DISCUSSION

FLUORESCENCE OF CELLS IN THE ULTRA-VIOLET

THE corpse-like appearance of human skin due to fluorescence in the ultra-violet is familiar to all who recall the mercury lamps formerly used in photography.

Fluorescence of living tissues may have been observed long ago, but it was not understood until after Stokes explained the nature of fluorescence in physical systems in 1852.[1] As early as 1855 Helmholtz[2] had made a careful study of fluorescence of the lens of the eye and considered the wave-lengths 3000–4000A most effective in producing fluorescence. Hertel[3] in 1905 showed that fluorescence of the cornea of the rabbit and of human skin is apparently strongest at 2320A, less distinct from 2320 to 2800A, rather feeble at 3830A, but can be detected even in the visible light at 4480A through a yellow-green filter which absorbs all the reflected 4480A. In 1909 Schanz and Stockhousen[4] reported that fluorescence of the lens of the eye begins in the blue, is more intense in the violet, and becomes most intense in the ultra-violet between 3700 and 4000A, with a maximum at 3850A. Walker[5] summarizes studies on the fluorescence of eye tissues. Stübel[6] in 1911 studied the fluorescence of a great number of other tissues, tissue products, protozoa, bacteria and various organic materials in light screened to give the wave-lengths 3000–4000A. He finds that the color of fluorescent light for different tissues varies from yellow to blue.

It seems of interest to report fluorescence of Paramecium, Oxytricha and a bacterium in the monochromatic light of the wave-lengths of the quartz mercury arc.

Paramecium multimicronucleata and Oxytricha fallax, cultured on a 0.1 per cent. lettuce infusion, were concentrated by centrifuging gently, washed in a balanced salt solution,[7] and again concentrated by centrifuging. They were then pipetted into a quartz-faced cell. Observations were made through a binocular microscope (20x) at right angles to the incident beam of light coming through a quartz monochromator from the arc.[8] The whole set-up was enclosed in a black drape to exclude stray light during observations.

The protozoans fluoresced at all the ultra-violet lines used. At 3660A the fluorescence was of a pale greenish gray shade. At 3350, 3130 and 3020A the color was more of a pale greenish blue. At 2804 and 2654A the protozoans fluoresce with a more whitish light, and as they dart into the beam of light appear like so many fireflies on a dark summer's night. At 2537A the color was again a pale greenish blue. Varying the size of the slit to change the intensity of the lines employed showed that at about equal intensities of incident radiation, fluorescence was of maximal intensity at 2804 and 2654A, of lesser but comparable intensity at 2537, 3020 and 3350A, and apparently minimal at 3130 and 3660A. The intensity of fluorescence was merely judged by the effects upon the eye of the observer.

A pseudomonad bacterium isolated from a lettuce infusion was observed in suspension in the quartz cell placed in the beam of ultra-violet light. A pale blue-green fluorescence occurred throughout the range of ultra-violet wave-lengths, but as the individual bacteria could not be seen in the set-up used, the degree of fluorescence at different wave-lengths could not be compared.

Hertel[3] states that the cornea ceases to fluoresce on death. Renschler[9] says Paramecia killed by the arc light fail to fluoresce. To determine whether death is always followed by cessation of fluorescence, protozoan cells were killed in several ways. Coagulated rapidly by the full light of the arc excluding only the heat, Paramecia showed decreased fluorescence. Killed by low intensities of monochromatic ultra-violet radiation, the Paramecia cytolized rapidly. The medium then became highly fluorescent and it was impossible to determine the nature of fluorescence of partially cytolized Paramecia or of the protoplasmic fragments. Coagulated by adding a few drops of 1.0 N HCl to the cell, the Paramecia showed greatly decreased fluorescence with apparent extinction at 3660A. But when heated until they just coagulated and studied in the ultra-violet light, Paramecia showed slightly increased fluorescence at 2537 and 2654A and little change from 2804 to 3660A. It appears then that fluorescence, of dead protozoan cells at least, depends upon the method employed in killing.

Fluorescence is chiefly of interest as one of the secondary processes following excitation of the electrons in the molecules of protoplasm by absorption of radiation. It will be noted that the abiotic wave-lengths (3000A and shorter) are strongly fluorescent. Part of the energy absorbed is emitted as light of longer wave-lengths probably without producing photochemical effects. Therefore fluorescence should

[1] Stokes, *Phil. Trans.*, p. 558, 1852.
[2] Helmholtz, *Pogg. Ann.*, 94: 205, 1855.
[3] Hertel, *Zeitschr. f. Allgem. Physiol.* 5: 95, 1905.
[4] Schanz and Stockhausen, *Arch. f. Ophthal.*, 73: 561, 1909.
[5] Walker, *Proc. Am. Acad. Sci.*, 51: 760, 1915–1916.
[6] Stübel, *Pflüger's Arch. f. d. Ges. Physiol.*, 142: 1, 1911.
[7] Barker and Taylor, *Physiol. Zool.*, 4: 620, 1931.
[8] Leighton and Blacet, *Jour. Am. Chem. Soc.*, 54: 3165, 1932.
[9] Renschler, SCIENCE, 73: 480, 1931.

be taken into account in any quantitative theory of the effects of ultra-violet light.

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THE BORON CONTENT OF SEA WATER

DURING the last few years the concentration of boron in sea water has been the subject of a great deal of interest and not a little speculation on the part of workers concerned with certain phases of oceanographic chemistry. Attention to this element has been directed principally by a number of investigations of the buffer mechanism, *i.e.*, the factors that determine and regulate the hydrogen-ion concentration of sea water. In this mechanism are involved the salts of the various weak acids known to occur in sea water, namely, carbonates, bicarbonates, phosphates, arsenates, silicates and borates. However, according to the information available until very recently, only the salts of carbonic acid, and in a few localities also the silicates, are present in sufficient quantity to require notice in studying the buffer system. Nevertheless, it has been found that the behavior of the buffer mechanism in sea water is not in accord with the theory of carbonate solutions, even when allowance is made for the activity of the ions of the strong electrolytes present. To explain this discrepancy the possibility of the occurrence of buffer salts other than those of carbonic acid in appreciable quantities has been considered but, as previously stated, this possibility has not been supported by the existing analytical data. For the phosphate, arsenate and silicate reliable data have been available for a number of years, but for the borate content only one value has been reported, namely, in 1877 by Dieulafait[1] who estimated that water from the Mediterranean contains approximately 0.0002 g. per liter of boron. This is equivalent to about 0.02 millimoles of boric acid, a quantity of no significance in the buffer mechanism, since it is only 1 per cent. of the molar concentration of the total carbon dioxide.

There was also reason to suspect that the boron content of sea water varied considerably with locality because in 1859 Veatch[2] reported to the California Academy of Sciences that he had detected boron in water from along the coast between San Diego and the Strait of Juan de Fuca, but not in water from beyond fifty or sixty miles off the California coast. He found boron most abundant toward the South and concluded that in certain localities it enters the sea from submarine volcanic sources. The absolute quantities of boron found were not indicated.

[1] L. Dieulafait, *Comptes Rendus*, 85: 605–608, 1877.
[2] J. A. Veatch, *Proc. Calif. Acad. Sci.*, 2: 7, 1859.

The reason that the concentration of boron in sea water has not been adequately investigated long ago is that until recently there has been no satisfactory method for its determination. In 1932 Foote[3] described a titration method suitable for determining small quantities of boron in water but this was soon thereafter modified by Wilcox[4] who used a quinhydrone electrode for detecting the titration endpoint. Foote also determined the boron content of a sample of sea water from Ventura, California, and found it to be 4.27 parts per million.

In this laboratory the electrometric titration method of Wilcox has been used, with minor modifications necessitated by the difference in salt content between sea water and the water for which the method was developed, for analyzing about fifty samples of sea water obtained from various depths from the surface down to 500 meters off La Jolla and to 3,200 meters near the Hawaiian Islands and from the surface in several other localities, including northern California and Tortugas, Florida. In these samples the boron content varied from 4.30 to 4.80 mg. per kilogram, the average being close to 4.50 mg. This variation corresponded very closely with the variation in the salinity of the water, the average ratio between boron and halides being 0.000239, or, stated in other words, boron constitutes about 0.013 per cent. of the total solids in sea water. As boric acid this corresponds to about 0.4 millimoles per liter or, if boric acid is regarded as monobasic, to nearly 20 per cent. of the equivalent concentration of all the weak acid radicals present.

It is therefore probable that boron compounds play an important part in regulating the hydrogen-ion concentration of sea water and that some of the conclusions drawn from previous studies of the buffer system will need to be revised. These matters will be discussed in a subsequent paper.

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PERSISTENT STRANDS OF THE ROOT-ROT FUNGUS IN TEXAS

THE ability of the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar,[1] to remain in the soil in a viable and infectious condition for a period of years, even when the fields are planted to

[3] F. J. Foote, *Ind. and Eng. Chem., Anal. Ed.*, 4: 39–42, 1932.
[4] L. V. Wilcox, *Ind. and Eng. Chem., Anal. Ed.*, 4: 38, 1932.
[1] B. M. Duggar, "The Texas Root-Rot Fungus and Its Conidial Stage," *Ann. Missouri Bot. Gard.*, 3: 11–23, illus., 1916.