Pacific Coast Branch.

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Further Studies on Penetration of Methylene Blue.*

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In a former paper[1] it was shown that when the marine alga, *Valonia,* was placed in solutions of methylene blue dissolved in sea water at pH's 5.8 and 9.0, the dye penetrating into the sap was methylene blue itself and not some lower homolog, such as trimethyl thionin, formed by the oxidation of methylene blue in the outside solution. The identification of the dye was made by means of the spectrophotometer.

In order to test this conclusion still further, another set of experiments was made using the fresh water alga, *Nitella.* The method was similar to that used in the experiments with *Valonia.* The same sample of dye was also used. All necessary precautions were taken during extraction to keep the sap from becoming contaminated by dye from the rest of the cell. The plants were also tested for injury by transferring samples from the experimental solutions to tap water. These were found to be alive (green and turgid) 2 months after experimentation, when they were discarded.

In the experiments herewith reported selected *Nitella* cells of the same size were placed in solutions of methylene blue for 2 hours at 25° C., after which the sap was extracted, placed in a hollow ground slide with a flat-bottomed depression, covered by a cover slip, and spectrophotometric measurements made.

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[1] Brooks, M. M., *Univ. of Calif. Pub. in Zool.,* 1927, xxxi, 79; *Proc. Nat. Acad. Sciences,* 1927, xiii, 821.

It was found that the dye penetrating into the sap had a maximum absorption at 660 mμ and a secondary maximum at 610 mμ. These are the absorption maxima for methylene blue. It is therefore concluded that methylene blue penetrates *Nitella* sap as such. This corroborates the previous results obtained by the writer with *Valonia*.

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Persistence of Denatured Horse Proteins in the Canine Circulaton.*

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In previous papers[1, 2] it was shown that horse proteins injected intravenously into normal dogs are retained quantitatively in the canine circulation for at least 6 days. During this retention, however, the proteins undergo marked denaturization. By the end of 4 days they will call forth no recognizable anaphylactic reaction even on massive transfusion into hypersensitive dogs. By the end of 6

TABLE I. *High Dilution Tests.*
Dogs injected intravenously on different days with 2 cc. horse serum per kg. of body weight. Blood samples withdrawn on the same day. Sera from these samples tested in parallel dilutions with rabbit precipitin. (For details of technic see previous papers.) +++, ++, +, (+), visible precipitate; ttt, tt, t, (t), (ƭ), turbidity or opalescence; 0, no demonstrable reaction.

Dilution	6-day Sample	30-day Sample
1: 32	++(+)	tt
1: 64	++(+)	(+)
1: 128	++	+
1: 256	++	+
1: 512	+(+)	+
1: 1,024	+	(+)
1: 2,048	+	(+)
1: 4,096	(+)	(+)
1: 8,192	tt(t)	(+)
1: 16,384	t(t)	tt
1: 32,768	t	t
1: 65,536	(t)	(t)
1:131,072	0	0

* Work aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Manwaring, W. H., Marino, H. D., McCleave, T. C., and Boone, T. H., *J. Immunol.*, 1927, xiii, 357.

[2] Manwaring, W. H., Marino, H. D., and Azevedo, J. L., *J. Immunol.*, 1928, xiv, in press.