PERMEABILITY DIFFERENCES BETWEEN NUCLEAR AND CYTOPLASMIC SURFACES IN *AMŒBA DUBIA.*

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Fresh water amebæ readily stain with the pH indicator, methyl red (1). Not only is the hyaline cytoplasm colored yellow with the dye but also the nucleus, the contractile vacuole and the various cytoplasmic inclusions. This occurs within a few minutes in a concentration prepared by adding 1 cc. of the standard 0.4 per cent. Clark and Lubs solution of the dye (Hynson, Westcott and Dunning) to about 8 cc. of the culture water. In this mixture the amebæ survive for over twenty-four hours with no appearance of being injured.

The fact that the yellow color is the color of the alkaline range of the dye [1] suggested the possibility of testing the penetrability of hydrochloric acid into the nucleus from the cytoplasm of the ameba. It is already known that the living cell-membrane is impermeable to hydrochloric acid but it is not known whether the acid, when once introduced into the cytoplasm, will pass through the surface membrane of the nucleus.

The various structural components of amebæ immersed in a solution of methyl red take up the dye at different rates. The hyaline cytoplasmic matrix is the first to color yellow, then the nucleus, and finally the contractile vacuole and the food vacuoles. The food vacuoles, however, always remain distinctly paler than the contractile vacuole.

EXPERIMENTAL RESULTS.

1. *Amebæ Stained with Methyl Red and Immersed in Solutions of Hydrochloric Acid.*

Amebæ, stained yellow with methyl red and then immersed in concentrations of N/1,600 and *N*/3,200 of HCl, usually became

[1] An aqueous solution of methyl red is yellow in its alkaline and red in its acid range. The turning point of the color is at the pH of about 5.5.

injured within 2–5 minutes. The injury was made apparent by a sudden change in color from yellow to red of a localized area which was quickly pinched off. Every few minutes this process was repeated so that the healthy remnant of the Ameba became successively smaller until the entire ameba was killed. In a moving ameba the first part to be injured was the tip of an extending pseudopodium. This phenomenon was best observed when the amebæ are placed in concentrations of $N/400$ to $N/800$ HCl, where the injury usually manifested itself within a couple of minutes, and in amebæ which put out large pseudopodia. In such cases there was a rapid flow of the yellow protoplasm to the tip of the pseudopodium where a small red spot suddenly appeared. The red color increased in extent as the injury effect spread. Usually the injured area is pinched off before the entire ameba becomes involved.

In strong solutions of the acid, *e.g.*, $N/200$, the injury quickly spreads from several spots on the surface of the ameba with the result that the entire ameba is killed before any pinching-off process can occur.

2. *Injection of Hydrochloric Acid into Stained Amebæ.*

An amount of approximately the volume of the nucleus of $N/200$ HCl or stronger was injected into the cytoplasm in the vicinity of the colored nucleus. The nucleus and the cytoplasm, about the site of the injection, immediately changed in color from yellow to red and the entire red mass, consisting of the injured cytoplasm and the nucleus, were then pinched off and discarded by the ameba.

With a more dilute concentration of HCl ($N/400$) the effect is not so drastic so that a reversible reaction could frequently be obtained. A small amount of $N/400$ HCl was micro-injected into a stained ameba, the tip of the micropipet being directed toward the nucleus at a distance of about half the diameter of the nucleus. Immediately after the injection the cytoplasm about the tip of the pipet turned red, after which the color of the nucleus likewise became red. The yellow color of the nucleus changed to red in a wave which spread from the border near the site of the injection. Within one or two seconds the red

color of both cytoplasm and nucleus returned to the original yellow. The reversion in color always occurred more rapidly in the nucleus than in the cytoplasm. The change in color of the nucleus from yellow to red and back to yellow was unaccompanied by any appreciable morphological change such as occurs when the nucleus is irreversibly injured.

Amebæ, in which the reversible reaction had been noted, were kept under observation for several days during which time they appeared to be quite normal.

3. *Injection of the Acid into Stained Amebæ in the Vicinity of the Contractile Vacuole.*

Injection of hydrochloric acid in the vicinity of the contractile vacuole produced no change in color of the interior of the vacuole unless the concentration of the acid is enough to cause irreversible injury. This is in marked contrast to the ease with which the nucleus changes in color and suggests that the membrane of the contractile vacuole, in this one respect, differs from that of the nucleus but is similar to that on the surface of the ameba. An alternative to this suggestion is that the HCl does enter but that the content of the vacuole is too highly buffered to be affected by the acid. However, the results of recent work (2) indicate that the concentration of dissolved substances in the vacuole is fairly low and that its content is mainly water.

Summary.

1. *Immersion Experiments.*

Amebæ, stained yellow with methyl red and immersed in solutions of HCl, exhibit injury effects by a sudden change in color from yellow to red in localized regions on the periphery. These regions are pinched off and discarded. In a moving ameba this injury first occurs at the tip of the extending pseudopodia.

2. *Injection Experiments.*

Sublethel concentrations of HCl which do not penetrate the ameba from without, will, when injected into the cytoplasm, readily diffuse into the nucleus without causing irreversible injury. On the other hand, the wall of the contractile vacuole

appears to be similar to that of the plasmalemma of the ameba in regard to the nonpenetrability of HCl.

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