A BIOCHEMICAL STUDY OF THE MARINE ANNELID WORM, *THORACOPHELIA MUCRONATA*

Its Food, Biochromes and Carotenoid Metabolism [1, 2]

By

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INTRODUCTION

Comparative biochemical studies of numerous marine invertebrates and fishes have indicated that the majority of such species so far examined selectively assimilate and store xanthophyllic or allied oxygenated carotenoids rather than carotenes, when consuming food containing both types of pigment. In some forms there is complete exclusion of carotenes, *e. g.*, in several fishes, in the sea mussel (*Mytilus californianus*), in at least three brittle stars, and in some color-variants of the anemone *Metridium senile*. Some asteroid echinoderms store carotenes, but in far lower concentrations than xanthophylls, while four species of echinoids appear to assimilate relatively greater quantities of carotenes (Fox, Updegraff and Novelli, 1944).

The marine polychaete worm *Thoracophelia mucronata* represents a metabolic departure from the above groups, since, while consuming a basic diet of finely particulate marine detritus similar to that ingested by mussels, brittle stars and sand dollars, it apparently stores only the carotene type (Sumner and Fox, 1933; Fox, 1936) and seems to destroy much of the xanthophyllic material. This is in direct contrast to *Mytilus* (Scheer, 1940) and to the ophiuroids *Ophiopteris papillosa*, *Ophiothrix spiculata* and *O. rudis*, which store xanthophylls but not carotenes (Fox and Scheer, 1941). *Thoracophelia* also exhibits more complete xanthophyll exclusion than does the sand dollar, *Dendraster*

[1] Contributions from the Scripps Institution of Oceanography, New Series, No. 378.

[2] This research was aided in part by a grant from the Penrose Fund of the American Philosophical Society and more recently by a grant from The Rockefeller Foundation. To the officers of each Institution grateful acknowledgment is made.

[3] The authors take pleasure in acknowledging the technical assistance of Mr. Arthur S. Lockley, and helpful information from Professor Wesley R. Coe.

excentricus, for example, whose body carotenoids are some 24% xanthophylls. Other echinoids likewise exhibit a comparable preponderance of carotenes (Fox and Scheer, 1941).

Reference should be made also to the sponges. For while these must ingest much organic detritus, certain of them further resemble *Thoracophelia* in assimilating carotenes with relative exclusion of xanthophylls. *Suberites domuncula* and *Ficulina ficus* seem to yield no acidogenic carotenoids, questionable amounts of xanthophylls and large quantities of persistently epiphasic carotenoids with spectra resembling those of torulene, lycopene (*in Suberites*), α-, β-, and γ-carotenes (Lederer, 1938). The red sponge *Hymeniacidon sanguineum* was found to contain α-, β-, and γ-carotenes, as well as the ketonic carotenoid echinenone, more commonly encountered in sea urchins (Drum and O'Connor, 1940; Drumm, O'Connor and Renouf, 1945).

Because of its vast numbers, *Thoracophelia mucronata* is an undoubted factor in the physical and chemical turnover of organic matter on local sandy shores, in a manner somewhat analogous to the function of earthworms in terrestrial soils. Furthermore, this species has long served, and is still employed, as an important and economical source of food for experimental marine animals, including some collections maintained upon diets designed to provide carotene. Finally, the species constitutes an interesting case of an unusual biochemical ability—that of absorbing only carotene, with seemingly complete exclusion of xanthophylls. For the above reasons, and with improved methods and equipment available, it was decided to reinvestigate the pigments of *Thoracophelia* and their metabolism in a more searching manner than was previously possible.

GENERAL DESCRIPTION AND HABITAT

Thoracophelia mucronata is a blood-red worm of some 0.5 to 2.0 mm. in diameter and from about 30 to 50 mm. or more in length when mature. Sexes are separate. Adults examined in May, July and September contained ripe eggs and sperm. The animals live buried in intertidal sand, where they may be dug in large and concentrated numbers from a fraction of an inch to a foot or more beneath the sand surface during low or medium tides. The worms swallow much sand, readily seen to constitute the gut-contents and feces of freshly collected specimens. When collected in their wet sand and placed on damp paper or in sea water, the worms crawl out of the sand in a few hours, clearing their bodies externally and partially internally of sand and other detritus, thus preparing themselves for analysis.

Sand grains in the gut of the worm and in its feces do not differ

significantly in size or appearance from the grains of the general environment; minute algal cells and detrital matter may be observed on the surface of such grains. The worms' biology, food and migrations within the sand constitute the subject of a separate research project.

The bright red and purplish colors of the worms are due to the presence of hemoglobin, which is not enclosed in erythrocytes, but is free in colloidal solution. The shed blood of this species was not observed to clot on exposure to air. Oxyhemoglobin was identified by its two characteristic sharp absorption bands, centering at 576 and 540 mμ respectively. Reduction with sodium hydrosulphite gave the single broad band of hemoglobin at 565 mμ (Hartridge reversion spectroscope). A strong positive test for hemoglobin was obtained with the benzidine reaction.

CAROTENOIDS AND CHROMOLIPOIDS

Methods. The worms were usually permitted the minimum interval of an overnight period in which to emerge and clear their bodies largely of sand and detritus. And, since we wished to minimize both incomplete digestive changes and aberrant post-mortem bacterial operations, final procedures sometimes involved the tedious step of hand-picking the emerged survivors. After blotting on towels, masses of the animals were weighted and comminuted to a fine mush under acetone in a blender.

Carotenoids were analyzed in accordance with regular methods applied to animal tissues (cf. Fox and Pantin, 1941). Exhaustive acetone extracts of the ground tissues were pooled, transferred to petroleum ether by dilution and extraction, then partitioned into epiphasic fractions (remaining in the petroleum ether phase) and hypophasic portions (migrating into the 90% methanol layer below). Epiphasic fractions were treated separately with warm alcoholic NaOH and subsequently repartitioned. Petroleum ether solutions of the final epiphasic and hypophasic pigments were separately washed, dried with solid NaCl and chromatographed. Samples of whole pigment extracts or of various separated fractions were examined spectroscopically in CS_2 or in "Skellysolve B," a refined hydrocarbon boiling between 60° and 70° C.

Skellysolve solutions of the pigments were chromatographed on Tswett columns consisting of MgO mixed with Hyflo Supercel (2 : 1 by weight) overlaid by similar columns of $CaCO_3$—Supercel mixtures of like proportions. The only colored zones from either epiphasic or hypophasic pigments were formed in the MgO portions of such columns.

Carotenoid material was measured in mg. per 100 g. of sand (dry

basis) or worms (wet), with the use of a Bausch and Lomb spectrophotometer, the absorption of light at 485 mμ by CS_2 solutions of pigment giving values in β-carotene equivalents. It is to be remembered, however, that recorded determinations may depart from true values, since we were dealing with lipoidally contaminated extracts rather than with solutions of pure carotenoids.

Results. Samples of sand, containing none of the worms, but from the same environment, were analyzed for pigments. The first 3.5 kg. sample yielded approximately 0.06 mg. of collective xanthophylls (in "lutein" units) per 100 g. of sand on the dry basis. The xanthophylls were accompanied by unmeasured amounts of carotenes, probable chlorophylls and greenish degradation products of the latter. A second sample of sand from the environment of a worm colony was given more thorough study. It weighed 4.1 kg. when dried, and contained 0.70% of organic matter (by combustion). Extracts of the fresh, undried sand yielded the following approximate quantities of biochromes, separated by partition and chromatography:

Epiphase	*In whole sand (mg. per 100 g.)*	*In organic matter of sand (mg. per 100 g.)*
"Chlorophyll"	0.041	5.85
"Phaeophytin"	0.016	2.30
β-carotene	0.002	0.28
α-carotene	0.001	0.14
Hypophase		
Green breakdown products (in "chlorophyll" units)	0.025	3.60
Total carotenoids (*i. e.*, five xanthophyllic fractions, preponderantly fucoxanthins, with chief maxima at 508–510 and 477 mμ in CS_2)	0.064	9.04

It is observed that β-carotene occurred in twice the concentration of the α-isomer, and that collective xanthophylls constituted 95.5% of the total carotenoids.

While actual amounts and ratios of the pigments in the sand vary with locality, depth and season, this general condition is characteristic of fresh, mixed marine detritus, and lends emphasis to the biochemically selective behavior of the worm, which selectively stores the β-carotene, eliminates the α-carotene, and apparently destroys the xanthophylls at least in part.

TABLE I. Carotenoid and Chromolipoid Materials in *Thoracophelia mucronata*

Sample	History	Weight (g.)	Total extract (mg. %)	Total epiphasic material (mg. %)	Total xanthophyllic material (mg. %)	Carotene (from hydrolysate) (mg. %)
I	Recently collected, but some dead included. Crude extract hydrolyzed.	218.7	——	——	0.01 (with chromolipoids)	0.15
II	Recently collected but some dead included.	75.5	0.30	0.24	0.01 (with chromolipoids)	0.215
III	Blood only, from freshly collected worms.	12 ml.	0.11	——	none (only chromolipoids)	0.04
IV	Collected previous day. Only live worms used.	75.5	0.25	0.18	none (only chromolipoids)	0.11
V	Starved one week in clean gravel and running, filtered sea water at 20° C.; only surviving worms used.	35.6	0.315	0.24	0.015 (with chromolipoids)	0.12
VI	Collected, washed and immersed in acetone within 3 hours.*	57.9	0.50	0.36*	0.01 (with chromolipoids)	0.24

* This extract contained some α-carotene (*i. e.*, about 13% of the total carotenes, the other 87% being β-carotene) due to the presence of undigested food residues in the gut, since the animals were purposely analyzed while the gut was still filled with sand.

That extracellular chlorophylls and xanthophylls are largely or completely decomposed in the gut of the worm was indicated first by analyzing fresh worms within three hours of collecting them, when their alimentary tracts still contained considerable sand, and second by a preliminary analysis of about 11 g. of sand voided by previously washed and segregated worms.

It will be noted (Table I, Sample VI) that there was an increase in the average amount of total extracted yellow pigment and of β-carotene itself in the fresh worms. Furthermore, traces of α-carotene were encountered, doubtless derived from the intestinal contents of the fresh sample. But no significant quantities of xanthophylls were observed.

The concentrated extract of the fecal sand was yellow, rather than green, in contrast to extracts of the fresh sand. On a chromatogram it yielded small amounts of chlorophyll-degradation products, carotene in a concentration of the same order of magnitude as that encountered in fresh sand (*i. e.*, 0.004 mg. per 100 g. sand) and, on the $CaCO_3$ portion of the column, no xanthophylls, but some yellow pigment which may have been derived from xanthophyll. It was hypophasic, but gave no positive tests for carotenoid material either with $SbCl_3$ or with concentrated H_2SO_4; furthermore, its absorption spectrum in CS_2 (Beckman instrument) showed maxima displaced farther toward the violet than those of fucoxanthin, their centers lying at 441 and 474 mμ, with a slight inflection at 498 to 499 mμ. While it would thus seem that fucoxanthins were destroyed in the gut of the worm, the bare possibility has not been excluded that such carotenoids may be desorbed from the sand (while β-carotene is not) and discharged in fluid feces.

The worms' bodies yielded yellow pigments which were chiefly epiphasic. The only consistently encountered carotenoid was a persistently epiphasic, alkali-resisting carotene whose absorption maxima were found to agree with those of β-carotene from plant sources, *i. e.*, 427–430, 450, and 476 mμ in Skellysolve (Fig. 1). The identity of the *Thoracophelia* pigments fraction with β-carotene was further confirmed by the results of the three-tube mixed chromatogram method: (1) β-carotene; (2) worm carotene; (3) a mixture of these two; in each of the three Tswett columns of MgO there appeared a single, identical orange zone of adsorbed pigment. Traces of more readily adsorbed pigments usually appeared on primary chromatograms of the persistently epiphasic fraction. Such material probably represented artifactual isomers, colored degradation products or other contaminants. But no α-carotene, which accompanies the β-isomer in this and all other marine sediments and commonly appears as a yellow zone be-

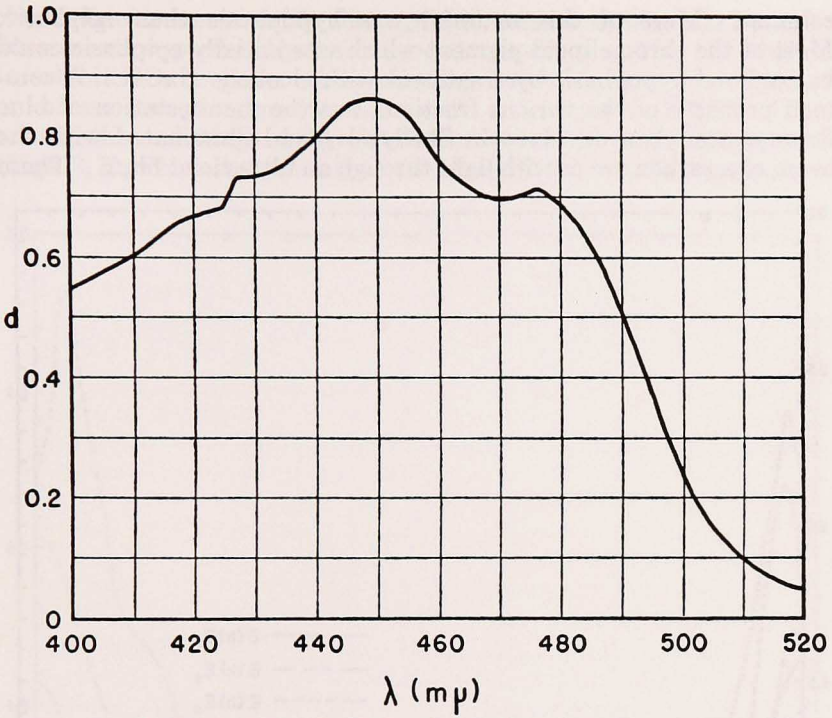

Figure 1. Absorption curve (Beckman) of β-carotene from *Thoracophelia*. Solvent Skellysolve B.

neath the orange band of the latter, was ever detected in extracts of the worm, save in traces when extracts were prepared from the fresh, actively feeding animals (Table I, Sample VI). The worm's assimilation of β-carotene, while excluding or destroying not only xanthophylls but even the isomeric α-carotene, all of which occur in the natural diet (Fox, *et al.*, 1944), seems remarkable. Quantitative spectrophotometric estimations of carotene in the worms' tissues are summarized in Table I.

The total yellow pigment values of 0.30, 0.25, 0.315, and 0.50 mg. %, measured as β-carotene equivalents on the raw extracts, agree rather well with that of 0.38 mg. % found earlier by a less refined, nonspectroscopic photometric method (Sumner and Fox, 1933).

Both epiphasic and hypophasic fractions yielded small amounts of yellow noncarotenoids which exhibited properties characteristic of the so-called chromolipoids or lipofuscines. Such pigments passed unadsorbed through $CaCO_3$, but could be chromatographed on MgO

columns. More of this material was hypophasic than epiphasic. Most of the chromolipoid pigment which was initially epiphasic could be rendered hypophasic by treatment with alcoholic NaOH. A common property of the various fractions was the manifestation of blue fluorescence when dissolved in Skellysolve and illuminated with the beam of a carbon arc or with light through an ultraviolet filter. These

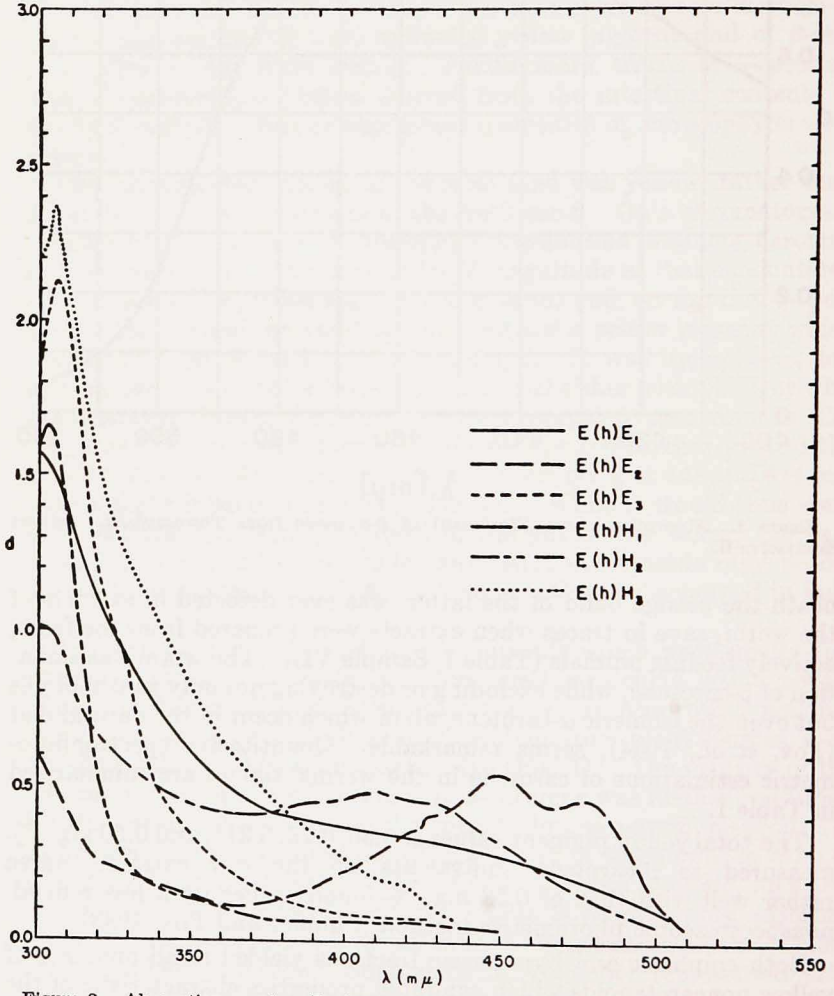

Figure 2. Absorption spectra (Beckman) of the persistently and initially epiphasic pigment (β-carotene and chromolipoids) from *Thoracophelia*. Solvent: Skellysolve B. Lettered fractions as in Table II.

chromolipoids were readily distinguishable from carotenoids by their fluorescent properties, by their absorption spectra, and by their failure to exhibit blue or green colors, showing instead brown, brown-yellow or brown-orange when treated with concentrated H_2SO_4. Chief among these distinguishing characters were the absorption spectra (Figs. 2, 3), which showed only slight absorption in the blue-green, blue

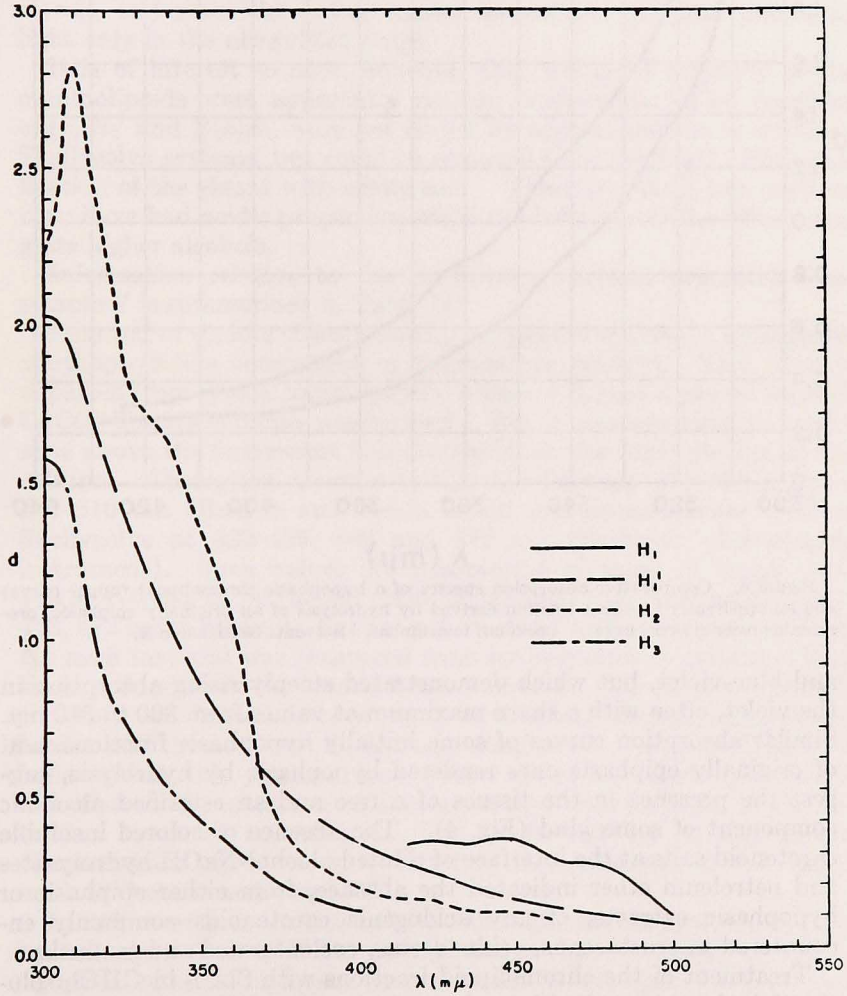

Figure 3. Absorption spectra (Beckman) of the hypophasic chromolipoids of *Thoracophelia*. Solvent: Skellysolve B. Lettered fractions as in Table II.

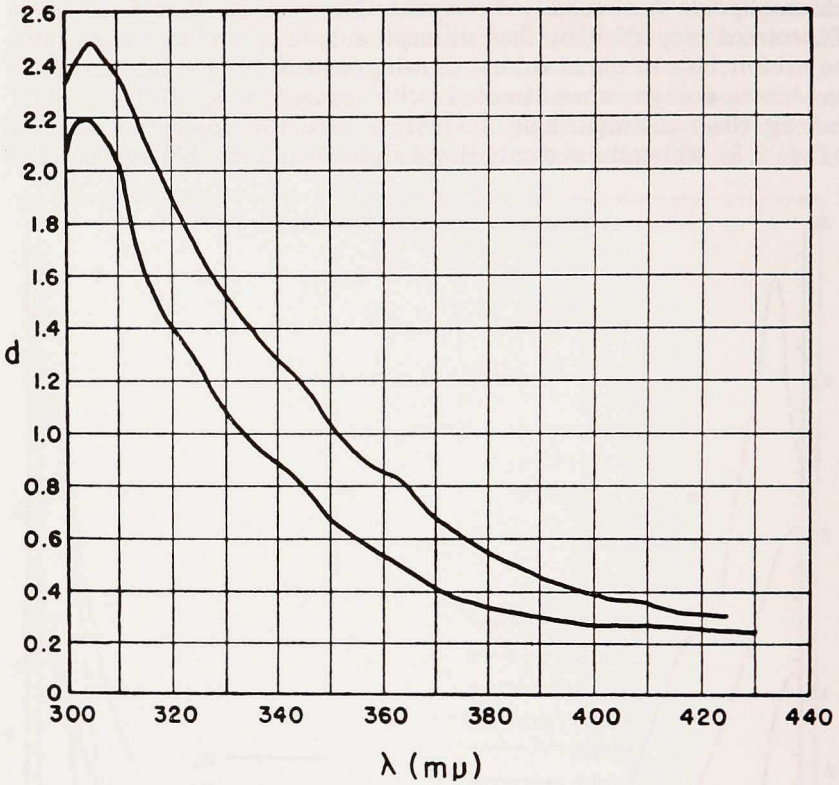

Figure 4. Comparative absorption spectra of a hypophasic chromolipoid (upper curve) and an apparently identical fraction derived by hydrolysis of an originally epiphasic, presumable ester (lower curve). Beckman instrument. Solvent: Skellysolve B.

and blue-violet, but which demonstrated steeply rising absorption in the violet, often with a sharp maximum at values from 300 to 310 mμ. Similar absorption curves of some initially hypophasic fractions, and of originally epiphasic ones rendered hypophasic by hydrolysis, suggest the presence in the tissues of a free and an esterified alcoholic component of some kind (Fig. 4). The absence of colored insoluble carotenoid salts at the interface of diluted alcohol-NaOH hydrolysates and petroleum ether indicated the absence, from either epiphasic or hypophasic extracts, of any acidogenic carotenoids commonly encountered in crustaceans, echinoderms, coelenterates and mollusks.

Treatment of the chromolipoid fractions with $SbCl_3$ in $CHCl_3$ solution failed to show the blue colors which characterize Vitamin A and numerous colored carotenoids, but resulted instead in pink to mauve

colors. The absorption spectra, color of fluorescence, and hypophasic behavior of our chromolipoid fractions eliminate any identity with phytofluene, the widely occurring plant polyene hydrocarbon described by Zechmeister and Sandoval (1946). While the Liebermann-Burchard reaction demonstrated the presence of sterols in a number of the fractions, it is not believed that such compounds were responsible for the yellow color, since some yellow fractions seemed to contain no sterols, and since the better known sterols are colorless, absorbing light only in the ultraviolet range.

It is of interest to note, however, that the great majority of the chromolipoids were apparently neutral compounds. Two fractions only, H_1' and $E(h)E_1$, were not eluted by neutral acetone or acetone-Skellysolve systems, but could be removed from the MgO after acidification of the eluant with acetic acid. Therefore, these two portions may have had acidic properties, while the behavior of the others suggests higher alcohols.

Information relative to the lipofuscine fractions separated from sample V is summarized in Table II.

Four out of six lots of worms analyzed yielded traces of a hypophasic xanthophyll-like component or degradation product. This fraction departed from typical xanthophyllic behavior in that it passed through $CaCO_3$ Tswett columns unadsorbed. But it was adsorbed as a pink zone above the fluorescent noncarotenoid on the MgO portion of the column. Absorption maxima in CS_2 lay between 475–485 mμ and 505–510 mμ (Bausch and Lomb visual spectrophotometer) and in Skellysolve at 422–425, 446 and 472 mμ (Beckman photoelectric instrument). Such values are reminiscent of those of lutein (420, 446.5, 476 mμ in petroleum ether or ethanol) and taraxanthin (417, 443, 472 mμ in ethanol), but are not identical with either set of values. No such material was recovered from hydrolysates of epiphasic fractions. Because of its inconsistent occurrence, the minute traces in which it was found when present, its atypical chromatographic behavior and the fact that no corresponding esters were ever found, we believe that this pigment may reflect either the decomposition of carotene or the presence of undegenerated food-residues in the intestinal tract of the animal. The inadsorbability of any yellow *Thoracophelia* pigments on $CaCO_3$ had been observed in the earlier work of Sumner and Fox (1935).

In its prevention of xanthophylls from entering the system, whether by not permitting diffusion through the intestinal mucosa or by destruction of such pigments in the lumen of the gut, *Thoracophelia* resembles some higher herbivores or omnivores rather than many of the lower forms. Cattle are known to absorb a part of the dietary

TABLE II. Physical and Chemical Properties of Chromatographic Pigment Fractions from *Thoracophelia*

Fraction (color on MgO column)	*Blue fluorescence*	*Absorption max.* ($m\mu$)	$SbCl_3$ *reaction*	*Liebermann-Burchard reaction for sterols*		*Acid treatment*	
				H_2SO_4 *layer*	$CHCl_3$ *layer*	H_2SO_4 *layer*	$CHCl_3$ *layer*
H_1 } (pink)	+	<300	pink	purple	green	....	....
H_1' }	+	ca. 300; 423; 446; 472	murky	brick-red	brown	....	....
H_2 (purplish-gray)	+	310 (sharp)	red-orange	purple	blue-green	....	....
H_3 (no colored zone)	+	ca. 300	pink (±)	faint purple	pale yellow	....	....
$E(h)E_1$ (orange)	+	<300	pale pink	purple	blue-green	....	....
$E(h)E_2$ (orange)	+ (impurity)	(427); 450; 477 (β-carotene)	blue	green or purple	colorless to blue	green	brown (impurities)
$E(h)E_3$ (diffuse yellow)	+	306 (sharp)	pink (±)	purple	brown	....	....
$E(h)H_1$ (reddish)	(+)	ca. 300; (390);	–	–	–	pale brown	bleached
$E(h)H_2$ (yellow)	(+)	(410); (428) 302.5 (sharp)	–	–	–	pale yellow-brown	bleached
$E(h)H_3$ (yellow filtrate)	(+)	305 (sharp)	pink	deep blue	green	orange	brown-orange

H = hypophasic pigment
E = epiphasic pigment
(h) = hydrolytic operation
$E(h)E$ = persistently epiphasic pigment
$E(h)H$ = originally epiphasic pigment rendered hypophasic by hydrolysis.
Subscript numbers refer to chromatographic position, beginning at top.

H_1' = fraction of H, elutable with acetic acid; similarly with $E(h)E_1$.
(+) = very faint positive
± = questionable
– = no reaction detected.

Maxima recorded in parentheses indicate very weak ones or points of inflection.

carotenes but very little xanthophyllic compounds (Palmer and Eckles, 1914; Goodwin, Dewar and Gregory, 1946). Sheep store mere traces of carotene and Vitamin A in their blood and other tissues (Palmer, 1916; Paulsen, Hilmoe and Moxon, 1944; Peirce, 1945) while excreting in their feces 70% of ingested carotenes and 80% of the xanthophylls consumed (Rogozinski, 1937). Vitamin A introduced by abdominal cannula into the digestive tract of sheep and goats produces a rise of Vitamin A levels in both systemic and portal blood, but colored carotenoids so introduced are not reflected in the blood in this way (Goodwin, *et al.*, 1946). It is believed that sheep must destroy xanthophylls in the digestive tract (Rogozinski, 1937). The same view is held regarding the horse, which assimilates only carotenes from green food, but excretes 70–80% of the dietary carotenes and 60% of the xanthophylls ingested. Since none of the latter are assimilated as such, they must be partially destroyed in the gut (Zechmeister and Tuzson, 1934; Zechmeister, Tuzson and Ernst, 1935). Hove (1943) found that water-extracts of minced rat stomachs destroyed carotene rapidly in vitro, in the presence of methyl linolate. He suggests the possible presence of carotene oxidase or lipoxidases. Sherman (1947) has shown that gastro-intestinal destruction of dietary carotene in the rat may be decreased by the administration of lutein. He suggests that the effect of the lutein may be due to its preferential destruction by a nonspecific enzyme which may be responsible for the degradation of carotenoids generally. The cod, *Gadus callarias*, and the lobster, *Homarus americanus*, are believed to slowly convert carotene into Vitamin A, as do mammals and birds (Neilands, 1947; Mattson, *et al.*, 1947; Wiese, *et al.*, 1947).

No destruction of xanthophylls from sand were demonstrable in preliminary experiments involving the incubation of such material (adsorbed in $CaCO_3$) with pastes of ground *Thoracophelia*. Partially starved worms (Table I, Sample V) exhibited no significant changes in carotene content, but did show slightly elevated quantities of the yellow noncarotenoid chromolipoid material (absorption at 305 mμ about 3 : 1).

In its assimilation of β-carotene without the accompanying α-isomer, *Thoracophelia* seems to be unique. This finding is contrary to an earlier provisional conclusion that the carotene in this species was preponderantly the α-isomer (Fox, 1936).

The reinvestigation of carotenoids in *Thoracophelia* lends emphasis to a provisional conclusion reached earlier by Sumner and Fox (1935) regarding the probable conversion of carotene into xanthophyll by the Pacific killifish, *Fundulus parvipinnis*, which stores only esterified members of the latter class. Fishes maintained for three months upon

carotenoid-free food neither lost nor gained xanthophyll, while parallel groups fed upon xanthophyll-rich flesh, and those kept on a diet of *Thoracophelia* underwent increases in total amounts of stored xanthophyll. Chemical examinations made at the time of those experiments and subsequently (Fox, 1936) revealed none of the yellow noncarotenoid chromolipoids of *Thoracophelia* in the fishes which ate the worms, since hydrolyzed extracts of the fish pigments were quantitatively adsorbed upon $CaCO_3$ columns and exhibited well defined absorption spectra.

Nor could the increase of xanthophyll stored by the worm-fed fishes have been logically assigned to the traces of "xanthophyll" sometimes encountered in the worms, for the average amount of residual "xanthophyll" revealed by the present survey is, according to Table I, about 0.031 mg. from the collective 475.2 g. of worms, or some 0.006 mg. % on the average. Sumner and Fox (1935) fed their worm-eating lot of *Fundulus* about 2000 g. of this food, corresponding to roughly 0.12 mg. of "xanthophylls" (and 6 mg. of carotene) over the three month experimental period. During that interval the growing consumers, while undergoing a decrease in *concentration* of stored xanthophylls, nevertheless achieved an actual collective augmentation of approximately 0.20 mg. of this class of carotenoid. Even quantitative absorption of all available dietary xanthophyll (which is not known to occur in any animal, and certainly was not achieved by the xanthophyll-fed lot in the experiment cited) would have fallen 40% short of supplying the observed increment. Since synthesis of xanthophylls from colorless precursors did not appear to take place in the fishes receiving the carotenoid-free diet of halibut flesh, we are still left with the conclusion that *Fundulus* must be able to convert nonxanthophyllic into xanthophyllic carotenoids. Furthermore, the only carotenoid which the fishes could have received from *Thoracophelia* was β-carotene.

COMPOSITION, FOOD AND METABOLISM

The finding in the worm's gut of little else of a microscopically visible nature save sand grains led to a consideration of the basic food of the animal.

The gut wall consists of deep and multiple longitudinal folds throughout its anterior portion and is provided throughout its length with rapidly sweeping cilia upon its inner surfaces. Peristalsis is marked. These factors, added to the rich blood supply in the intestinal walls, provide for intimate contact with the ingested sand grains in the region of digestion and absorption.

Beyond question, the organic material present in the sand exists

largely as adsorbed colloidal micelles and other fine particles. This adsorptive property of sand, which had previously been chemically cleaned, was demonstrated in the laboratory by gentle agitation with colloidal material prepared by the fine comminution of marine plants, followed by centrifugal removal of the coarser particles. Extraction of the treated and subsequently water-rinsed sand with acetone yielded green and yellow plant pigments. Egg albumin, the colloidal dye congo red, and the crystalloid organic dyes night blue, methyl violet and methylene blue were also observed to be readily and firmly adsorbed to sand. Congo red was adsorbed more readily to raw, untreated sand, wherefrom it could be removed incompletely and with difficulty by vigorous shaking, while the other dyes became adsorbed in greater quantities to the previously cleaned sand. These observations suggested that the marine organic colloids already adsorbed upon the raw sand must inhibit somewhat the additional adsorption of crystalloidal dyes, while increasing that of the electrically charged colloid dye congo red.

Use was made of the firm adsorbability of the nontoxic dye, methylene blue, upon sand to demonstrate the approximate rate of sand-ingestion by the worms. The dyed sand was washed with sea water until no further dye was thus desorbed and washed out, but still leaving the sand a deep blue color. Freshly collected *Thoracophelia* were then buried in the damp blue sand contained in earthenware plant-pots, standing partially immersed in sea water. Subsequent examination disclosed that numerous specimens had evacuated the raw sand from the gut and had replaced this with the dyed sand within 15-minute intervals. The dyed sand was in turn readily replaced by normal sand when the worms were given the opportunity. They seem to swallow either substrate equally readily.

The proportions of water, ash and total organic matter in fresh worms were determined, as well as the relative quantities of sand which had been ingested (all as listed in Table III). The values for sand were determined by treatment of the ash with dilute HCl, followed by reweighing the dried insoluble residue. The results obtained are doubtless conservative, since carbonates and iron sulfide are soluble in dilute acids and therefore would have been removed by the treatment accorded the ash.

Some rough calculations may be useful in suggesting the quantities of sand and organic matter adsorbed thereto, which the worms may ingest and utilize in order to build up or maintain the organic material of their tissues.

TABLE III. Composition of Fresh Worms Corrected for Quantities of Ingested Sand (Silica)

	Sample 1	*Sample 2*	*Average*
Wet weight, g	40.97	37.33	.....
Dry matter, *corrected for silica*, %	19.82	19.70	19.76
Water (by difference), %	80.18	80.30	80.24
Ash, *corrected for silica*, %	2.02	2.02	2.02
Organic matter (by difference), %	17.80	17.68	17.74
Silica in gut (g. per 100 g. of fresh worm tissue)	5.84	5.29	5.56

The whole worms contain close to 18% organic matter and swallow some 6% of their own weight of sand. This sand may contain about 1% of organic matter, *i. e.*, by taking a rough average between our figure of 0.7% and the value of 0.1% of organic nitrogen (given by Revelle and Shepard, 1939), the latter value being multiplied by a factor of 14 to 18 to give the total organic matter (Trask, 1939). Thus, if 100 g. of the worms fill their alimentary tracts each quarter hour with 6 g. of sand, this represents a cycle of more than 210 kg. of sand per annum, or of somewhat more than 2000 g. of organic matter adsorbed to the sand. Grown specimens weigh about 40 mg. each and therefore ingest on the average some 9 mg. of sand per hour, 0.23 g. per day, or about 84 g. per year. Adult animals would appear to operate at a biochemical efficiency of 18/2000 x 100 or 0.9% during a year's time. And it is worth re-emphasizing that the worm, while assimilating none of the relatively plentiful xanthophylls or α-carotene from the organic matter in the sand, contrives to concentrate the β-carotene by some five-fold, if we take 0.28 mg. of β-carotene as its concentration in the organic matter of the sand and 100/18 x 0.25 or 1.4 mg. as its approximate concentration in the worm's organic matter.

Some approximate values are given in tabular form below, to indicate the relative magnitude of local operations of the beach-worm. Populations are based upon counts of samples, and values for the density of sand, the rate of its ingestion, etc., are based upon measurements of samples.

Volume of damp sand in worm-bed, 1 mile long, 10 ft. wide, 1 ft. thick ..... 5.28×10^4 ft.^{3}
Weight of damp sand in this volume (density ca 131 lbs/ft.^{3}) ..... 3.46×10^3 tons
Weight of actual sand (subtracting ca 18.5% water) ..... 2.82×10^3 tons
Total number of worms in worm-bed (ca 3×10^3/ft.^{3}) ..... 1.58×10^8
Total weight of worms in worm-bed (ca 0.04 g. each) ..... 7.0 tons
Weight of sand cycled annually by worms (ca 84 g. each) ..... 1.46×10^4 tons

Ratio of sand cycled annually by worms to sand in worm-bed $\frac{1.46 \times 10^4}{2.82 \times 10^3} = 5.2$

(equivalent to all the sand in the worm-bed about once every 10 weeks)

Organic matter in sand cycled annually by worms (ca 1%).....1.46×10^2 tons
Organic material in worms in the colony (ca 20%).....1.4 tons
Relative efficiency of worms in maintaining a standing population, assuming an annual life-cycle.....$\frac{1.4 \times 10^2}{1.46 \times 10^2} = 0.96\%$

Quantity of carotene in sand of worm-bed (ca 2.8×10^{-4}%)..... 16 lbs.
Quantity of carotene in worms of the colony (ca 2.5×10^{-4}%)..... 0.035 lbs.
Relative efficiency of carotene assimilation, assuming an annual life cycle.....$\frac{0.035 \times 10^2}{16 \times 5.2} = 0.042\%$

Volume of sand in wedge of beach, 1 mi. long, 200 ft. wide, 2.5 ft. high.....1.32×10^6 ft.^{3}
Weight of dry sand in wedge.....7.05×10^4 tons

Fraction of sand on beach cycled annually by worms.....$\frac{1.46 \times 10^4 \times 10^2}{7.05 \times 10^4} = 21\%$

SUMMARY

1. The blood-red, hemoglobin-containing, sand-eating marine polychaete worm, *Thoracophelia mucronata*, carries in its blood and other tissues appreciable quantities of β-carotene.

2. No α-carotene or other carotenoids could be detected in the worms' tissues, save for inconsistent traces of an unesterified, hypophasic, xanthophyll-like pigment or colored degradation product which was unadsorbed by $CaCO_3$.

3. Small quantities of yellow, noncarotenoid blue-fluorescent chromolipoids are extractable. Some of these are hypophasic initially, while others are rendered so by hydrolysis. These chromolipoids exhibit low light-absorption in the green, blue and blue-violet regions, but single sharp maxima in the ultraviolet at 310, 306, 305, 302.5, 300 mμ or below.

4. Although no carotenoid oxidase was demonstrated, it is suspected that the remarkable exclusion of xanthophylls from its system by *Thoracophelia* may be allied to the presence of lipoxidases, which must participate in the genesis of the animal's yellow chromolipoids.

5. *Thoracophelia* probably derives its subsistence from the colloidal organic matter adsorbed to the sand. Consuming some 24% of their own weight of sand per hour, 100 g. of the animals must ingest some 210 kg. of sand annually, representing about 2000 g. of organic matter. Since each adult worm weighs about 40 mg., this is equivalent to about 84 g. of sand (containing .84 g. organic matter) per worm per year.

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