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Does Methylene Blue Penetrate Into Living Cells?

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Experiments with dyes tend to favor the theory that the penetration of a basic dye into living cells depends on the fact that the dye has an apparent dissociation constant, and that in the form of free base, which predominates at higher pH values, it penetrates very readily, while in the form of salt it penetrates so slightly that its entrance may be neglected.

According to this theory, methylene blue, which behaves like a strong base,[1] should not penetrate, since it exists in the form of a salt. But the literature is full of statements that living cells are stained by methylene blue. No one, however, has heretofore tested the dye which has actually entered a living cell from a solution of methylene blue. Until this is done, we are in no position to conclude that the dye found inside a living cell is methylene blue, rather than one of its lower homologues (such as trimethyl thionin or "Azure B", which is less basic), since it is very difficult[2] to obtain methylene blue absolutely free from such dyes (especially at higher pH values where some of it changes very readily to the other forms).

Previously, the writer[3] has found that unless there was injury or contamination of the sap from the deeply stained cell wall, the vacuole of the living cell of *Valonia* took up practically no dye[4] from methylene blue dissolved in sea water at pH 5.4, while it took up more at pH 9. Chloroform behaved similarly, in that the higher the pH value of the aqueous solution, the more dye was taken up by the chloroform. The dye which had been taken up by the chloroform was dissolved in sea water after removal from chloroform, and was found to enter the vacuole of living *Valonia* much more readily than was the case with the dye from the methylene blue solution at the same concentration and pH value. The higher the pH value the more rapid was its rate of penetration.

It may be asked whether the dye found in the vacuole and in the chloroform is methylene blue or its homologues. To test this point a series of absorption curves were obtained by spectrophotometric analysis and the following results were obtained:

(1) Absorption curves showed that the sample of methylene blue employed, dissolved either in sea water at pH 9 or in sap of *Valonia* or in artificial sap of *Valonia,* was chiefly methylene blue.

Contamination by other dyes was insufficient to be detected by this method.

(2) The sap of *Valonia* collected from the living cells which had been placed for 1 hour in methylene blue dissolved in sea water at pH 9, contained a dye which was found to be chiefly trimethyl thionin. If methylene blue penetrated it was not present in detectable amount.

(3) The dye, which had been taken up by chloroform from a solution of methylene blue at pH 9, and which was then removed from the latter by shaking up with distilled water, was also found to be chiefly trimethyl thionin.. No detectable amount of methylene blue was present.

(4) The dye, which had been absorbed by the cell wall from a solution of methylene blue at pH 9, and which was then allowed to diffuse out into artificial sap, was found to be chiefly methylene blue. Other dyes were not present in sufficient quantity to be detected.

It may be added here that the cell wall is also capable of taking up trimethyl thionin.

That the methylene blue or other dyes were not present in the vacuole in their reduced forms was proved by the fact that no increase in the coloration of the sap took place on exposing the sap, which was made alkaline, to air.

The experiments were repeated with a fresh water alga, *Nitella** *flexilis,* and approximately the same results† were obtained.

The fact that no methylene blue can be detected in the vacuole of these cells does not prove that it does not enter the protoplasm, since it might not be able to enter the vacuole. We cannot be certain of this point unless we can prove (1) that there is some methylene blue inside the vacuole or inside the cytoplasm, or (2) that if there is any dye in the cytoplasm it is not methylene blue but a homologue.

The experimental results presented in this paper serve to point out the danger in drawing any theoretical conclusion regarding oxidation reduction potential or permeability based on the penetration of the dye from the methylene blue into the living cells.

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* The higher the pH value of the trimethyl thionin, the less rapidly is the cell wall of living *Nitella* stained, while the rate of staining with methylene blue does not seem to change appreciably with varying pH values between pH 5 and pH 9.

† These results are complicated by a greater possibility of contamination of the sap from stained cell wall.

ing all the spectrophotometric analyses mentioned in this paper. This is a preliminary report.

[1] For a discussion of the apparent dissociation constant of methylene blue *cf.* Clark, W. M., Cohen, B., and Gibbs, H. D., *Public Health Reports,* 1925, xl, 1131.

[2] Clark, W. M., and his collaborators (see footnote 1) state that methylene blue is difficult to purify. It is stated that another dye in the methylene blue may be active in staining (Scott, R. E., and French, R. W., *The Military Surgeon,* 1924, August, p. 1). The writer has tried to obtain methylene blue free from other dyes by repeated extraction with chloroform but at pH 9 it was impossible to obtain such a sample owing to the fact that a certain amount is being converted to another form at such a pH value.

[3] Irwin, M., *J. Gen. Physiol.,* 1925-26, ix, 561.

[4] This result is contrary to the result obtained by Brooks, M. M., *Am. J. Physiol.,* 1926, lxxvi, 360.

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Action of Narcotics on the Ameba by Means of Microinjection and Immersion.

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Comparative studies were made on the influence of the narcotics: ethyl alcohol, chloretone, ether, and chloroform on the protoplasm of *Amoeba dubia,* with special reference to their action on the plasmalemma.

Immersion Experiments: Very weak concentrations (subnarcotic) cause the Amebae to spread out and continue their movements in an expanded condition. This may be an effect of lowered surface tension. Ether in its narcotic concentration, approximately 2 per cent, produces a reversible gelation accompanied by the cessation of all movements. Lethal concentrations of all the narcotics cause the Ameba to round up followed by a sinking of its granules and a disintegration of the plasmalemma.

Injection Experiments: No narcotic effect was observed by injection into the interior of the Ameba. Chloretone in all concentrations increases the fluidity and streaming movements of the interior. Eighty per cent alcohol produces a coagulation which is localized and reversible, when small amounts are injected, and irreversible

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