	Roche (1952); Roche *et al.* (1963)
Cirrhipathes spiralis	5.43	0	0.43	Mörner (1908)
	4.07	0.2	ND	Roche (1952); Roche *et al.* (1963)
Cirrhipathes lütkeni	20.3	1.7	0.51	This paper

[a]*Gorgonia flabellum* may have been confused with *G. ventalina* in older literature.

amount. The inability to overcome such analytical difficulties may be reflected in the lack of a consistent interspecific trend between tip and base protein.

Halogenation of gorgonin and antipathin has been the focus of considerable interest in the past, although scant attention has been paid to its relationship to maturation. Using chemical techniques, Mörner (1907) concluded that no difference in halogen content could be distinguished in 5 gorgonian species as a function of skeletal age. Roche (1952), on the other hand, concluded that in specimens of *Eunicella verrucosa* Pallas, the halogen content (specifically iodine) decreased with age. The data presented here differ from both interpretations: not only can clear differences be seen in the distribution of halogens in tip versus base, but in the case of iodine the concentrations increase proximally. This is understandable, since both gorgonins and antipathins actively concentrate radioiodine at the growing tip with markedly decreased concentrations in older portions (Goldberg, 1977). Presumably, the iodine concentration will necessarily increase with age and continued growth.

Although intrageneric differences and geographic variation have not been accounted for, the data presented in Table 4 seem to indicate that, for the most part, higher values occur than reported previously. This is perhaps not surprising, considering the non-destructive nature of the activation analysis used in the present study. The concentration of iodine by the antipatharians studied here give the highest dry weight values yet reported for any organism (Binnerts and Das, 1974).

Halogens are usually thought to be associated with the proteins of gorgonin and antipathin in the form of iodo- and bromo-tyrosine (Roche *et al.*, 1963; Goldberg, 1976). Enough phenol is present to account for the amount of halogens found in most cases (Fig. 5). However, certain regions of *Swiftia exserta* and *Antipathes rhipidion* would require at least some of the phenols to be halogenated in two positions. In the bases of both antipatharians, phenol is not present in sufficient quantity to account for the total

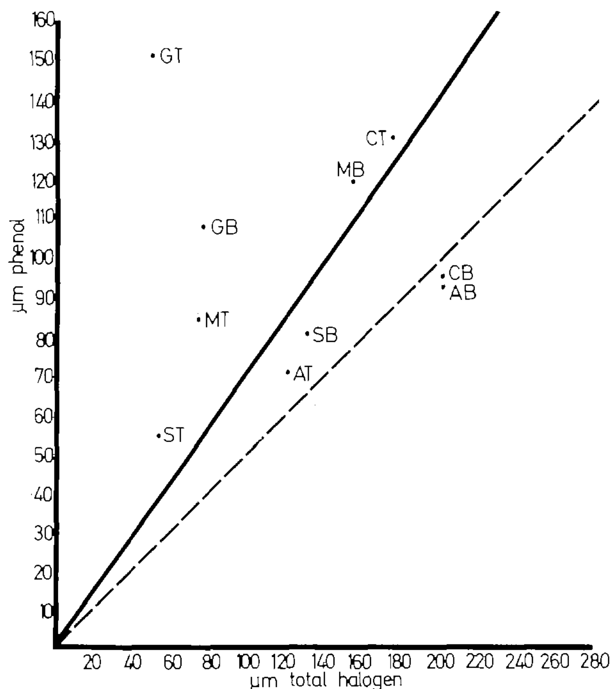

Fig. 5. Relationship between phenolic components and halogen in gorgonian and antipatharian skeletons. MT: *Muricea muricata* tip, MB: *M. muricata* base; ST: *Swiftia exserta* tip, GB: *G. ventabase*; GT: *Gorgonia ventalina* tip, SB: *G. ventalina* base; CT: *Cirrhipathes lütkeni* tip, CB: *C. lütkeni* base; AT: *Antipathes rhipidion* tip, AB: *A. rhipidion* base. Solid line indicates 1:1 relationship; species above this line are undersaturated with respect to halogenated phenol. Dotted line indicates saturation level of phenol; species below this line possess more iodine than can be accounted for by phenol alone

halogen content, even if it is assumed that all phenol is disubstituted. The difference may be explained by the presence of histidines. Histidine is known to account for 14 to 16% of all amino acid residues in these antipatharians (Goldberg, 1976); iodohistidine accounts for an additional 2%. However, due to the ease of dehalogenation in acid (Savoie *et al.*, 1973), this is probably an underestimate. An additional source of halogen could be the lipid material through addition reactions; preliminary thin-layer chromatographic analysis with *Muricea muricata* suggests that this is the case.

Halogenation of structural protein is generally thought to occur as a side effect of the phenolic oxidation required in cross-linking (Welinder, 1972; Hunt and Breuer, 1973; Hunt, 1976). However, more functional relationships including orientational effects of halogenated proteins (Roche, 1959) and an increase in reactivity of phenol after halogenation (Pryor, 1962) have also been considered as possibilities. The relationship between halogenation and protein tanning remains conjectural, but the distinct increase with age supports the view that halogen is involved in maturational phenomena.

Glucose and galactose represent over 90% of the carbohydrates in the collagen-bearing gorgonian skeleton; this is unusual, since invertebrate collagens are associated with a wide variety of saccharides (Gross *et al.*, 1958; Katzman and Jeanloz, 1970; Katzman and Oronsky, 1971; Barzansky *et al.*, 1975). It is the vertebrate collagens that are most often found with only glucose and galactose components.

The antipathins do not appear to contain collagen, although glycine accounts for about a third of the amino acids, and some proline (but no consistently demonstrable hydroxyproline) is present. The carbohydrate data support this view: the typical collagen hexoses, glucose and galactose, are entirely absent. The dominance of hexosamines (70 to 80% of the carbohydrate) may result in part from sialic acid degradation. However, a white, fibrous residue which remains after 24 h in hot N-NaOH constitutes the source of most, if not all of the amino sugar. The fibers compose 6 to 8% of the skeleton by weight (*N*=8), and contain 24 to 26% amino sugar, confirming the chitin-like x-ray diffraction patterns obtained by Ellis *et al.* (in preparation).

Acknowledgements. The activation analyses were performed at the Puerto Rico Nuclear Center while the author was an Oak Ridge Associated Universities faculty fellow. I am grateful to F. G. Lowman, R. Brown, and the staff of PRNC for making reactor facilities available to me. Computer analysis of data was facilitated by the expertise of B. Smith, whose help is greatly appreciated. P.L. Colin (Department of Marine Science, University of Puerto Rico) assisted with diving facilities at La Parguera Marine Laboratory, J.F. Woessner, Jr. provided the hydroxyproline determinations, and R.A. Gonzalez aided the author with the analysis of carbohydrate. I thank L. Ellis, R. Chandross and R.S. Bear (Department of Anatomy, University of North Carolina) for allowing me to cite their unpublished x-ray diffraction analyses. The critical reviews by both J.F. Woessner, Jr. and D.G. Anderson (Department of Biochemistry, University of Miami) improved the manuscript and are greatly appreciated.

Literature Cited

Adams, F. and R. Dams: Compilation of precisely determined γ-transition energies of radionuclides produced by reactor irradiation. J. radioanalyt. Chem. *3*, 99-125 (1969)

Allen, R.N.: Philippine black coral. Philipp. J. Sci. *41*, 65-71 (1930)

Barzansky, B., H.M. Lenhoff and H. Bode: Hydra mesoglea: similarity of its amino acid and neutral sugar composition to that of vertebrate basal lamina. Comp. Biochem. Physiol. *50B*, 419-424 (1975)

Binnerts, W.I. and H.A. Das: Determination of iodine in biological material. Meth. biochem. Analysis *22*, 251-306 (1974)

Cook, F.C.: The chemical composition of some gorgonian corals. Am. J. Physiol. *12*, 95-98 (1904)

DeStefanis, V.A. and J.G. Ponte: Separation of sugars by thin-layer chromatography. J. Chromat. *34*, 116-120 (1968)

DuBois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith: Colorimetric method for determination of sugars and related substances. Analyt. Chem. *28*, 350-356 (1956)

Ellis, L., R. Chandross and R.S. Bear: Detection of chitin in antipatharian skeletons. (In preparation)

Folch, J., I. Ascoli, M. Lees, J.A. Meath and F. N. LeBaron: Preparation of lipid extracts from brain tissue. J. biol. Chem. *191*, 833-841 (1951)

Goldberg, W.M.: Comparative study of the chemistry and structure of gorgonian and antipatharian coral skeletons. Mar. Biol. *35*, 253-267 (1976)

\- Radioiodine as an indicator of skeletogenesis in gorgonian and antipatharian corals. Proc. int. Symp. coral Reefs *1*, 511-516 (1977). (Third; ed. by D.L. Taylor. Miami: School of Marine and Atmospheric Sciences, University of Miami)

Gross, J., B. Dumsha and N. Glazer: Comparative biochemistry of collagen — some amino acids and carbohydrates. Biochim. biophys. Acta *30*, 293-297 (1958)

Hunt, S.: The gastropod operculum: a comparative study of the composition of gastropod opercular proteins. J. mollusc. Stud. *42*, 25-260 (1976)

\- and S.W. Breuer: Chlorinated and brominated tyrosine residues in molluscan scleroprotein. Trans. biochem. Soc. *1*, 215-216 (1973)

Katzman, R.L. and R.W. Jeanloz: The carbohydrate chemistry of invertebrate connective tissue. *In*: Chemistry and molecular biology of the intercellular matrix, Vol. 1. pp 217-227. Ed. by E.A. Balazs. New York: Academic Press 1970

\- and A.L. Oronsky: Evidence for a covalent linkage between heteropolysaccharide and an hydroxyproline-containing peptide from *Metridium dianthus* connective tissue. J. biol. Chem. *246*, 5107-5112 (1971)

Kirchner, J.G.: Thin-layer chromatography. *In*: Technique of organic chemistry, pp 1-788. Ed. by E.S. Perry and A. Weisberger. New York: Wiley Interscience 1967

Lederer, L.M., J.M. Hollander and I. Pearlman: Table of isotopes, 594 pp. New York: Wiley & Sons 1967

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall: Protein measurement with the Folin phenol reagent. J. biol. Chem. *193*, 265-275 (1951)

Mendel, L.B.: On the occurrence of iodine in corals. Am. J. Physiol. *4*, 243-246 (1900)

Mörner, C.T.: Zur Kenntnis der organischen Gerüstsubstanz des Anthozoenskeletts. I. Mitteilung. Hoppe-Seyler's Z. physiol. Chem. *51*, 33-63 (1907)

\- Zur Kenntnis der organischen Gerüstsubstanz des Anthozoenskeletts. II. Mitteilung. Hoppe-Seyler's Z. physiol. Chem. *55*, 77-83 (1908)

Neumann, H.: Untersuchungen über die Bildung des Achsenskelettes einiger Gorgonaceen. Jena. Z. Med. Naturw. *47*, 497-528 (1911)

Pryor, M.G.M.: Sclerotization. *In*: Comparative biochemistry, Vol. 4. pp 371-396. Ed. by M. Florkin and H.S. Mason. New York: Academic Press 1962

Roche, J.: Biochimie comparée des scléroprotéines iodées des anthozoaires et des spongiaires. Experientia *8*, 45-54 (1952)

\- On some aspects of iodine biochemistry in marine animals. Pubbl. Staz. zool. Napoli *31* (Suppl.), 176-189 (1959)

-, M. Fontaine and J. Leloup: Halides. *In*: Comparative biochemistry, Vol. 5. pp 493-548. Ed. by M. Florkin and H.S. Mason. New York: Academic Press 1963

Rondle, C.J.M. and W.T.J. Morgan: The determination of glucosamine and galactosamine. Biochem. J. *61*, 586-589 (1955)

Savoie, J.C., P. Thomopoulos and F. Savoie: Studies on mono- and diiodohistidine. I. The identification of iodohistidines from thyroidal iodoproteins and their peripheral metabolism in the normal man and rat. J. clin. Invest. *52*, 106-115 (1973)

Sugimoto, K.: Iodine in gorgonian corals. J. biol. Chem. *76*, 723-728 (1928)

Szmant-Froelich, A.: Structure, iodination and growth of the axial skeletons of *Muricea californica* and *M. fruticosa* (Coelenterata: Gorgonacea). Mar. Biol. *27*, 299-306 (1974)

Warren, L.: The thiobarbituric acid assay of sialic acids. J. biol. Chem. *234*, 1972-1975 (1959)

Welinder, B.S.: Halogenated tyrosines from the cuticle of *Limulus polyphemus* (L.). Biochem. biophys. Acta *279*, 491-497 (1972)

Woessner, J.F.: The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. Archs Biochem. Biophys. *93*, 440-447 (1961)

Dr. Walter M. Goldberg
Department of Biological Sciences
Florida International University
Miami, Florida 33199
USA

Date of final manuscript acceptance: June 30, 1978. Communicated by J.M. Lawrence, Tampa