Production of Red Tide in the Laboratory

GLADYS S. KING
Research Assistant, Marine Laboratory, University of Miami and Collaborator, U.S. Fish and Wildlife Service.

IN A PAPER presented at the Institute meeting last year, Dr. Gordon Gunter (1) described general aspects of the Red Tide in Florida. It was mentioned that the 1946-1947 Red Tide, which entailed the loss of millions of pounds of fish, was caused by an outburst of microscopic marine organisms. The organisms were identified as naked dinoflagellates and scientifically named *Gymnodinium brevis* (2,3). Reports of dinoflagellates which have caused similar water discolorations and fish mortalities are available from many parts of the world with recorded occurrences in Florida as early as 1840 (4).

The work to be discussed is a part of a comprehensive research program started about a year ago, on red water developments in this area. Cooperating in the program are the Gulf Investigations, U.S. Fish and Wildlife Service located in Sarasota and the University of Miami Marine Laboratory. One part of the program is a detailed investigation of biological, physical and chemical conditions of Gulf water to detect variations which may possibly be related to Red Tide outbreaks. Another part is the work to be described—a study of methods for growing Red Tide and related organisms in the laboratory. By means of such experimentation under controlled laboratory conditions, it is possible to learn factors which are essential for the growth of the organisms. These same factors may be expected to be required for their existence in the Gulf.

So far in the course of this research it has been impossible to locate specimens of *Gymnodinium brevis*. Another species of *Gymnodinium, G. simplex*, and also a Protozoan of another class, a ciliate, *Plagicampa marina*, are employed for the laboratory studies. The organisms were isolated from plankton samples collected in the Gulf. Both are microscopic and have conspicuous green chromatophores as described for *G. brevis*.

Early in the study it was found that *G. simplex* and *P. marina* grew very well in a nutrient composed of sea water, natural or artificial, supplemented with yeast extract. The organisms have been in continuous culture in this nutrient since first isolated nearly a year ago, without signs of waning vitality. The artificial sea water is prepared after a formula of McClendon (5) with minor elements introduced by additions of Hoagland's A-Z solution (6). The fact that other Protozoan taken from the Gulf also thrived in this yeast extract nutrient may recommend it as an enrichment medium for marine Protozoa.

Soon after isolation, *G. simplex* and *P. marina* were made bacteria-free: *Gymnodinium* by use of 2,4-dichlorophenoxyacetic acid and *Plagiocampa*, by particular exposures to ultra violet radiation. The cultures are at present maintained in test tubes incubated at 30° C and subjected to water-filtered light having an intensity of approximately 40 ft. candles for a photoperiod of 12 hours.

On the assumption that these plankton species having chloroplasts live essentially as green plants, with particular requirements of phosphates, nitrates and nitrites, extended studies were made to determine the effects of inorganic phosphorus and nitrogen on their growth. Phosphorus and nitrogen were added to both natural and artificial sea water, with and without yeast extract, in various forms: phosphorus as H_3PO_4, K_2 H PO_4 and Na H Po_4; nitrogen as NH_4OH,

$Ca(NO_3)_2$ and $NaNO_2$. Concentrations ranged from one to 200 times the amounts of these elements recorded to occur normally in sea water. No growth promoting effects were found for any of the additions made and it was demonstrated conclusively that the cells could not survive in a nutrient containing only inorganic constituents.

In a later phase of the study, the value of yeast extract in sea water for growing *G. simplex* and *P. marina* was found to be attributable to the amino acids of yeast. With this discovery, it is now possible to grow the organisms in a

TABLE I

COMPOSITION OF SYNTHETIC MEDIUM FOR GROWTH OF *G. simplex* AND *P. marina*.

CONSTITUENTS		G/LITER
*a 1. NaCl		26.0000
2. $MgCl_2$		2.2600
3. $MgSO_4$		3.2480
4. $CaCl_2$		1.1530
5. KCl		0.7210
6. $NaHCO_3$		0.1980
7. NaBr		0.0580
8. H_3BO_3		0.0580
9. Na_2SiO_3		0.0024
10. $Na_2Si_4O_9$		0.0015
11. H_3PO_4		0.0015
12. Al_2Cl_6		0.0130
13. NH_3 (as NH_4OH)		0.0020
14. $LiNO_3$		0.0013
*c 15. $FeCl_3$		
16. MoO_3		
*b 17. $Co(NO_3)2 \cdot 6H_2O$		
18. $CuSO_4 \cdot 5H_2O$		
19. $ZnSO_4$	Total	0.0010
20. $SnCl_3 \cdot 1H_2O$		
21. $MnSl \cdot 4H_2O$		
22. $NiSO_4 \cdot 6H_2O$		
23. Ti_2O		
24. KI		
25. Arginine		0.2000
26. Histidine		0.0500
27. Isoleucine		0.1500
28. Leucine		0.2000
29. Lysine		0.2000
30. Methionine		0.0500
31. Phenylalanine		0.1000
32. Threonine		0.1500
33. Trypotophane		0.0500
34. Tyrosine		0.1500
35. Valine		0.2000
36. Cystine		0.0500
	Total	35.2692
	Distilled water to 1000 ml.	
	pH = 8.4	

*a *Constituents 1-14 modified after McClendon (1917) formula for artificial sea water.*
*b *Constituents 17-24, from Hoagland's A-Z solution for green plants.*
*c *Preparation of constituents 15-24.*

15. $FeCl_3$	0.080g	per 100 ml, 1 ml per liter of nutrient.
16. MoO_3	0.007g	
17. $Co(NO_3)_2 \cdot 6H_2O$	0.10g	per 100 ml, dilute 1:18 and of this, use 1 ml per liter of nutrient solution.
18. $CuSO_4 \cdot 5H_2O$	0.10g	
19. $ZnSO_4$	0.10g	
20. $SnCl_3 \cdot 1H_2O$	0.05g	
21. $MnCl \cdot 4H_2O$	0.70g	
22. $NiSO_4 \cdot 6H_2O$	0.10g	
23. Ti_2O	0.10g	
24. KI	0.05g	

nutrient which is entirely synthetic. The nutrient contains artificial sea water and amino acids added as pure chemicals in ratios analyzed for yeast (7, 8, 9). The exact composition of this medium is given in Table 1.

The amino acid requirements demonstrated by *G. simplex* and *P. marina* lead to a consideration of the classic view of Pütter (10) who endeavored to show that dissolved organic nitrogenous compounds and other dissolved organic matter, play an important role in the nutrition of aquatic organisms. The arguments and observations, pro and con, concerning this important problem, have been reviewed by later investigators, particularly Krough (11). Krough concluded that the large quantity of dissolved organic nitrogenous matter shown by his own analyses to be in solution in sea water, provided there by excreta of animals, decomposition of dead animals, dissolving phytoplankton and other microorganisms, is unsuitable as food for living marine organisms. Thus the material is lost out of organic circulation. Krough does suggest the possibility that a microfauna as yet unknown may exist in the sea which is able to utilize the dissolved material.

In the present investigation it is shown clearly that the marine organisms studied, *G. simplex* and *P. marina,* are not only able to utilize certain organic nitrogenous material when dissolved in sea water, but actually require such substances in their nutrition. This organic nitrogen dependence suggests the possibility that the organisms may be members of that particular fauna in the sea, referred to by Krough, which functions as an important link between the abundant organic nitrogenous material in solution and particulate protein constituents fed on by more complex and larger marine animals. The apparent value of marine microorganisms in this role agrees with conclusions proposed by present-day planktologists (12), that nannoplankton have a critical part in the cycle of life in the sea.

Aside from this academic interpretation of results found to date, a practical application is the provision of a basis for recommending an investigation of dissolved organic nitrogenous matter in Gulf water as a clue to outbursts of Red Tide dinoflagellates.

REFERENCES

1. GUNTER, G. 1948. *The Red Tide and the Florida Fisheries.* Proc. Gulf and Caribbean Fisheries Inst. Inaugural Session: 31-32.
2. DAVIS, C. C. 1948. *Gymnodinium brevis* sp. nov., a cause of discolored water and animal mortality in the Gulf of Mexico. Bot. Gaz. 109:358-360.
3. FLORIDA STATE BOARD OF CONSERVATION AND UNIVERSITY OF MIAMI MARINE LABORATORY. 1948. *The Red Tide.* Educational Series No. 1.
4. GALTSOFF, PAUL S. 1949. *The mystery of the Red Tide.* Sci. Mo. 68:109-117.
5. SVERDRUP, H. U., et al. 1942. *The oceans, their physics, chemistry, and general biology.* Prentice Hall, Inc. Publ. p. 186.
6. MEYER, B. S. and ANDERSON, D. B. 1938. *Plant Physiology.* D. Van Noskand Co. Inc. Publ. p. 431.
7. BLOCK, R. J. and BOLLING, D. 1940. *The determination of amino acids.* Burgess Publ.
8. BLOCK, R. J. and BOLLING, D. 1943. *The amino acid yield from various animal and plant proteins after the hydrolysis of the fat free tissue.* Arch. Biochem. 3:217-226.
9. CSONKA, F. A. 1935. *Proteins of yeast.* Jour. Biol. Chem. 109:703-715.
10. PETERSON, W. H., FRED, E. P. and DOMOGALLA, B. P. 1925. *The occurrence of amino acids and other organic nitrogen compounds in lake water.* Jour. Biol. Chem. 63:287-295.
11. KROUGH, AUGUST. 1934. *Conditions of life in the ocean.* Ecol. Monog. 4:421-439.
12. RILEY, G. A. and BUMPUS, D. F. 1946. *Phytoplankton - zooplankton relationships on Georges Bank.* J. Mar. Res. 6:33-47.