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Some Biochemical Aspects of Red Tides and Related Oceanographic Problems[1]

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ABSTRACT

"Red Tides" are blooms of plankton organisms which may or may not be lethal to aquatic organisms. The sudden increase in multiplication rates of the organisms is caused by a complex of biological factors, and physical factors play a major role in effecting mechanical concentration.

This paper deals with the biological factors operative in the genesis of blooms. Nutritional studies show that organic factors which occur naturally in the water are important. The existence of biologically active organic compounds and possible modes of action are discussed. The method of measurement of "carbohydrates" is explained and their production by bacteria-free cultures of *Prorocentrum* is demonstrated. Such organic compounds could serve as substrates for vitamin B_{12}-producing bacteria and thus play a role in the conditioning of water for succeeding populations.

Gymnodinium breve, the causative organism of the Florida Red Tide, responds differentially to Gulf of Mexico waters of different origins. A suggested explanation is the interaction between environmental copper and hydrogen sulfide. Once started, a bloom may replenish the required nutrients by the decomposition of the fish that it kills.

INTRODUCTION

"Red Tide" is a popular term applied to unusual concentrations of freshwater or marine organisms—protozoans or unicellular algae—which may or may not cause a red discoloration of the water, and which may or may not be lethal to fish or other aquatic organisms. In recent years the phenomenon has become widely known and the term much used because of some highly publicized and spectacular fish kills. The explanation of the genesis of these blooms is to be found in the physiology and related environmental responses of the organisms concerned, while the extent of resulting fish kills is dependent upon their concentration. Biological and chemical factors are most critical in determining the environmental responses of the causative organisms, while physical factors are the most important concentrating agents.

The mechanics of concentration of semibuoyant particles drifting in the open sea can be explained by such devices as convergence of water masses, wind-driven surface currents, and convection cells. Various authors have treated these systems in theoretical terms, and Ryther (1955) has reviewed them in the light of the present knowledge of dinoflagellate nutrition. He concluded, "It is necessary only to have conditions favoring growth and dominance of a moderately large population of a given species and the proper hydrographic and meteorological conditions to permit the accumulation of organisms at the surface and to effect their further concentrations in localized areas."

The U. S. Fish and Wildlife Service has concentrated on the study of the biological processes that stimulate plankton blooms, and the collection of field data necessary for the interpretation of laboratory results. The following is a review of some of our more recent findings of general interest and a discussion of the relations between the natural organic compounds in sea water and plankton blooms. Specific oceanographic aspects will be emphasized.

I wish to acknowledge the work of my colleagues, Mr. J. O. Bell, Mr. Alexander Dragovich, Mr. K. T. Marvin, Dr. Sammy Ray, Dr. T. J. Starr, Mr. W. B. Wilson, and Mrs. Zoula Zein-Eldin, who have made

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contributions to the information used in this paper.

NUTRITIONAL STUDIES ON DINOFLAGELLATES

Although we are studying the nutrition of dinoflagellates in general, our initial attempts concerned the isolation and continued culture of *Gymnodinium breve*, the causative organism of the Florida Red Tide (Wilson and Collier 1955, Ray and Wilson 1957). The composition of the most useful medium, and one which we are still using, is given in Table 1.

With this as a starting point we progressed from unialgal cultures to bacteria-free cultures (Ray and Wilson 1957) and have worked intensively to derive a defined medium. Many workers have put much effort into this problem, using a variety of organisms (Pringsheim 1946, Barker 1935, Hutner and Provasoli 1955, and Provasoli and Pintner 1953). Optimum limits for inorganic salts, trace metals, vitamins, amino acids, and miscellaneous growth factors have been determined empirically. Even with all this information and the results of our own work we are still left with the conclusion that some sea waters contain organic materials that make the addition of soil extract or vitamin B_{12} unnecessary.

Thus we became concerned with the biological controls which systems of aquatic organisms can exert on each other through the intermediary of organic substances. Through metabolites exuded by living cells, or decomposition products from dead cells, a population may exclude other populations, or promote or inhibit the growth of succeeding populations (Stevens 1949, Wilson 1951, Lucas 1949, and Collier 1953).

TABLE 1. *Medium for the unialgal isolation of* G. breve *Davis*

After all additions are made, there is approximately 110 ml of medium. (After Wilson and Collier 1955)

Aged sea water (salinity, about 36.5 ‰)	95.0	ml
Distilled water*	5.0	ml
NH_4Cl†	0.1	mg
KH_2PO_4†	0.05	mg
$MgCl_2 \cdot 6H_2O$†	0.02	mg
$Na_2S \cdot 9H_2O$†	0.1	mg
$NaHCO_3$†	0.1	mg
Vitamin B_{12}	0.1	μg
Thiamine hydrochloride	1.0	mg
Biotin	0.05	μg
Soil extract‡	2.0	ml
EDTA·Na (6 ml of 0.25-percent solution)	15.0	mg

* More or less may be required, depending on the salinity.

† Added as 0.5 ml of the following solution of the components of van Niel's medium for sulfur bacteria: NH_4Cl, 0.2 g; KH_2PO_4, 0.1 g; $MgCl_2 \cdot 6H_2O$, 0.04 g; $NaHCO_3$, 0.1 g; and $Na_2S \cdot 9H_2O$, 0.2 g to 1 liter of distilled water.

‡ Simmer for 40 min a mixture of 500 g of garden soil and 1 liter of distilled water. Let it stand for 4 days and decant the supernatant. Repeat simmering and decantation until extract is clear. Use the supernatant.

THE DEMONSTRATION OF BIOLOGICALLY ACTIVE COMPOUNDS IN SEA WATER

Collier *et al.* (1953), using a modified Dische reaction (Erdman and Little 1950), reported carbohydrate-like materials which quantitatively affected the pumping rate of oysters, and whose concentration in untreated water was influenced by light. They varied in concentrations from zero to over 20 milligrams per liter in the estuary studied. Wangersky (1952) reported the identification of dehydro-ascorbic acid and a rhamnoside from natural sea water. High concentrations of carbohydrates were found in rich marsh waters and in extreme concentrations of plankton organisms associated with a red tide outbreak in Florida (Collier 1953). These latter values were on the order of 50 mg per liter.

Interesting results of the study of the specific effects of commercially prepared organic compounds on various organisms were the motor responses elicited in oysters and barnacles by niacinamide and ascorbic acid, respectively (Collier *et al.* 1956). The effective concentrations were 0.042 mg per liter of sea water for niacinamide and 0.014 mg per liter for ascorbic acid. This is a further demonstration of the potential role of organic compounds in the marine community.

THE MEASUREMENT OF "CARBOHYDRATES" IN NATURAL SEA WATER

Before discussing the distribution of organic substances in the Gulf of Mexico and their production in laboratory cultures some analytical details should be clarified. The use of N-ethyl carbazole for the quantitative estimation of carbohydrates in marine waters was originally described by Erdman and Little (1950) and has been improved by Zein-Eldin and May (1957). Some time ago we found that in the waters with which we were working anthrone did not provide the sensitivity of N-ethyl carbazole, and as a result we adopted the latter as our standard reagent.

The analytical results are reported in terms of "milligrams per liter (arabinose equivalent)," but will be referred to hereafter as simply "carbohydrates." The reason for this is that we do not know what specific compounds are being measured, but only that on the hydrolysis of a sample we get a color response which can be quantitated according to Beer's Law. Zein-Eldin has plotted a series of absorption curves for a variety of hexoses and pentoses, and she kindly provided me with the figures used here to compare hexose and pentose curves with those obtained from naturally produced substances.

The absorption curve for a bacteria-free culture of *Prorocentrum* sp. is compared with the curves for arabinose and sucrose in Figure 1. The original traces were made with a recording spectrophotometer. The curve made from the culture clearly indicates a mixture of pentoses and hexoses.

The origin, significance, and molecular structures of these compounds raise some questions which need to be answered. Lewis and Rakestraw (1955) found that filtered samples of sea water do not yield significant quantities of carbohydrate in the Pacific. On the other hand, Plunkett and Rakestraw (1955) show heterogeneous distribution of dissolved organic materials in the Pacific as determined by Krogh's method for organic carbon. They considered these differences significant and concluded that since these materials represent the largest fraction of total organic matter in the sea, more intensive investigation is needed. Kay (1954), using the determination of elemental organic carbon, found a situation in the Kielerbucht similar to that reported by Plunkett and Rakestraw. After discussing his findings and pointing out conflicting arguments, he concluded that the whole matter must await clarification of the exudation of organic metabolites by the plankton organisms, particularly the algae. He considered the latter to be a plausible source of dissolved organic compounds in the sea. Vallentyne (1957) has given a comprehensive review and classification of organic compounds reported in the literature.

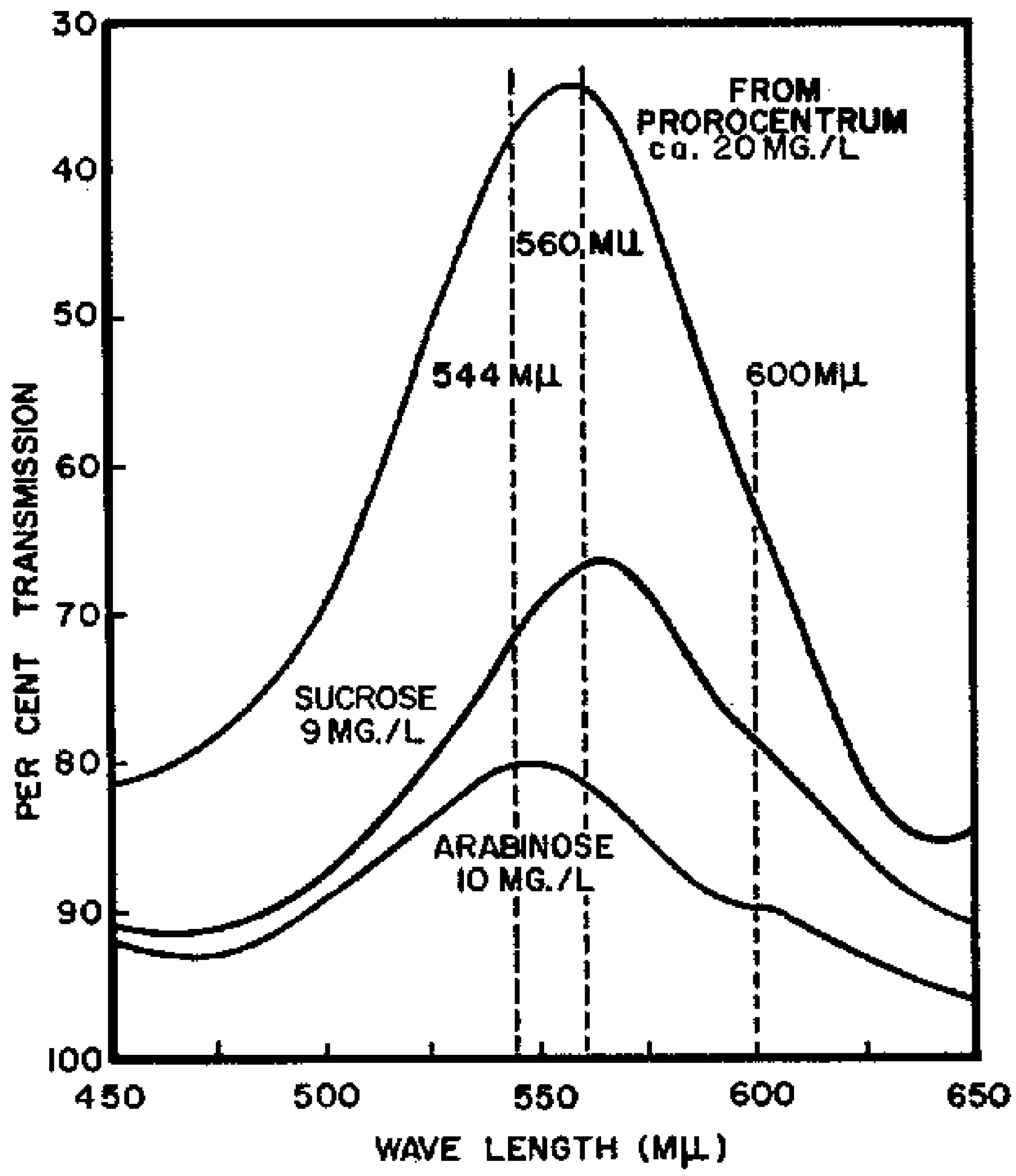

FIG. 1. Photometric responses of sucrose, arabinose, and culture of *Prorocentrum* sp. after treatment with N-ethyl carbazole. Concentrations are indicated on curves. Traces were made with recording spectrophotometer.

PRODUCTION OF ORGANIC SUBSTANCE IN LABORATORY CULTURES

In the preceding section I offered evidence that the carbohydrate responding to N-ethyl carbazole and produced by a single, specific organism in bacteria-free culture could be qualitatively classed with known sugars.

To understand the significance of measurements of these compounds made on samples from natural environments we should have some idea of the quantitative aspect.

For this purpose I present some results from studies on a 16-liter, unialgal (not bacteria-free) culture of *Prorocentrum* sp. which contained approximately 2.8 million cells per liter. The organisms were separated from the medium with a high-speed continuous-flow centrifuge. Although bacteria were present, it is doubtful that they contributed greatly to the carbohydrate values when *Prorocentrum* was by far the dominant organism. This situation is similar to that found by Goldberg *et al.* (1951) in their study on phosphate utilization by diatoms. Five independent carbohydrate determinations were made for the centrifugate, the residue, and the basal medium. The means of these determinations and their respective standard deviations are given here. A mean value of 0.72 ± .22 mg/L is given for the basal medium. This initial quantity is not subtracted from the final results because of the likelihood that it served as a carbon source for the organisms in the culture and was redistributed between them and their metabolites. The mean carbohydrate value for the whole culture was 9.58 ± .48 mg/L, and for the centrifugate 5.84 ± .44 mg/L. The mean difference of 3.74 ± .46 mg/L represented the portion of the material retained by those organisms not passing through the centrifuge. The total carbohydrate in the centrifugate was about 60% of that in the original culture, and estimating 10% of this as originating in crushed organisms or those not retained in the residue, this culture produced approximately 2 mg of non-particulate carbohydrate per million cells. In terms of the whole culture the production was 3.4 mg of carbohydrate per million cells. Similar experiments with *G. breve* and *Gymnodinium splendens* gave values of 2 mg and 1.6 mg, respectively, for the centrifugates. We cannot rely on either of the latter because the organisms were too fragile for an evaluation of their resistance to the mechanical effects of centrifuging. *Prorocentrum* is an armoured dinoflagellate and is more resistant than the naked forms mentioned above.

Lewin (1956) found that *Chlamydomonas mexicana* nom. prov., a mucilaginous freshwater flagellate, liberated as much as 0.1 gram per liter of soluble polysaccharide into the culture medium. This material constituted 20–25% of the total organic matter produced. While these values cannot be compared to our results from the *Prorocentrum* cultures, they indicate the magnitude of the potential role of algae in producing free organic substances in the aquatic environment.

THE DISTRIBUTION OF CARBOHYDRATES IN THE COASTAL AND ESTUARIAL WATERS OF THE GULF OF MEXICO

In earlier work Collier *et al.* (1953) found that carbohydrates in marsh waters fluctuated between zero and 25 mg/L. Such a range should have been expected in an area which was influenced both by the carbohydrate-poor waters of the open Gulf of Mexico and some highly productive marsh areas. The same work also showed a clear diurnal cycle of concentrations, with the maximum coming at midafternoon and the minimum after midnight. In samples taken from the high concentrations of *G. breve* in a red tide I reported (1953) some 50 mg of crystalline material per liter of water. This is not out of line with 44 mg/L of carbohydrates measured in a 16-liter unialgal (not bacteria-free) culture of *Prorocentrum*. Our evidence supports the idea that, in surface waters at least, some of these substances are produced by photosynthetic organisms, and when such organisms are in sufficiently dense aggregations, significant quantities of these substances will be indicated by the N-ethyl carbazole reaction.

The patchiness or highly uneven distribution of organisms in marine waters is well established (Barnes and Marshall 1951, Bainbridge 1953), and if the carbohydrates are associated with such aggregates of photosynthetic organisms we should find them similarly distributed.

In a series of oceanographic cruises in the Gulf of Mexico by the U. S. Fish and Wildlife Service research vessel ALASKA samples for the determination of carbohydrates, tyrosine, and organic phosphorus were collected at depths ranging from the surface to 2500 meters at stations distributed over the entire Gulf. There were over 800 determinations each of carbohydrate, tyrosine, and organic phosphorus in this series. A detailed study of this investigation will appear elsewhere, but it is helpful to present some of this information here to indicate how consistent are the results of organic analyses with heterogeneous plankton distribution.

On one of the cruises ALASKA encountered an area of steep salinity gradient that was indicative of a convergence of two dissimilar water masses. In the area there was "red water" caused by a heavy concentration of a ciliate, probably *Mesodinium pulex* (Slobodkin 1953). This bloom was well offshore and bore no resemblance to a fish-killing red tide such as is caused by *G. breve*. The concentration of the organisms is consistent with the mechanisms surveyed by Ryther (1955). In samples of water collected from this station (preserved by quick freezing and neither filtered nor centrifuged) we got the following values: carbohydrates 3.6 mg/L, tyrosine 1.4 mg/L, and organic phosphorus 6.7 microgram-atoms PO_4-P/L. At a station in clear water outside of the ciliate concentration we obtained the following values: carbohydrates 0.0 mg/L, tyrosine 0.3 mg/L, and organic phosphorus 1.0 microgram-atoms PO_4-P/L. These differences demonstrate the association of high carbohydrates, tyrosine, and organic phosphorus values with high concentration of organisms.

Table 2 illustrates the frequency distribution of carbohydrates, tyrosine, and organic phosphorus in all samples taken within the 100-fathom curve by M/V ALASKA. By inspection one can see that these distributions are similar to those shown by Barnes and Marshall (1951) to characterize plankton aggregations. This similarity may be interpreted to mean that the concentration of organic substances represented by the carbohydrate, tyrosine, and organic phosphorus tests were generally low, but when they did occur the concentrations were relatively high, or, in other words, there was a tendency towards the contagious distribution (Barnes and Marshall 1951).

TABLE 2. *Frequency distributions of carbohydrates, tyrosine, and organic phosphorus for samples collected over continental shelf of Gulf of Mexico by M/V Alaska*

Carbohydrates			Tyrosine			Organic Phosphorus		
mg/L	n	%	mg/L	n	%	µg-at. PO_4-P/L	n	%
.0	68	23.2	.0	63	19.7	.0	13	6.7
.2	37	12.6	.2	73	22.8	.2	35	18.1
.4	41	14.0	.4	63	19.7	.4	34	17.6
.6	32	11.0	.6	41	12.8	.6	32	16.6
.8	24	8.2	.8	32	10.0	.8	31	16.1
1.0	22	7.5	1.0	11	3.4	1.0	15	7.8
1.2	20	6.8	1.2	16	5.0	1.2	11	5.7
1.4	10	3.4	1.4	10	3.1	1.4	3	1.6
1.6	14	4.8	1.6	3	0.9	1.6	0	—
1.8	8	2.7	1.8	6	1.9	1.8	4	2.1
2.0	7	2.4	2.0	2	0.6	2.0	4	2.1
2.2	1	0.3	—	—	—	2.2	5	2.6
2.4	2	0.7	—	—	—	2.4	2	1.0
2.6	3	1.0	—	—	—	2.6	2	1.0
2.8	2	0.7	—	—	—	2.8	0	—
3.0	2	0.7	—	—	—	3.0	2	1.0
	293	100.0		320	99.9		193	100.0

ORGANIC COMPOUNDS AND RED TIDES

We may now deal more specifically with the Florida Red Tide. Over a period of several months a series of stations along the Florida coast has been sampled for carbohydrates, proteins, and other chemical entities. These data will receive a more detailed treatment elsewhere, but I would like to mention some of the outstanding features of the analysis in terms of the genesis of red tides.

There were 184 pairs of protein and carbohydrate determinations used in this study. The extremes of carbohydrate values were 0.0 and 19.4 mg per liter; of tyrosine, 0.0 and 2.9 mg per liter. The highest concentrations in both cases were found in rivers, tidal streams, and marshes bordering the estuaries; the concentration generally diminished in a seaward direction, with the lowest values occurring in the offshore waters of the Gulf. The latter were within the general range of the quantities reported from the ALASKA cruises.

At this point the most important feature is the erratic nature of the distributions of these two classes of compounds and, as pointed out above, the probability that they are directly related to the distribution of microplankton.

Organic compounds provide a substrate for bacterial activity and can serve as growth promoters for animal and plant components of the plankton. A bloom of algae can charge the water with sufficient organic material to support a growth of bacteria and/or to condition the water for succeeding species. We have found that a particular bacterial colony type always associated with our unialgal cultures of *G. breve* as the dominant bacterium is a producer of vitamin B_{12}, a growth promoter. This bacterium will not develop to a position of dominance in the absence of *G. breve*. Here, then, is at least an experimental system in which the alga is probably supplying an organic substrate which indirectly conditions the water for its own growth. In combination with the distribution of organic compounds detailed above, this observation leads to some experiments similar to those conducted by Wilson (1951). The following is the summary of his paper: "Eggs and larvae of *Echinus esculentus*, *Ophelia bicornis* and *Sabellaria alveolata* developed abnormally, or were in poor health, in seawater collected from the English Channel in the region of the Eddystone, although in water collected from the Celtic Sea development was generally normal and healthy. Experiments indicated that the Channel water lacked some unknown constituent, essential for healthy development of these species, present in the Celtic Sea. The results accord with some of the hydrographical and biological changes in the area which have taken place within recent years."

Mr. W. B. Wilson of our laboratory at Galveston has been conducting growth experiments with cultures of *G. breve* using sea water collected at different times from various points as a base for his culture media. At one extreme some of these waters are outright toxic to the organisms, and at the other the waters will support luxuriant growth with no supplementary additions. There are intermediate types which cause response after treatment with additives ranging from a single item of the above formula (Table 1) to the whole list. The extremes may come from samples collected at the same time only a few miles apart.

We have found that sulfides will substitute for organic chelators and that copper is highly toxic to *G. breve*. Sulfides were used in the original medium for the artificial culture of *G. breve* (Table 1). The mangrove-covered marshes that border the estuaries of the Florida west coast are prolific producers of hydrogen sulfide, and the natural copper concentration in the coastal waters is extremely variable. The interplay of these two factors is possibly active in establishing the level of toxicity of untreated sea water to this and other organisms. An additional factor may be the action of natural organic compounds as chelators, because they themselves can act as copper binders. Thus it may be possible for them simultaneously to detoxify water and to furnish growth promoters for the more sensitive members of the marine plankton.

A constant surveillance in Florida waters over the past three years has shown that *G. breve* is present in very low concentrations at one point or another along the Florida coast most of the time. Mr. W. B. Wilson has successfully isolated and cultured the organism from waters within the city limits of Galveston, Texas. Wilson and Ray (1956) found blooms of the same organism associated with large fish kills off the South Texas and Mexican east coasts, and again it was successfully isolated and cultured. From these findings we conclude that the organism may normally exist over a considerable geographic range in very low numbers.

Its sensitivity to unfavorable conditions keeps it in check and prevents a more frequent occurrence of Red Tides. However during the rainy season the introduction of large volumes of land drainage with heavy loads of organic materials can provide the initial stimulus for heavy blooms of the organism, which in turn may augment themselves with both inorganic and organic materials produced by the decomposition products of dead fish (Wilson and Collier 1955).

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