Adaptations to Sulfide in Sulfide-System Meiofauna. Endproducts of Sulfide Detoxification in Three Turbellarians and a Gastrotrich

E. N. Powell*, M. A. Crenshaw and R. M. Rieger

Curriculum in Marine Science, Dental Research Center, Department of Zoology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514, USA

ABSTRACT: End-products of sulfide detoxification have been compared in three turbellarians and a gastrotrich typical of lenitic beaches with a well-developed sulfide system. Oxidation products were correlated with the oxygen concentration in the animal's habitat indicating that adaptation to life in the sulfide system involves reduction of dependency on molecular oxygen in metabolism. Archiloa wilsoni, a surface dwelling turbellarian, made sulfite and sulfate, which are rich in oxygen. Of the three sulfide-system species examined, the primary end-product in two (the turbellarian Pseudohaplogonaria sp. and the gastrotrich Dolichodasys carolinensis) was elemental sulfur which lacks oxygen. The third species (the turbellarian Solenofilomorpha funilis) made thiosulfate, a compound with a high S : O ratio, plus sulfate and sulfite. The presence of an important pathway of sulfide oxidation with elemental sulfur as the major end-product was heretofore unknown in the animal kingdom. Its presence in two phyla, Platyhelminthes and Gastrotricha, however, indicates that it is widespread in the lower invertebrates. Similarly, the formation of thiosulfate as a major product of sulfide oxidation has not been reported previously in the invertebrates. The discoveries of two new pathways for sulfide detoxification and of major differences in sulfide metabolism within a single taxon, the turbellarian order Acoela, indicate that invertebrates have a variety of detoxification mechanisms based on sulfide oxidation and that a substantial degree of plasticity in the detoxification method can be expected even between closely related organisms. The possibility that the observed sulfide oxidation is by symbiotic prokaryotes, rather than the animals themselves, is discussed.

INTRODUCTION

The interstitial fauna of most sandy marine sediments exhibits a vertical zonation pattern that is correlated with the redox potential of the sediments. Two distinct communities exist: one typical of the oxidized surface sediments and the second, termed the sulfide system by Fenchel and Riedl (1970), typical of the deeper reduced sediments (Fenchel and Riedl, 1970; Boaden and Platt, 1971; Ott, 1972; Crezee, 1976). The presence of these deeper-living metazoans is unexpected since their environment is characterized by both the absence of oxygen and the presence of hydrogen sulfide, either one of which has been considered a lethal condition for all free-living metazoa (Fenchel and Riedl, 1970).

Hydrogen sulfide is a metabolic poison (Evans, 1967), which is lethal at low concentrations (less than 1 ppm) to most vertebrates (Bonn and Follis, 1967; Colby and Smith, 1967; Smith et al., 1976) and invertebrates (Oseid and Smith, 1974a, b). To live in the sulfide system, an organism must withstand a longterm exposure to hydrogen sulfide at concentrations between 1 and 300 ppm (Berner, 1963; Fenchel and Riedl, 1970; Wharfe, 1977), which is well above these lethal limits. Powell et al. (1979) have shown that typical sulfide-system turbellarians and gastrotrichs incorporate less ^{35}S-sulfide during a 2-h exposure than their surface dwelling counterparts. Data from autoradiographs of a number of the sulfide-system turbellarians showed that much of the incorporated ^{35}S was present in the body wall and relatively little was found in the internal tissues. If the animals were exposed continuously to ^{35}S-sulfide, the incorporation pattern persisted. Nevertheless, the label in the body wall was not permanently bound to cellular components because, if the ^{35}S-sulfide was replaced with ^{32}S-sulfide (unlabelled), the label in the body wall was rapidly lost. Based on this evidence, Powell et al. (1979) proposed that a sulfide detoxification system traps and detoxifies sulfide in the body wall.

* Present address: Texas A & M University, Department of Oceanography, College Station, Texas 77843, USA.

The present study was undertaken to investigate the biochemical pathways by which sulfide is detoxified by these invertebrates. Four species have been investigated. One of these, *Archiloa wilsoni*, is an epifaunal proseriate turbellarian characteristic of the oxic surface layer of marine sediments where exposure to sulfide is occasional and short term (Ott and Machan, 1971; Jorgensen, 1977). The other three, the acoel turbellarians *Solenofilomorpha funilis* and *Pseudohaplogonaria* sp. and the gastrotrich *Dolichodasys carolinensis*, are representatives of the sulfide system where exposure to sulfide is continuous and where oxygen is scarce or completely absent. Powell et al. (1979) have already described the results of autoradiography using ^{35}S-sulfide for the three turbellarians and have concluded that all three possess a sulfide detoxification system in the body wall. The detoxification mechanism, if confirmed to be present in the animals themselves rather than, for example, symbiotic bacteria, would indicate that a number of metabolic pathways considered to be typical of bacteria are also found in the invertebrates.

MATERIALS AND METHODS

Hydrogen sulfide exists in seawater as three chemical species – H_2S, HS^-, and S^{2-} – depending on the pH (Goldhaber and Kaplan, 1974). The experiments were performed at a typical sediment pH of 7.3 (Farris, 1976). Under these conditions, significant quantities of H_2S and HS^- are expected (Goldhaber and Kaplan, 1974). For the purposes of this paper, no distinction is made and both are referred to simply as 'sulfide'.

The animals were collected from the White Oak River, North Carolina sandflat described by Farris (1976) and Crezee (1976) at Crezee's 40 m collection site. For the purposes of this study, the sedimentary environment has been divided into two zones, (a) the surface, oxic layer and (b) the sulfide system which includes both the redox potential discontinuity layer and the reduced zone beneath. The animals studied will be referred to as surface animals and sulfide-system animals. *Archiloa wilsoni*, the surface animal examined, is restricted to the upper 2 cm of the sediment and, in particular, is always above the redox potential discontinuity (Crezee, 1976). The population maxima for the three sulfide-system animals, *Solenofilomorpha funilis*, *Pseudohaplogonaria* sp., and *Dolichodasys carolinensis*, are usually found in or near the redox potential discontinuity. Furthermore, a substantial fraction of each population is usually found to extend well into the reduced zone (see Crezee, 1976 for *S. funilis* and *Pseudohaplogonaria* sp.; Fenchel and Riedl. 1970 Fig. 9 # 17 for *D. carolinensis*), whereas

few individuals are ever found in the upper 1–2 cm where *A. wilsoni* occurs. The vertical distributions published for these species are, for the most part, based on collection intervals of 1–2 cm. Unpublished data of E. Powell and R. Rieger indicate that important differences in vertical zonation occur in intervals of 0.5 cm or less. Furthermore, Reise and Ax (1979) have shown that infaunal tubes and burrows complicate the distributional picture considerably by introducing microoxic zones below the redox potential discontinuity. Therefore, the distributional data available is not sufficient to determine clearly whether the distributions of these three species is identical. The published data does indicate however that each belongs to the community of the sulfide system as described by Fenchel and Riedl (1970).

For the turbellarians, the names and designations are the same as used by Crezee (1976), although current investigations by R. Rieger indicate that *Pseudohaplogonaria* sp. is probably an undescribed genus close to *Haploposthia*. The identification of *Dolichodasys carolinensis* is from Ruppert and Shaw (1977).

Animals were always used within 2 weeks of collection (usually within 1 week) and were kept in the sand in which they were collected until used. After extraction by magnesium chloride decantation (Crezee, 1976), the animals were pipetted into a 3-ml plexiglass chamber with two openings, one of which was covered with a 32-μm nylon mesh to prevent the animals from being flushed out of the chamber during water exchanges. The chamber was flushed with 100 ml of nitrogen-bubbled seawater ([0$_2$] < 0.2 ppm) buffered to pH 7.3 with 10 mM Hepes buffer. 12 μCi^{35} S-sulfide ([S^{-2}] = 30 ppm) was added. At least 50 animals (usually over 100) were used in each experiment. At the end of 5 h, the animals were hardened briefly (about 30 s) in 10 % formalin so that they could be washed without damage. They were then washed well and placed into a homogenizer with about 0.2 ml 10 mM phosphate buffer (pH = 7.0) containing 0.2 % formaldehyde.

The homogenization conditions were chosen to preserve a number of commonly occurring, but reactive, sulfur species (i. e., SO_3^{2-}, $S_2O_3^{2-}$, S°). Reactions between S^O and $S_2O_3^{2-}$ are too slow to be important to this study (Roy and Trudinger, 1970). S^O and $S_2O_3^{2-}$ are relatively stable at pH 7.0 when SO_3^{2-} is not present. Sulfite was stabilized by adding formaldehyde, which reacts selectively with SO_3^{2-} to form formaldehyde bisulfite (Pollard et al., 1964). Thus, all three compounds were relatively stable in the above solution.

The animals were homogenized and the entire homogenate was immediately spotted onto Gelman SA thin layer chromatography paper (Kelly, 1970). The

solvents and conditions used for the various compounds were:

Solvent system	Compound separated
heptane (Banaszkiewicz, 1976)	S°
benzene : ethyl acetate (4:1) (v/v)	S°
toluene : n-propanol : methanol (2 : 2 : 1) (v/v)	polythionates, formaldehyde bisulfite, S°
toluene : methanol (2 : 1)	$S_2O_3^{2-}$, formaldehyde bisulfite, S°
toluene : methanol (1 : 1) twice	SO_4^{2-}, $S_2O_3^{2-}$, formaldehyde bisulfite, S°

Each compound, except sulfate, was separated in at least two different solvent systems to reconfirm each indentification. Standards were run with each experiment. The standards were localized by spraying with a mixture of silver nitrate and sodium fluoresceinate (Pollard et al., 1962). The experimental chromatograms were sliced into 1 cm sections and counted in a liquid scintillation counter using tritisol (Fricke, 1975) as the scintillation cocktail.

RESULTS

The results of the thin-layer chromatography experiments are shown in Table 1. For each species, the data obtained using toluene : methanol (1:1) as the solvent system, which alone separated all four compounds identified, are given in full to indicate the percentage of cpm present in all the oxidation products resolved relative to those still remaining at the origin and, therefore, unidentified and to show that all significant compounds moved from the origin were identified. The other solvent systems, which were used to reconfirm these identifications, failed to move one or more of the four identified compounds from the origin and also failed to resolve any additional compounds. Thus, data for each of these runs is given only as the percentage of the total cpm present on the chromatogram.

In the surface-dwelling turbellarian *Archiloa wilsoni*, sulfate and sulfite were the major compounds formed. Some elemental sulfur could be detected. Thiosulfate was never found. In the acoel turbellarian *Pseudohaplogonaria* sp., the major end-product of sulfide metabolism was elemental sulfur. Some sulfite

Table 1. Products of sulfide metabolism as determined by thin-layer chromatography of ^{35}S-labeled compounds. Each horizontal sequence represents one chromatogram of about 50–100 animals (see methods section). A complete breakdown of all cpm on a chromatogram run using toluene : methanol (1:1) as the solvent system, which separated all four compounds identified, is given. Data from other chromatograms using other solvent systems are given only as percent of total cpm (see results section). –: Compounds not resolved from the baseline in the solvent system used. a: % of all cpm spotted on the chromatogram using any solvent system; b: % of all cpm which migrated from the origin when toluene : methanol (1 : 1) was used; c: cpm present where no peak occurred, probably resulting from counting statistics and/or spot trailing; d: cpm remaining at the origin when toluene : methanol (1 : 1) was used. Solvent systems: TM, toluene : methanol; TPM, toluene: n-propanol: methanol; Hep: heptane; BEA: benzene : ethyl acetate (see methods section)

Animal	SO_4^{2-}		SO_3^{2-}		$S_2O_3^{2-}$		S°		Other[c]	cpm remaining at the origin[d]	Solvent system used
	% of total cpm[a]	% of total cpm moved[b]	% of total cpm[a]	% of total cpm moved[b]	% of total cpm[a]	% of total cpm moved[b]	% of total cpm[a]	% of total cpm moved[b]	% of total cpm moved[b]	% of total cpm[a]	
Archiloa wilsoni	29	53	16	29	0	0	6	11	7	44	TM (1:1)
	23		21		0		2				TM (1:1)
	–		16		–		9				TPM
	–		–		–		7				Hep
	–		–		–		5				Hep
	–		–		–		0				BEA
Solenofilmorpha funilis	25	27	23	25	37	40	1	2	6	6	TM (1:1)
	–		35		–		6				TPM
	–		12		21		18				TM (2:1)
Pseudohoplogonaria sp.	11	15	12	16	0	0	38	52	15	33	TM (1:1)
	–		14		–		44				TPM
	–		–		–		52				Hep
	–		–		–		19				BEA
Dolichodasys carolinensis	12	16	13	17	0	0	44	57	10	24	TM (1:1)
	–		9		–		41				TPM
	–		12		0		44				TM (2:1)
	–		–		–		40				Hep
	–		–		–		34				BEA

and sulfate were also detected. The compounds and proportions detected in the sulfide system gastrotrich, *Dolichodasys carolinensis*, were nearly identical to those found in *Pseudohaplogonaria* sp. In *Solenofilmorpha funilis*, sulfite, sulfate, and elemental sulfur were present. In addition, a significant amount of thiosulfate was found.

For the surface animal *Archiloa wilsoni*, a higher percentage of radioactivity remained at the origin with the toluene : methanol (1:1) solvent system (Table 1) than for the sulfide system forms. In contrast, very little radioactivity remained at the origin for *Solenofilomorpha funilis*. Interestingly, for the two animals in which elemental sulfur was the predominate product, very similar percentages remained at the origin.

DISCUSSION

Mechanistic Considerations

Very little is known about sulfur biochemistry in invertebrates. In the only study on sulfide detoxification known to the authors, Patel and Spencer (1963) have shown that the hemoglobin of *Arenicola marina* possesses a detoxification capability. In fact, the information on hydrogen sulfide metabolism in vertebrates is also limited. Such pathways must exist, since the intestinal flora produces sulfide (10^{-4} M in the cow rumen; Wellinger and Wuhrmann, 1977; Baxter and van Reen, 1958; Huisingh et al., 1974), but the pathways of detoxification remain unclear (e. g. Baxter and van Reen, 1958; Sörbo, 1958; Curtis et al., 1972). Oxidation through sulfite to sulfate certainly occurs (Curtis et al., 1972; Siegel, 1975) and thiosulfate is often found as an intermediate. Some elemental sulfur is formed, but this is viewed as the breakdown product of a polysulfide (Sörbo, 1960). A similar pathway is common in bacteria (Peck, 1962; Aminuddin and Nicholas, 1973; Kuenen, 1975). Here, thiosulfate and elemental sulfur are viewed as either direct intermediates or side products depending on the bacteria and author involved (e. g., Peck, 1962; Kuenen, 1975; Pfennig, 1975; Trüper, 1975). A second pathway, which involves the simple oxidation of sulfide to elemental sulfur, is well known in bacteria (e. g. Hansen and van Gemerden, 1972; Oltmann and Stouthamer, 1975; Pfennig, 1975) but remains unreported in the animal kingdom. In addition, sulfide is inorganically oxidized by oxygen to thiosulfate, sulfite and sulfate (Cline and Richards, 1969; Kuenen, 1975).

All the pathways discussed above, be they bacterial, invertebrate, or vertebrate pathways, involve an oxidation of sulfide to another inorganic sulfur compound of some kind. For the purposes of this article, these compounds can be divided into two groups. One group contains compounds composed of both sulfur and oxygen atoms and includes sulfate, sulfite, thiosulfate, and a variety of polythionates. The second group is composed of the non-oxygen containing compounds elemental sulfur and polysulfide. Within the sulfide system, with the possible exception of the redox potential discontinuity layer, oxygen is probably not sufficient to supply pathways yielding Group one compounds (e. g. SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, etc.). A priori, these pathways appear to be precluded, leaving, as the only alternative, an oxidation, yielding elemental sulfur ($S°$) or polysulfide as the major end-product.

Since elemental sulfur was among the compounds detected and since some of the sulfide-system biota are known to harbor bacterial symbionts (e. g. Fenchel et al., 1977; Powell et al., 1979), we were concerned that symbiotic bacteria might be responsible for the formation of this compound. Transmission EM studies of *Dolichodasys carolinensis* and *Pseudohaplogonaria* sp., however, did not reveal any symbiotic bacteria and support the interpretation that the animals, themselves, make the detected compounds. Low and high-power electron micrographs of several cross sections through the body wall of *D. carolinensis*, in the region of the gonad, revealed only one profile within the basal layer of the cuticle which might be identified as a bacterium. In addition, Ruppert and Shaw (1977) did not report the presence of symbiotic bacteria in this species. An EM survey of some cross sections in the region of the mouth opening (mid body) of a Bouin-fixed specimen of *Pseudohaplogonaria* sp., also failed to demonstrate any bacterial associations (courtesy Dr. Stephen Gardiner and Julian Smith). Since sulfide oxidation pathways are well characterized in prokaryotes, but, excepting the study of Patel and Spencer (1963), are unreported in eukaryotes, the possibility that the sulfide oxidation reported by us is due to activity of symbiotic bacteria must still be taken seriously. Confirmation of these findings by other workers is clearly required to establish beyond doubt that these pathways are non-bacterial. The above data strongly suggests this to be the case. Even if there is a contribution from prokaryotes, however, the results are ecologically very significant.

The pathway utilized by *Archiloa wilsoni* involves an oxidation to sulfate via sulfite. This pathway resembles the typical sulfide oxidation pathway with an oxygen atom source that is described for mammals (Siegel, 1975) and bacteria (e. g., Kuenen, 1975). Since no thiosulfate was detected, this pathway evidently differs from the one described by Sörbo (1960). The small amount of elemental sulfur detected may be

interpreted as the breakdown product of a polysulfide (e. g., Sörbo, 1960) or a side reaction. Sulfite is certainly the major intermediate as has been found by most workers (e. g., Aleem, 1975; Kuenen, 1975). The pathway is clearly oxygen atom intensive, as might be expected, since A. wilsoni is restricted to the surface layer.

Elemental sulfur is the end-product found in two of the three sulfide-system animals examined, Dolichodasys carolinensis and Pseudohaplogonaria sp. Pathways yielding elemental sulfur as the principle end-product are common in prokaryotes (e. g., Aleem, 1975; Trüper, 1975), known in some higher plants (e. g., Knobloch 1966a, b), and may be present in fungi (Pezet and Pont, 1977). Only two instances of elemental sulfur formation have been reported in the animal kingdom to our knowledge. Meister et al. (1954) reported that rat liver extracts could oxidize cysteine to pyruvate and elemental sulfur. Patel and Spencer (1963) suggested that one of the end-products of sulfide oxidation by the hemoglobin of Arenicola marina might be elemental sulfur. The presence of elemental sulfur as a major end-product of sulfide metabolism, however, has not been demonstrated previously in the animal kingdom to our knowledge. The formation of elemental sulfur is, however, predictable since elemental sulfur is a nontoxic form of sulfur not requiring oxygen atoms and oxygen atoms are certainly in limited supply in the sulfide system. In comparison to Archiloa wilsoni, much lower percentages of sulfite and sulfate are present in Pseudohaplogonaria sp. and D. carolinensis. Their presence may be evidence for a second pathway used when an oxygen atom source is available or the oxidation of some sulfide present in the animal during post-incubation preparation. Homogenization, for example, was not done in an oxygen-free system. The absence of thiosulfate suggests the former since thiosulfate is usually a product of inorganic sulfide oxidation reactions with oxygen (Cline and Richards, 1969; O'brien and Birkner, 1977).

A more complicated situation is present in the third sulfide-system animal, Solenofilomorpha funilis. Elemental sulfur is formed in varying amounts but is not a major end-product. Sulfite and sulfate are present in quantities substantially larger than found in either Dolichodasys carolinensis or Pseudohaplogonaria sp., and almost certainly represent a functional pathway ostensibly similar to the one found in Archiloa wilsoni. The primary end-product, however, appears to be thiosulfate. Thus S. funilis is the only sulfide-system animal studied whose primary end-product contains oxygen atoms. The formation of thiosulfate in an invertebrate has not been reported previously, and the formation of thiosulfate as the primary end-product of sulfide oxidation is quite rare even in bacteria. Nor-

mally it occurs as an intermediate (e. g. Sörbo, 1960; Trüper, 1975; and others referenced previously). It is particularly interesting that thiosulfate was undetectable in A. wilsoni even though sulfate was the end-product, yet the primary end-product in S. funilis even with sulfate formed in considerable quantities.

Exploitation of the sulfide system requires the ability to live under low oxygen tensions. One possible adaptational strategy, the reduction of dependency on molecular oxygen in metabolism, is clearly demonstrated by these data. The end-product formed by Archiloa wilsoni, sulfate, contains sulfur at its highest oxidation state and four atoms of oxygen per sulfur atom. The presence of an oxygen intensive pathway of this sort should require an abundant oxygen supply such as occurs in the surface layer where A. wilsoni is found. On the other hand, in the sulfide system, oxygen is present in only small amounts in the redox potential discontinuity and absent below it. The end-products formed by the three animals examined consistently contain sulfur at a lower oxidation state than sulfate and, in two species, the end-product, $S°$, contains no oxygen atoms at all. In the third species, the oxidation products contain oxygen, but one of them, $S_2O_3^{2-}$, has the highest S : O ratio of any commonly occurring oxyacid of sulfur.

The formation of thiosulfate, which requires oxygen, by Solenofilomorpha funilis but not by Pseudohaplogonaria sp. or Dolichodasys carolinensis suggests a fundamental difference in oxygen availability either environmentally or metabolically between S. funilis and the other two. Is S. funilis adapted to living in the low oxygen environment of the redox potential discontinuity, where oxygen atoms are present but often in short supply? In addition, migrations of the discontinuity observed by Ott and Machan (1971) and Ankar and Jansson (1973) imply a significant change in oxygen concentration with the tidal cycle and over longer periods. Animals living under these conditions might have a number of pathways available for use depending on the ambient oxygen concentration, including those with oxygen dependent end-products. Based on the above considerations, one might predict that S. funilis lives nearer to the oxic layer, on the average, than either Pseudohaplogonaria sp. or Dolichodasys carolinensis. On the other hand, Reise and Ax (1979) have shown that many so-called sulfide-system species are associated with the micro-oxic zones of infaunal burrows. Perhaps S. funilis is one of these. Further distributional studies are needed to determine whether the complicated detoxification system of S. funilis actually has an environmental correlation.

Since elemental sulfur formation from sulfide does not require oxygen atoms, why is this pathway relatively unimportant in Solenofilomorpha funilis? Ele-

mental sulfur is extremely insoluble in sea-water. Bacteria that form sulfur often contain large vacuoles filled with it in their cytoplasm. The excretion of elemental sulfur from cells may be more difficult and require more energy than the removal of an inorganic ion such as thiosulfate or sulfate, both of which are water soluble and easily removed simply by diffusion. Interestingly, the two animals making elemental sulfur have less extractable ^{35}S than *S. funilis* (i. e. more cpm remained at the origin; Table 1). This may be more than just coincidence and may indicate that this pathway requires the incorporation of some sulfur in a tightly bound form. The binding of sulfide to a membrane-bound polysulfide either as an intermediate or as a mechanism to handle the insoluble elemental sulfur could be responsible. A membrane-bound polysulfide has been implicated in a number of bacterial systems (Aminuddin and Nicholas, 1973; Aleem, 1975). Thus, one might expect the formation of a soluble salt when possible. Thiosulfate has a low toxicity (Sörbo, 1972; Stine et al., 1976). It also has the best sulfur   oxygen (S : O) ratio of any common oxyacid and, therefore, might be expected to replace elemental sulfur as an end-product under low oxygen conditions.

The presence of oxygen atoms in some of the compounds produced during these anoxic experiments implies the availability of oxygen atoms in some form during the experiments. This oxygen atom supply could be of two kinds. A storage system may be present. Oxygen storage is relatively common in invertebrates (e. g., Mangum and van Winkle, 1973). The $MgCl_2$ decantation process used to extract the animals from the sediment results in the exposure of these animals to molecular oxygen. Therefore, sulfide-system animals certainly had the opportunity to acquire and store oxygen. The presence of oxyacids as end-products in *Solenofilomorpha funilis* and *Archiloa wilsoni* might indicate that such a phenomenon exists. Alternatively, a compound such as nitrate or carbonate may be the source. Bacteria certainly use nitrate reduction as an oxygen atom source (Aminuddin and Nicholas, 1973; Aleem, 1975). Such a mechanism has been proposed as a possible pathway present in animals of the sulfide system to account for the presence of cytochromes in some of these animals (Maguire and Boaden, 1975).

In contrast to *Archiloa wilsoni* and *Solenofilomorpha funilis*, the ability to acquire and use oxygen atoms seems to be poorly developed in *Pseudohaplogonaria* sp. and *Dolichodasys carolinensis* even though they went through the same extraction process. At least they do not make use of oxygen atoms in sulfide metabolism to any great degree. The possibility exists that the sulfite and sulfate observed in these animals may be simply a side reaction with oxygen that occurred during the post-incubation procedure. Sulfide system animals should be euryoxic or facultatively anaerobic. (See Hammen, 1976 for the correct usage of these terms). The evidence here certainly supports this hypothesis.

Phylogenetic Considerations

When Fenchel and Riedl (1970) described the sulfide system, they noted the remarkable similarity of its chemical environment to the anoxic, reducing environment of the primitive earth (Cloud, 1968). They stressed the number of supposedly primitive groups of metazoans either restricted to or well represented in the sulfide system (e. g. various types of bacteria, protozoa, fungi, and primitive metazoa) and suggested that this habitat might, therefore, be a relict one, containing direct descendants of the most primitive metazoa. Boaden (1975) and Maguire and Boaden (1975) have expanded on this concept by suggesting that the first metazoa were anaerobes living in reducing conditions and that direct descendants of these early metazoans may be found within the sulfide system. Some animals of the sulfide system might, therefore, retain the biochemical pathways of the earliest metazoa.

Studies on the intermediary metabolism of invertebrates have shown that many invertebrates are excellent anaerobes (e. g., molluscs: de Zwaan et al., 1976; annelids: Zebe, 1975; parasitic Platyhelminthes and Aschelminthes: Saz, 1971), and the evidence from the present study indicates that sulfide-system forms are, as expected, well adapted for life without oxygen. On the other hand, a variety of important chemical pathways are obligatorily aerobic. These include sterol synthesis (Goldfine, 1965; Morris, 1978) and collagen synthesis (Towe, 1970). If the data presented here and elsewhere (e. g., Schiemer, 1973; Wieser, et al., 1974) is interpreted as evidence for either facultative or obligate anaerobiosis, how are these apparently oxygen-requiring processes performed in these animals? An obligatorily anaerobic metazoan may be impossible (see also Reise and Ax, 1979). If Boaden (1975) is correct, however, then this ability to withstand anoxia is a primitive feature and the utilization of oxygen has been acquired or enhanced during invertebrate evolution. Since sulfide is accepted as a major constituent of both the primitive earth and anoxic environments of today's ocean, since the presence of sulfide detoxification pathways appears to be widespread among the invertebrates, and since both oxygen dependent and oxygen independent pathways are clearly present, the

pattern of occurence of the pathways of sulfide metabolism in the lower metazoa may provide the kind of data needed to test Boaden's hypothesis. If, for example, the formation of elemental sulfur is judged to be a primitive feature, and not an environmentally dictated convergence, then one might deduce that the original metazoan had an anaerobic detoxification system and to this extent, at least, that it functioned as an anaerobe.

Elemental sulfur is formed by two different phyla, the Platyhelminthes and the Gastrotricha, which are not now viewed as being particularly closely related within the lower metazoa (Rieger, 1976). If both *Pseudohaplogonaria* sp. and *Dolichodasys carolinensis* were judged to be primitive within their respective phyla, then an interpretation that the pathways ending in elemental sulfur were primitive might be tenable. *Pseudohaplogonaria* sp. may be considered by some as a primitive turbellarian since it is an acoel (Westblad, 1948; Steinboch, 1966; but see Ax, 1963 for contrary views). On the other hand, based on the presence of a complex U-shaped caudal organ, highly aberrant sperm morphology (Ruppert and Shaw, 1977), and the occurrence of multiciliated cells in the epidermis (Rieger, 1976), *D. carolinensis* can be considered an evolutionarily advanced gastrotrich. Thus two animals having quite different evolutionary histories make elemental sulfur. In addition, within the relatively circumscribed group, the Acoela (Tyler and Rieger, 1977 and references therein), are two animals, *Solenofilomorpha funilis* and *Pseudohaplogonaria* sp. with different types of sulfide metabolism as judged from the end-products produced. One makes elemental sulfur and essentially no thiosulfate, whereas the other makes thiosulfate and much less elemental sulfur. There appears to be a substantial amount of plasticity in the pathways and end-products of sulfide metabolism even within one turbellarian order, the Acoela. These facts indicate that the formation of elemental sulfur observed here is probably the result of an evolutionary convergence dictated by the anoxic interstitial environment.

A second species of *Dolichodasys*, *D. delicatus*, occurs in the shallow subtidal (Ruppert and Shaw, 1977) and intertidally (Rieger, unpublished) on lotic beaches. This environment contains oxygen and resembles, in this regard, the surface layer inhabited by *Archiloa wilsoni*. If the type of sulfide metabolism is environmentally determined, than *D. delicatus* should closely resemble *A. wilsoni*, but not *D. carolinensis*, in this respect. The investigation of two very closely related species such as these, which occur in two very different habitats, should provide a test of the phylogenetic and environmental interpretations of these pathways as discussed here.

CONCLUSIONS

Life in the sulfide system requires the ability to tolerate continuous sulfide stress. In addition, since most of the sulfide produced in marine sediments is reoxidized at the surface (Jorgensen, 1977), the surface dwelling animals must also be expected to have adaptations to withstand short-term sulfide stress. Powell et al. (1979) have demonstrated that a sulfide detoxification system exists in the body wall of a number of interstitial metazoans. The data presented here show that this detoxification always consists of an oxidation, with a number of compounds formed depending on the animal and habitat in question. These compounds include sulfate, sulfite, thiosulfate, and elemental sulfur. The discovery of the formation of two compounds, thiosulfate and elemental sulfur, neither of which, to our knowledge, has been reported before in the invertebrates, underscores how little is really known about sulfur metabolism in the invertebrates. There can be no doubt that the ability to detoxify sulfide must play an important role in determining the observed vertical distribution patterns and the resulting community structure of soft-sediment meiofaunal populations. The greater sulfide tolerance in the macrofauna living on or in sediments with a sulfide system (Jacubowa and Malm, 1931; Theede et al., 1969), and the changes in macrofaunal community structure accompanying long-term vertical shifts in the location of the redox potential discontinuity (Ankar and Jansson, 1973; Reimers, 1976) suggests that adaptations for sulfide detoxification may play an important role in macrofaunal community composition as well.

LITERATURE CITED

Aleem, M. (1975). Biochemical reaction mechanisms in sulfur oxidation by chemosynthetic bacteria. Pl. Soil 43: 587–607

Aminuddin, A., Nicholas, D. (1973). Sulphide oxidation linked to the reduction of nitrate and nitrite in *Thiobacillus denitrificans*. Biochim. biophys. Acta 325: 81–93

Ankar, S., Jansson, B. (1973). Effect of an unusual natural temperature increase on a Baltic soft-bottom community. Mar. Biol. 18: 9–18

Ax, P. (1963). Relationships and phylogeny of the Turbellaria. In: Dougherty, E. (ed.) The lower Metazoa, comparative biology and phylogeny. University of California Press, Berkeley, pp. 191–224

Banaszkiewicz, S. (1976). Determination of free sulfur in chemical reagents by means of thin layer chromatography. Microchem. J. 21: 306–308

Baxter, C., van Reen, R. (1958). The oxidation of sulfide to thiosulfate by metallo-protein complexes and by ferritin. Biochim. biophys. Acta 28: 573–578

Berner, R. (1963). Electrode studies of hydrogen sulfide in marine sediments. Geochim. cosmochim. Acta 27: 563–575

Boaden, P. (1975). Anaerobiosis, meiofauna, and early metazoan evolution. Zool. Scr. 4: 21–24

Boaden, P., Platt, H. (1971). Daily migration patterns in an intertidal meiobenthic community. Thalassia jugosl. 7: 1–12

Bonn, E., Follis, B. (1967). Effects of hydrogen sulfide on channel catfish Ictalurus punctatus. Trans. Am. Fish. Soc. 96: 31–36

Cline, J., Richards, F. (1967). Oxygenation of hydrogen sulfide in seawater at constant salinity, temperature, and pH. Environ. Sci. Technol. 3: 838–843

Cloud, P. (1968). Atmospheric and hydrospheric evolution on the primitive earth. Science, N. Y. 160: 729–736

Colby, P., Smith, L. (1967). Survival of walleye eggs and fry on paper fiber sludge deposits in Rainy River, Minnesota. Trans. Am. Fish. Soc. 96: 278–296

Crezee, M. (1976). Solenofilomorphidae (Acoela), major component of a new turbellarian association in the sulfide system. Int. Revue ges. Hydrobiol. 61: 105–129

Curtis, C., Bartholomew, T., Rose, F., Dodgson, K. (1972). Detoxification of sodium ^{35}S-sulphide in the rat. Biochem. Pharmac. 21: 2313–2321

De Zwaan, A., Kluytmans, J., Zandee, D. (1976). Facultative anaerobiosis in molluscs. Biochem. Soc. Symp. 41: 133–168

Evans, C. (1967). The toxicity of hydrogen sulphide and other sulphides. Q. Jl exp. Physiol. 52: 231–248

Farris, R. (1976). Systematics and ecology of Gnathostomulida from North Carolina and Bermuda. Ph. D. thesis, University of North Carolina at Chapel Hill

Fenchel, T., Perry, T., Thane, A. (1977). Anaerobiosis and symbiosis with bacteria in free-living ciliates. J. Protozool. 24: 154–163

Fenchel, T., Riedl, R. (1970). The sulfide system: A new biotic community underneath the oxidized layer of marine sand bottoms. Mar. Biol. 7: 255–268

Fricke, U. (1975). Tritisol: A new scintillation cocktail based on Triton X–100. Analyt. Biochem. 63: 555–558

Goldfine, H. (1965). The evolution of oxygen as a biosynthetic reagent. J. gen. Physiol. 49: 253–268

Goldhaber, M., Kaplan, I. (1974). The sulfur cycle. In: Goldberg, E. (ed.) The Sea, Vol. 5, Marine Chemistry. Wiley, New York, pp. 569–655

Hammen, C. (1976). Respiratory adaptations: Invertebrates. In: Wiley, M. (ed.) Estuarine processes, uses, stresses, and adaptations to the estuary, Vol. I. Plenum Press, New York, pp. 347–355

Hansen, T., van Gemerden, H. (1972). Sulfide utilization by purple nonsulfur bacteria. Arch. Mikrobiol. 86: 49–56

Huisingh, J., McNeill, J., Matrone, G. (1974). Sulfate reduction by a Desulfovibrio species isolated from sheep rumen. Appl. Microbiol. 28: 489–497

Jacubowa, L., Malm, E. (1931). Die Beziehungen einiger Benthos-Formen des Schwarzen Meeres zum Medium. Biol. Zentralbl. 51: 105–116

Jørgensen, B. (1977). The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). Limnol. Oceanogr. 22: 814–832

Jørgensen, B., Cohen, Y (1977). Solar Lake (Sinai). 5. The sulfur cycle of the benthic cyanobacterial mats. Limnol. Oceanogr. 22: 657–666

Kelly, D. (1970). Thin-layer chromatography of inorganic sulphur compounds. J. Chromatogr. 51: 343–345

Knobloch, K. (1966a). Photosynthetische Sulfid-oxydation grüner Pflanzen. I. Mitteilung. Planta 70: 73–86

Knobloch, K. (1966b). Photosynthetische Sulfid-oxydation grüner Pflanzen. II. Mitteilung Wirkung von Stoffwechselinhibitoren. Planta 70: 172–186

Kuenen, J. (1975). Colourless sulfur bacteria and their role in the sulfur cycle. Pl. Soil 43: 49–76

Maguire, C., Boaden, P. (1975). Energy and evolution in the thiobios: an extrapolation from the marine gastrotrich Thiodasys sterreri. Cah. Bio. mar. 16 (suppl.): 636–646

Mangum, C., van Winkle, W. (1973). Responses of aquatic invertebrates to declining oxygen conditions. Am. Zool. 13: 529–541

Meister, A., Fraser, P., Tice, S. (1954). Enzymatic desulfuration of β-mercaptopyruvate to pyruvate. J. Biol. Chem. 206: 561–575

Morris, J. (1978). The biochemistry of anaerobiosis. Biochem. Soc. Trans. 6: 353–356

O'brien, D., Birkner, F. (1977). Kinetics of oxygenation of reduced sulfur species in aqueous solution. Environ. Sci. Technol. 11: 1114–1120

Oltmann, L., Stouthamer, A. (1975). Reduction of tetrathionate, trithionate, and thiosulphate and oxidation of sulphide in Proteus mirabilis. Arch. Mikrobiol. 105: 135–142

Oseid, D., Smith, L. (1974a). Factors influencing acute toxicity estimates of hydrogen sulfide to freshwater invertebrates. Water Res. 8: 739–746

Oseid, D., Smith, L. (1974b). Chronic toxicity of hydrogen sulfide to Gammarus pseudolimnaeus. Trans. Am. Fish. Soc. 103: 819–822

Ott, A. (1972). Determination of faunal boundaries of nematodes in an intertidal sandflat. Int. Revue ges. Hydrobiol. 57: 645–663

Ott, A., Machan, R. (1971). Dynamics of climatic parameters in intertidal sediments. Thalassia jugosl. 7: 219–229

Patel, S., Spencer, C. (1963). The oxidation of sulphide by the haem compounds from the blood of Arenicola marina: J. mar. biol. Ass. U. K. 43: 167–175

Peck, H. (1962). Symposium on metabolism of inorganic compounds V. Comparative metabolism of inorganic sulfur compounds in micro-organisms. Bacteriol. Rev. 26: 67–94

Pezet, R., Pont V. (1977). Elemental sulfur: accumulation in different species of fungi. Science, N. Y. 196: 428–429

Pfennig, N. (1975). The phototrophic bacteria and their role in the sulfur cycle. Pl. Soil 43: 1–16

Pollard, F., Nickless, G., Burton, K. (1962). A spraying reagent for anions. J. Chromatogr 8: 507–509

Pollard, F., Nickless, G., Glover, R. (1964). Chromatographic studies on sulphur compounds Part IV. The decomposition of acidified thiosulphate and polythionate solutions. J. Chromatogr. 15: 518–532

Powell, E., Crenshaw, M., Rieger, R. (1979). Adaptations to sulfide in the meiofauna of the sulfide system. I. ^{35}S-accumulation and the presence of a sulfide detoxification system. J. exp. mar. Biol. Ecol. 37: 57–76

Reimers, T (1976). Anoxische Lebensräume. Struktur und Entwicklung der Mikrobiozönose an der Grenzfläche Meer/Meeresboden. Ph. D. thesis, Christian-Albrechts-Universität zu Kiel

Reise, K., Ax, P. (1979). A meiofaunal 'Thiobios' limited to the anaerobic sulfide system of marine sand does not exist. Mar Biol. 54: 225–237

Rieger, R. (1976). Monociliated epidermal cells in Gastrotricha: Significance for concepts of early metazoan evolution. Z. zool. Syst. Evolutionsforsch. 14: 198–226

Roy, A., Trudinger, P. (1970). The biochemistry of inorganic compounds of sulphur, Cambridge University Press, Cambridge

Ruppert, E., Shaw, K. (1977). The reproductive system of gastrotrichs. I. Introduction with morphological data for two new *Dolichodasys* species. Zool. Scr 6: 185–195

Saz, H. (1971). Facultative anaerobiosis in the invertebrates: Pathways and control systems. Am. Zool. 11: 125–135

Schiemer, F. (1973). Respiration rates of two species of gnathostomulids. Oecologia 13: 403–406

Siegel, L. (1975). Biochemistry of the sulfur cycle. In: Greenberg, D. (ed.) Metabolic pathways Vol. 7, Metabolism of sulfur compounds. Academic Press, New York, pp. 217–286

Smith, L., Oseid, D., Olson, L. (1976). Acute and chronic toxicity of hydrogen sulfide to the fathead minnow *Pimephales promelas*. Environ. Sci. Technol. 10: 565–568

Sörbo, B. (1958). On the formation of thiosulfate from inorganic sulfide by liver tissue and heme compounds. Biochim. biophys. Acta 27: 324–329

Sörbo, B. (1960). On the mechanism of sulfide oxidation in biological systems. Biochim. biophys. Acta 38: 349–351

Sörbo, B. (1972). The pharamacology and toxicology of inorganic sulfur compounds. In: Senning, A. (ed.) Sulfur in organic and inorganic chemistry, Vol. 2. Marcel Dekker Inc., New York, pp. 143–170

Steinboch, O. (1966). Die Hofsteniidae (Turbellaria, Acoela). Grundsätzliches zur Evolution der Turbellaria. Z. zool. Syst. Evolutionsforsch. 4: 58–195

Stine, R., Slosberg, B., Beachum, B. (1976). Hydrogen sulfide intoxication, a case report and discussion of treatment. Ann. intern. Med. 85: 756–758

Theede, H., Ponat, A., Hiroki, K., Schlieper, C. (1969). Studies on the resistance of marine bottom invertebrates to oxygen-deficiency and hydrogen sulfide. Mar Biol. 2: 325–337

Towe, D. (1970). Oxygen-collagen priority and the early metazoan fossil record. Proc. Nat. Acad. Sci. 65: 781–788

Trüper, H. (1975). The enzymology of sulfur metabolism in phototropic bacteria – a review. Pl. Soil 43: 29–39

Tyler, S., Rieger, R. (1977). Ultrastructural evidence for the systematic position of the Nemertodermatida (Turbellaria). In: Karling, T. G., Meinander, M. (eds) The Alex Luther centennial symposium on Turbellaria. Acta Zool. Fennica 154: 193–207

Warfe, J. (1977). The intertidal sediment habitats of the lower Medway Estuary. Kent. Environ. Pollut. 13: 79–91

Wellinger, A., Wuhrmann, K. (1977). Influence of sulfide compounds on the metabolism of *Methanobacterium* strain AZ. Arch. Microbiol. 115: 13–17

Westblad, E. (1948). Studien über Skandinavische Turbellaria Acoela, V. Ark. Zool. 41A: 1–82

Wieser, W., Ott, J., Schiemer, F., Gnaiger, E. (1974). An ecophysiological study of some meiofauna species inhabiting a sandy beach at Bermuda. Mar. Biol. 26: 235–248

Zebe, E. (1975). In vivo-Untersuchungen über den Glucose-Abbau bei *Arenicola marina* (Annelida, Polychaeta). J. Comp. Physiol. 101: 133–145