measurements at 340 mμ also indicate that FMN is essential for DPNH oxidation, although most of this activity depends upon a nonluminescent reaction. In the presence of excess DPNH and FMN, the partially purified preparations rapidly lose their ability to emit a bright light. However, the supernatant from a fresh, boiled enzyme preparation rapidly restores light production, thus indicating the presence of a heat-stable factor that is essential for luminescence. This is analogous to the destruction of both firefly and *Cypridina* luciferins during luminescence, suggesting, therefore, that this substance can be legitimately classified as bacterial luciferin.

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Extinction of Light by Filter Passing Matter in Chesapeake Bay Waters[1, 2]

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Over one hundred water samples were tested for the presence of dissolved coloring matter (*1*) in order to determine whether coloring matter in solution is of importance in the extinction of light in Chesapeake Bay waters. These samples were taken during the months of April and August, 1951, at stations located in Chesapeake Bay and adjacent sounds and rivers. Most of the samples were obtained from the surface, a few from a 10-ft depth, and, at four stations, from the surface to the bottom at 10-ft intervals. To avoid changes due to biological activity during storage (*2*), the samples were processed and analyzed immediately after being pumped aboard. As soon as filtering with a fine Berkefeld filter was completed, the absorbency (A_s) (*3*) of the sample was determined at 20- to 25-mμ intervals of wavelength with a Beckman model DU spectrophotometer having a 50-cm liquid absorption cell.

Examination of the typical absorbency curves for filtered samples in Figs. 1 and 2 shows a lack of significant color absorption peaks. The regular increase in absorbency with decreasing wavelength becomes of measurable magnitude at approximately 550 mμ and can be partially accounted for by the presence of fine filter passing suspensoids which, when combined with whatever dissolved coloring matter is present, act as a yellow substance (*4*) by absorbing and scattering light from the shorter wavelength end of the visible spectrum. The filter passing materials caused the greatest extinction in the violet, less in the blue, and tend to become unimportant in the green.

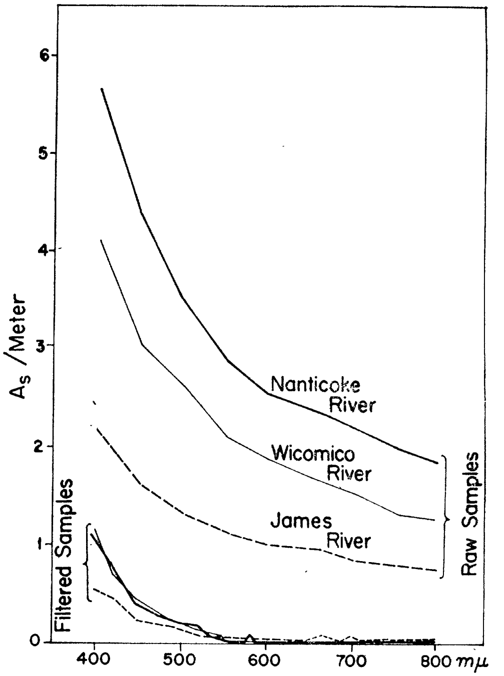

FIG. 1.

It should be noted that the color curves for samples

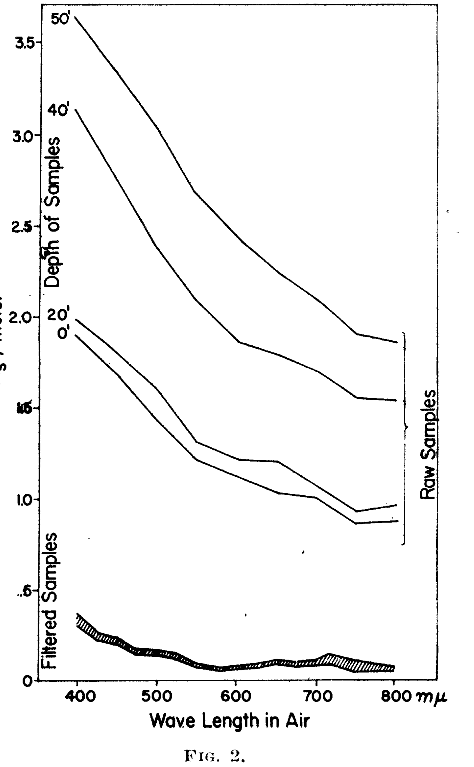

FIG. 2.

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[1] Contribution No. 13 from the Chesapeake Bay Institute.

[2] Results of work carried out for the Office of Naval Research of the Navy Department, the State of Maryland (Dept. of Research and Education), and the Commonwealth of Virginia (Virginia Fisheries Laboratory).

from the Wicomico and Nanticoke rivers, both of which drain extensive swamp areas, indicate the presence of greater amounts of filter passing material than found in the samples from the lower Bay and the James River. This seems reasonable since we would expect greater amounts of organic material to be present in these two rivers.

Each time a sample was drawn for color determination, part of it was left unfiltered and was placed in a second spectrophotometer fitted with 10-cm cells. Measurements comparable to absorbency per meter were obtained at 50-mμ intervals of wavelength across the same part of the spectrum as that for which color determinations had been made. These measurements, which take into account both the filter passing material and the nonfilter passing suspended matter, were then compared with the absorbency values for the corresponding filtered samples. The upper sets of curves in Figs. 1 and 2 are typical examples of untreated samples. The narrowness of the envelope for the color curves on Fig. 2 shows that filtering effectively eliminates the optical difference between samples taken at various depths.

The primary factor in light extinction in Chesapeake Bay waters is the particulate matter which can be readily filtered out. The river data in Fig. 1 show filterable material to be at least four times as effective at all wavelengths as the filter passing material. In open bay samples, maximum effectiveness of the filter passing material was approximately one-fifth that of the filterable material.

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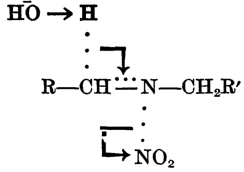
Comments and Communications

The Second Order Elimination of Nitrous Acid from Secondary Nitramines

In a recent discussion (*1*) of the Hofmann and Saytzeff rules for elimination reactions it has been proposed that the operation of the Hofmann rule in bimolecular eliminations may signify the dominant importance of steric effects, the Saytzeff rule being more general and implying electromeric control. A case of Hofmann elimination in an E1 reaction has also been attributed to steric factors, and this has led to the generalization that all types of eliminations may be governed by the Hofmann rule when steric factors are important (*2*). This contrasts with the view of Hughes, Ingold, and co-workers (*3*) that the Hofmann rule implies inductive control of elimination and is in force in E2 reactions in which the atom of the departing group which is involved in the bond-breaking carries a positive charge, as in $-\overset{+}{N}R_3$, $-\overset{+}{S}R_2$, $-\overset{+}{P}R_3$, and $-\overset{+}{S}\overset{-}{O}_2R$; the Saytzeff rule is then considered to be applicable to all other cases of E2 and to "unimolecular" eliminations. Swain and Scott's (*4*) interpretation of the Hofmann rule also involves attack on the most acidic hydrogen, and thus refers to inductive control.

An interesting case of E2 elimination which has apparently been generally overlooked and which may be pertinent to this discussion is that of the loss of nitrous acid from secondary nitramines under the influence of alkali. These reactions were studied some years ago (*5–9*), and of the mixed aliphatic secondary nitramines employed, the predominant products were always such as to lead to the enunciation of the rule that the larger group formed an amine, the other radical resulting in an aldehyde. With benzyl and substituted benzyl derivatives the rule was reversed. The reactions were assumed to involve an intermediate imine, and it was shown (*8*) that under the experimental conditions an imine would decompose to give the products. Further, the reaction of alkali with a secondary nitramine is second order and nitrite ion is produced in the rate determining step, or, conceivably, in a subsequent rapid reaction (*10*). Hence, by analogy with other second order eliminations, such as the structurally similar alkaline decomposition of *p*-toluenesulfonbenzylmethylamide (*11*),[1] it seems quite reasonable that the transition state may be represented by:



If this is the case, the results of the orientation experiments are all interpretable by the Hofmann rule, the benzyl group reversing the results, as in other systems (*3*). The geometries of the $> C=C <$ and $> C=N\diagup$ systems seem sufficiently similar to permit comparisons, and hence it appears difficult to attribute

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[1] It may be noted that the present system is a nitrogen analogue of the carbonyl elimination reaction ($E_{CO}2$) of organic nitrates investigated by J. W. Baker and D. M. Easty (*J. Chem. Soc.*, 1208 [1952]). In the latter instance there was no possible alternative product, and no obvious steric difficulty; there was evidence of inductive influences in the relative rates of the methyl, ethyl, and isopropyl compounds.