tions in phytoplankton stock would develop during a 4-hour nocturnal grazing period. This is consistent with the observed small-scale coherences. Greater depletions of phytoplankton accompanied zooplankton patches of larger spatial scale, implying more prolonged contact between the zooplankton peaks and phytoplankton troughs (although the coherence was weaker for these larger features).

A possible mechanism for maintaining the observed zooplankton and phytoplankton patterns is the intense (in this region) diel vertical migration of the zooplankton. Pearre (*11*) has suggested in a study based on gut content analyses that the daily downward migration of carnivorous zooplankton can be triggered by satiation. Mackas and Bohrer (*12*) found similar evidence for herbivorous copepods. Evans *et al.* (*13*) constructed a mathematical model coupling this migratory pattern with a vertical current shear. Their model output showed intensifying zooplankton patches that are negatively correlated with the spatial pattern of the phytoplankton, a result that is consistent with (although not proved by) our observations.

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Mercury Distribution in the Gulf Stream

Abstract. *Measurements of reactive mercury in ocean water in the Gulf Stream at depths from 250 to 4460 meters revealed lower and more consistent concentrations than have been reported in the western Atlantic. The mean and standard deviation of 24 values in a vertical profile were 4.1 ± 1.0 nanograms per liter. A correlation was found between variations in mercury and silicate, suggesting a common link in their marine geochemistry.*

Information on the natural occurrence and cycle of Hg in the ocean is important in evaluations of man's role in mobilizing this potentially toxic metal. Contamination has been a major obstacle to obtaining reliable concentrations of Hg in seawater (*1*). Earlier studies have not shown a consistent correlation between the Hg concentration and the distributions of other chemicals in the ocean (*2–7*). We have used a sensitive method for the shipboard analysis of Hg in seawater which minimizes contamination. Results for a vertical profile of samples taken from the Gulf Stream show lower concentrations than those measured in earlier work and systematic variations with silicate.

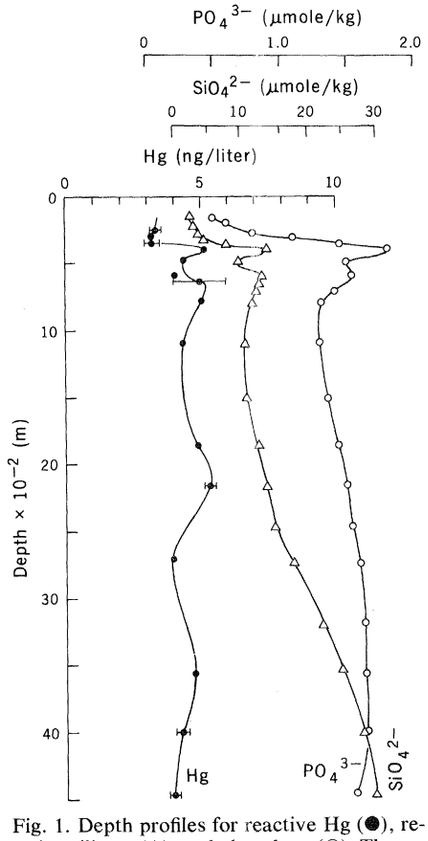

Fig. 1. Depth profiles for reactive Hg (●), reactive silicate (△), and phosphate (○). The error bars for Hg are equal to 2σ in total magnitude, average 1σ = 0.5 ng/liter. The sea floor is 4670 m deep at this station.

With this analytical technique it was possible to measure the so-called reactive Hg (inorganic Hg plus a portion of the organic Hg) in seawater. Five milliliters of 10 percent stannous chloride were added to 250 ml of seawater, which had been acidified with 2 ml of concentrated Hg-free HNO_3 to avoid loss after collection; we brought the sample into equilibrium with room temperature by allowing the sample to sit for approximately 2 hours before analysis. Nitrogen was bubbled through the sample at the rate of 290 ml/min for 15 minutes to transfer the elemental Hg to a preconcentration trap consisting of Au-coated quartz sand. The Hg was released from the Au by heating to 350° ± 25°C as measured by an iron-constantan thermocouple placed inside the preconcentration trap. The Hg then was swept through a cold vapor atomic absorption instrument (Spectro Products HG-3). Further bubbling of the sample and heating of the preconcentration trap did not show any detectable signal; this result indicated the complete recovery of the Hg. Analyses were carried out within about 6 hours of collection to minimize loss or contamination of Hg associated with storage. The method provided a 100-fold concentration factor between the Hg initially stripped from the seawater and that passing through the analysis instrument. The technique is a modification of the one given by Fitzgerald *et al.* (*5*).

In the calibration of the method we

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used a $HgCl_2$ solution (1 part per million) stabilized with 0.01 percent potassium dichromate and 5 percent HNO_3. We determined a standard curve by adding 1, 2, and 3 μl of the $HgCl_2$ solution to 250 ml of Hg-free seawater and analyzing it in the same manner as the sample. We verified the accuracy of the method by determining the Hg in National Bureau of Standards standard reference material (SRM) 1641. Our analysis yielded 1.50 ± 0.03 ng of Hg for a 1-μl spike of SRM 1641 added to 250 ml of seawater. The certified value was 1.49 ng/μl. The 1-standard-deviation precision of the analysis was ± 0.5 ng/liter, based on five measurements from two 5-liter Niskin sampling bottles which were tripped at 1530 ± 10 m. The mean concentration of these five analyses was 5.4 ng/liter. The detection limit of the analysis (two times the instrumental noise) was 1 ng/liter. At the beginning of the cruise the blank value was ≤ 1 ng/liter, but it increased to 4 ± 1 ng/liter near the end of the cruise at which time the Gulf Stream samples were collected and analyzed. The reported values have been corrected for this blank concentration.

The sampling and analysis for this work was done in November 1977 aboard the R.V. *Knorr* at a location in the Gulf Stream (39°10.2′N, 65°30.0′W) where the 15°C isotherm was at a depth of 256 m. A 12-position rosette sampler with 5-liter Niskin bottles was used with in situ temperature, conductivity, and pressure sensors to obtain the samples. In most cases two samples were drawn for Hg analysis from each Niskin bottle in order to provide a check against spurious values associated with sample processing. The nutrients (phosphate, nitrate, and silicate) were measured aboard ship with a Technicon Auto-Analyzer II.

The vertical profile of Hg in the Gulf Stream shows relatively consistent values from 250 to 4460 m (Fig. 1). The average reactive Hg concentration through the water column and its standard deviation (24 analyses) are 4.1 ± 1.0 ng/liter. This result may be compared with the values for reactive Hg of 8 ± 4 ng/liter reported by Fitzgerald (*6*) for depths from 0 to 750 m in the Sargasso Sea and 3 to 4 ng/liter reported by Baker (*8*) in the waters around the United Kingdom. An earlier investigation (*2*) of total Hg in the Gulf Stream indicated values of 149 ± 70 ng/liter; this value is larger by an order of magnitude than the value for total Hg (6 to 11 ng/liter) determined by Fitzgerald and Hunt (*4*). Most investigators indicate that 25 to 75 percent of the Hg in ocean water is reactive (*4, 8, 9*); hence our measurements of 4.1 ± 1.0 ng/liter for reactive Hg are substantially different from the earlier values of 149 ± 70 ng/liter for total Hg (*2*). None of the earlier investigators have observed systematic Hg variations with depth or hydrographic properties.

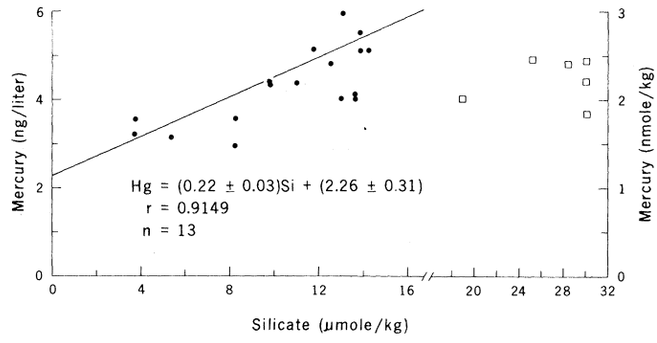

Fig. 2. Correlation diagram for reactive Hg and silicate. The regression line was based on the Hg values in the depth interval from 0 to 2200 m (●); values for the depth region from 2200 to 4460 m are represented by the squares.

A correlation between Hg and silicate shows a systematic variation in these two parameters in the upper 2200 m of the water column (Fig. 2). Below 2200 m silicate increased without a corresponding increase in Hg. The correlation coefficient of Hg versus silicate in the upper 2200 m indicates that 80 percent of the variability in Hg is associated with variations in silicate, and the standard deviation of the regression slope is significantly different from zero at the 99 percent confidence limit. As far as we can determine, this is the first time that Hg concentrations have been shown to vary significantly with other chemical parameters in oceanic waters. Mercury correlates better with silicate than with phosphate. The correlation coefficient with silicate is $r = .91$, whereas with phosphate $r = .50$ (in the region from 0 to 2200 m). This correlation suggests that inorganic Hg is removed from surface waters and is regenerated in the intermediate waters at rates similar to silicate.

Recent work by Matsunaga *et al.* (*7*) shows Hg concentrations of 5.0 ± 0.5 ng/liter for waters from the Kuroshio and Oyashio regions of the northwest Pacific. Our results are in good agreement with these concentrations. Matsunaga *et al.* did not observe a systematic variation with depth or with silicate. They did not report nutrient data along with their Hg values, and with their vertical sampling procedure it would not have been possible to obtain high resolution of nutrient gradients. More information must be obtained in order to understand why a mercury-silicate relationship might be found in the northwest Atlantic Ocean but not in the northwest Pacific Ocean.

Our results suggest reactive Hg concentrations on the order of 3 ng/liter for waters in and above the thermocline of the Gulf Stream. Rainfall is considered to be a major source of Hg to the ocean globally, with submarine volcanic input being important in specific areas such as near Iceland (*10*). A residence time for this Hg in surface waters may be estimated from this value and from the rate of input by rainfall. A typical annual rate of rainfall for the northwest Atlantic Ocean is 90 cm/year (*11*). The concentration of Hg in rain is not well established. Fitzgerald (*10*) found a Hg concentration of 11 ng/liter in rainfall at coastal locations in Connecticut and Massachusetts, whereas Matsunaga and Goto (*12*) reported an average value of 1 ng/liter in coastal rainwater over eastern Japan. These values suggest a flux of Hg to the ocean due to rainfall in the range of 0.1 to 1.0 ng cm^{-2} $year^{-1}$. If this input of Hg is distributed in the upper 100 m containing about 3 ng/liter, a residence time of 30 to 300 years for Hg in surface waters is implied.

This analysis may overestimate the residence time because it does not consider additional possible inputs in the region such as continental runoff or the transfer of volatile Hg between the atmosphere and ocean. On the other hand, the mixing depth in this portion of the ocean is probably greater than 100 m during a 30-year period. The upper limit of 300 years for the residence time of Hg is, however, less by an order of magnitude than the 3200 years accepted by the National Academy of Sciences (*13*). Most estimates indicate that man's activities contribute a flux of Hg to the atmosphere that is comparable in magnitude to natural processes (*11, 14*). Our results showing the concentration of Hg in ocean water and its variation with silicate provide information on the importance in the marine environment of man's mobilization of Hg.

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Chemical Evidence for Production of Hydroxyl Radicals during Microsomal Electron Transfer

Abstract. *Rat liver microsomes generate methane from dimethyl sulfoxide and ethylene from either methional or 2-keto-4-thiomethylbutyric acid during electron transfer initiated by reduced nicotinamide-adenine dinucleotide phosphate (NADPH). Hydrocarbon gas production is suppressed by hydroxyl radical scavenging agents. Azide, an inhibitor of catalase, augments the production of hydrocarbon gases. These observations constitute chemical evidence for the generation of hydroxyl radicals by microsomes.*

Reduction of oxygen during microsomal electron transfer reactions has been the subject of many investigations. Hydrogen peroxide has been identified as an end product (*1*) and the superoxide radical as an intermediate (*2*). Iron-peroxy complexes have also been implicated (*3*). Further knowledge of products or intermediates of oxygen metabolism could help to elucidate aspects of microsomal function and of cytochrome P-450 mediated reactions.

We now report chemical evidence for the formation of hydroxyl radicals (·OH) during microsomal electron transfer. Generation of hydroxyl radicals is not unexpected in systems that generate H_2O_2 (Eq. 1)

$$H_2O_2 + \text{iron catalyst} \rightarrow \cdot OH \quad (1)$$

particularly when catalysts such as iron or iron chelates are present (*4*). Cytochrome P-450, an endogenous heme protein in microsomes, may play an important role and deserves further investigation. In our experiments, no external iron was added.

Three agents were used to detect hydroxyl radicals: methional (3-thiomethylpropanal), 2-keto-4-thiomethylbutyric acid (KTBA, the keto-acid analog of methional), and dimethyl sulfoxide (DMSO). These agents scavenge hydroxyl radicals and form ethylene gas from methional or KTBA and methane gas from DMSO. Generation of ethylene has been used to detect hydroxyl radicals, for example, during the xanthine-xanthine oxidase reaction (*5*), the autoxidation of certain organ-specific cell toxins (*6*), and the phagocytic process (*7*). Dimethyl sulfoxide reacts with hydroxyl radicals to generate methyl radicals ($\cdot CH_3$) (*8*), which give rise to methane by hydrogen abstraction. Methane generation from DMSO during oxidant hemolysis of vitamin E-deficient rat erythrocytes has been reported (*9*). The reactions involved in the formation of hydrocarbon gases from methional, KTBA, and DMSO can be summarized by Eqs. 2 to 4, respectively (*5*, *8*, *10*):

$$CH_3SCH_2CH_2CHO + \cdot OH \rightarrow CH_2{=}CH_2 + HCOOH + {}^1/_2(SCH_3)_2 \quad (2)$$

$$CH_3SCH_2CH_2COCOOH + \cdot OH \rightarrow CH_2{=}CH_2 + 2CO_2 + {}^1/_2(SCH_3)_2 \quad (3)$$

$$CH_3SOCH_3 + \cdot OH \rightarrow \cdot CH_3 + CH_3SOOH \quad (4)$$

Liver microsomes were prepared from male Sprague-Dawley rats (*11*), washed once, and suspended in 125 m*M* KCl. All solutions were prepared with Millipore water subsequently distilled in an all-glass apparatus. Microsomes were incubated at 37°C in a medium consisting of 83 m*M* potassium phosphate buffer (*p*H 7.4), 10 m*M* potassium pyrophosphate, 10 m*M* $MgCl_2$, 0.3 m*M* $NADP^+$, 0.5 m*M* azide, 10 m*M* glucose-6-phosphate (G-6-P), and 7 units of glucose-6-phosphate dehydrogenase (G-6-PD) in a total volume of 3 ml. The $NADP^+$, G-6-P, and G-6-PD functioned as an NADPH-generating system. Drug metabolism as well as ethanol oxidation by microsomes requires NADPH (*12*), whose role appears to be reduction of cytochrome P-450 from the ferric to the ferrous state. The role of azide was to inhibit microsomal catalase. Samples were prepared in the cold and microsomes (approximately 7.5 mg of protein) were added. The $NADP^+$ was added last to initiate the reaction, and the samples were then placed into a water bath at 37°C. Samples were contained in sealed 18-ml screw cap tubes. The caps were fitted with silicone septa, which were punctured for removal of a sample of gas phase. A gas-tight, plastic and rubber, disposable 1 ml syringe (Becton-Dickinson) was used. Before removing a sample of gas, the plunger was drawn back and forth ten times to ensure that the gas content of the tube was adequately mixed. The gas sample (0.5 ml) was then injected directly into a Hewlett-Packard model 5750 gas chromatograph for measurement of the hydrocarbon gases (*6*, *13*).

Table 1 shows that time-dependent production of ethylene was observed with either methional or KTBA as substrate. Methane production was observed with DMSO. The yield of methane was less than that of ethylene. In control studies, no hydrocarbon gases were generated when G-6-P, G-6-PD, or $NADP^+$ was omitted. Therefore, the generation of NADPH was an absolute requirement. None of the latter constituents, either alone or together, generated hydrocarbon gases in the absence of microsomes. Therefore, an absolute requirement for microsomes was demonstrated, and hydrocarbon gas production could not be attributed directly to the NADPH-generating system or to an interaction between this system and the buffer. Lastly, no gas was formed when methional, KTBA, or DMSO was omitted. Thus, the appearance of ethylene or methane required the presence of substrate and the simultaneous initiation of microsomal electron transfer reactions.

Ethanol and *n*-butanol are good scavengers for hydroxyl radicals, whereas urea is not (*13*, *14*). When either ethanol or *n*-butanol was added, the formation of hydrocarbon gas was suppressed. For example, 50 m*M* ethanol suppressed eth-

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