THE RELATION OF pH TO THE ABSORPTION OF DYES BY BACTERIA

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According to the theory of Stearn and Stearn[2], bacteria behave as conjugated proteins, i.e., they are amphoteric in nature and are, therefore, able to react with either acidic or basic substances. If this is the case, the absorption of acidic and basic dyes by bacteria would depend not only on the nature of the dye but also on the hydrogen-ion concentration of the solution in which the absorption took place. At high pH values, or in basic solution, the bacterial cells would tend to ionize as acids and would readily combine with basic dyes but not with acid dyes. At low pH values the conditions would be reverse.

Stearn and Stearn, and other investigators, have carried out a number of experiments which indicate the validity of this theory. They also explain the function of mordants in staining as being one of oxidation. Oxidation renders the protoplasm more acid in nature and, therefore, increased its capacity for combining with a basic dye.

If it is assumed that the Stearn hypothesis holds true for living organisms as well as for dead, the pH value of the medium should be a determining factor in the absorption of a given dye by a living organism provided, of course, that the pH is either above or below the isoelectric range of the organism and, provided further, that the organism can life and grow in such a medium. The lower the pH value of the medium, the greater should be the tendency of the organism to absorb an acid dye and the higher the pH the greater should be the tendency to absorb a basic dye.

During the course of an attempt to find some differentiating characteristics between *Pseudomonas tumefaciens* and *Radiobacter* some interesting reactions of these organisms with acid and basic dyes were noted. While no differenting characteristics were found yet all of these organisms reacted with one of the dyes used in a manner opposite to that which might be expected if the Stearn hypothesis were correct. A brief outline will be given of the work done and an attempt will be made to explain the apparent anomalous behavious of these organisms with the dye, congo red.

Strains of *Pseudomonas tumefaciens* and *Radiobacter* were grown on agar plate cultures containing congo red. After nine days growth most of the plates contained colonies which were either all red, all pink, or all colorless. A few plates contained all three types. On re-innoculation from individual colonies using similar media the resulting growths were found in every case to have the same color as the original colony. *Since congo red is an acid dye, it should, according to the Stearn hypothesis, be best absorbed when the colonies had produced an acid reaction in the medium.* Accordingly, innoculations were made from colonies which showed the greatest degree of color absorption, in litmus milk. However, after fifteen days no fermentation of the milk could be discerned and the pH recorded was 7.6. Several other strains which gave pink or colorless colonies in the plate culture lowered the pH of litmus milk cultures considerably more than the colonies of decided red color.

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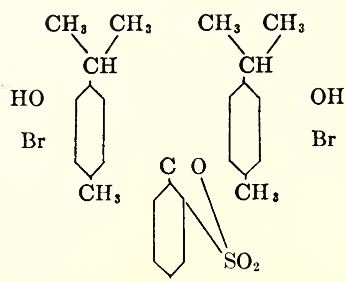
[1]Deceased.
[2]Stearn & Stearn. Jour. Bact. 9:5. 1924.

The absorption of the acid dye, from thymol blue, by these organisms was also studied. Strains were placed on mannitol agar slants. In several cases the reaction became quite acid and in such case considerable quantities of the dye were absorbed by the colonies, coloring the clumps a decided orange-yellow. Using several different sugars the same phenomenon was noted, this dye being best absorbed when the reaction had become somewhat acid. Therefore, this dye behaved as would be expected if the Stearn hypothesis is held.

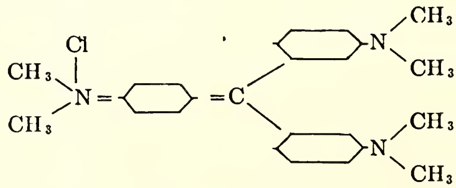
Strains of these organisms were next grown in peptone broth medium. To one set of tubes was added crystal violet, a basic dye, to another set, brom thymol blue and to the third, congo red. The pH values were also adjusted so as to give cultures with each of the dyes having pH values of 3, 4, 5, 6, 7, and 8. It would be expected in accordance with the theory that the two acid dyes would be best absorbed at low pH values while the basic dye would be best absorbed at a high pH value. Both congo red and brom thymol blue precipitated out of the solution at pH values of 3 and 4 so that no results could be obtained for these values. At pH 5 brom thymol blue was obviously absorbed by the organisms leaving the solution lighter. At higher pH values the absorption was progressively less. On the other hand, congo red seemed to be equally well absorbed at pH 6, 7, and 8. Crystal violet was definitely better absorbed at pH values of 7 and 8 than at lower values. Thus, the two dyes brom thymol blue and crystal violet behave in accordance with the theory while congo red seems to have little correlation with pH.

A study of the structural formulae of these three dyes throws some light on the apparently peculiar behavior of congo red. Brom thymol blue is unquestionably acid in nature since it contains both a sulfonic group and an acid hydroxy group. It is not amphoteric in nature and is not an indicator in the ordinary sense of the word. It is difficult to see how it could play any other than an acid role in its reaction.



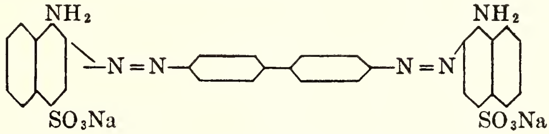
Brom Thymol Blue (an acid dye)

Crystal violet is commonly classed as a basic dye. The dye radical combines with an acid radical to form a salt. It could, therefore combine with bacteria only when the organisms are playing an acid role, i.e., in solutions of high pH.



Crystal Violet

Congo red is commonly classed as an acid dye. The molecule contains two sulfonic acid groups and *two amino groups.*



Congo Red

It is obvious that this dye is unlike the others in that it is itself an amphoteric compound. Its acid properties are slightly more pronounced than its basic properties for its isoelectric range if from pH 5 to pH 8. Since it is amphoteric it should have little tendency to combine with bacteria for both would dissociate basically at low pH values and as acids at high pH values. However, this would be the case only if the isoelectric ranges of both dye and bacteria were the same. If the isoelectric range or point of the bacteria were lower than that of the congo red then combination could take place above that point for the bacteria would be more acidic than the dye. Likewise combination should take place if the isoelectric point of the bacteria were higher than that of the dye. The roles of the reactants would then be reversed. It is quite probable that the isoelectric point of the bacteria is lower than that of the dye for many bacteria are known to have isoelectric points as low as pH 4 and 3. Furthermore, those colonies which showed the greatest absorption of congo red on agar plates developed a pH of 7.6 in litmus milk. This is close to the upper limit of the isoelectric range of congo red where absorption should be the greatest if the bacteria were playing an acid role, i.e., had a lower isoelectric range than the dye. If this is the case, then congo red is playing the role of a basic rather than an acid dye toward these bacteria. Nevertheless, its behavior is quite in harmony the theory.

These results suggest the necessity for carefully studying the possible amphoteric nature of a dye before predicting its reaction with bacteria. The superficial classification of a dye as acidic or basic is not sufficient to determine how it may behave with bacteria at various pH values. These results also suggest the advisability of a similar study with other dyes having both acid and basic groups in the molecule to determine whether they behave in a similar manner or not.

It was noted in the course of this study that congo red even when present in considerable concentration in the medium had little if any inhibitory effect on the growth of the organisms while both of the other dyes when present in higher concentrations seemed to retard growth considerably. If the conclusions reached above are correct the organism has united with the amino group of the congo red rather than with the sulfonic acid group. The chemical stimulus of the organism has driven it in this case to that portion of the dye molecule which is least harmful or most beneficial.