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A PECULIAR STAINING REACTION IN *EUGLENA RUBRA* HARDY 1911

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During experimental work in which routine temporary stains were applied to various species of Euglena, an unusual reaction was found to occur in *Euglena rubra* when subjected to Noland's 1928 stain and fixative.

Hardy (1911) described *Euglena rubra* as possessing red pigment granules, haematochrome, in addition to chlorophyll. The organism possesses a pellicle with discontinuous spiral striations and two types of cysts—a temporary thin-walled cyst and a more permanent thick-walled cyst (Johnson, 1939).

The purpose of the present investigation is to describe the reaction of *Euglena rubra* to Noland's 1928 stain and fixative and to offer a possible explanation of the phenomenon.

Method

A drop of the medium containing the organisms was placed on a slide, and two drops of Noland's temporary stain for demonstrating flagella and cilia were added to the original drop. The composition of the fixative and stain includes 80 cc. of a saturated solution of phenol in water, 20 cc. of a 40 percent solution of formaldehyde in water, 4 cc. of glycerine, and 20 mgms. of gentian violet, moistened before adding (Noland, 1928).

Observations

From approximately forty species of Euglena treated with Noland's fixative and stain, the pellicle separates from the protoplast only in *Euglena rubra*. The flagellar attachment remains intact and the striations of the separated pellicle are prominent in all organisms. The distance separating the pellicle from the protoplast is one-fourth or more the diameter of the protoplast (Fig. 1, 2). The space between the plasma membrane and pellicle may or may not appear vacuolated (Fig. 1, 2). Any pressure which tends to separate the pellicle from the protoplast may be relieved by forcibly breaking the pellicle, and under these conditions no contraction of the pellicle occurs after release of the internal pressure. Measurements comparing living and stained organisms

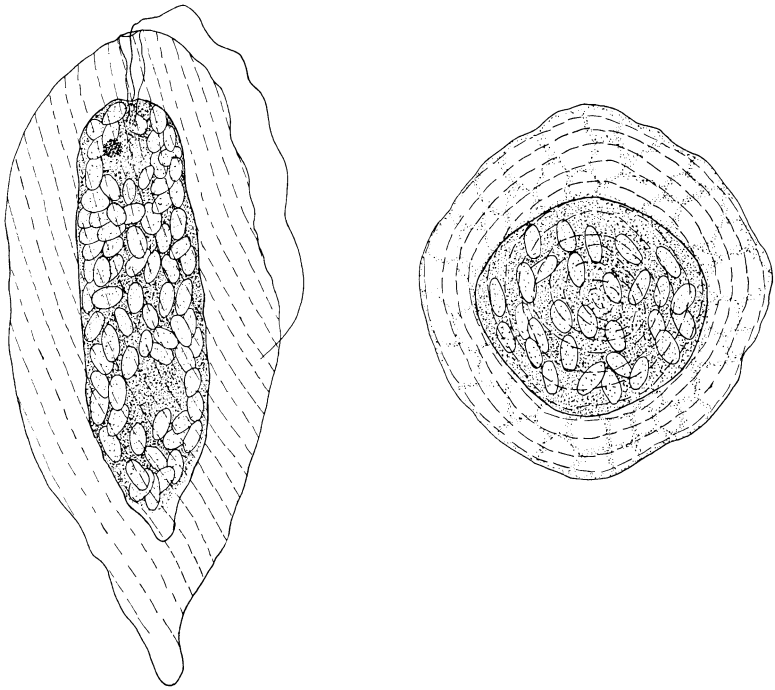

Fig. 1. Trophozoite of *Euglena rubra* in which the pellicle has become separated from the protoplast after treatment with Noland's 1928 temporary fixative. (330X).

Fig. 2. Resting cyst of *Euglena rubra* after treatment with Noland's 1928 temporary fixative, showing vacuolated area between protoplast and pellicle. (330X).

indicate that the volume of the protoplast is decreased only slightly, if at all.

The separation occurs in resting cysts and trophozoites, but never in the more permanent cysts in which the gelatinous wall beneath the pellicle is much thicker than in resting cysts. The reaction seldom occurs in every organism of any one preparation.

Discussion

Euglena rubra, like most Euglenoids, possesses two means of locomotion, flagellar and metabolic (euglenoid) movement. The striations of the pellicle are generally thought to contain elastic elements, which tend to retain the form of the organism during and after contraction of the superficial layers of the body. *Euglena rubra* when immersed in Noland's stain and fixative apparently lose their normal elasticity and therefore are unable to return to their original condition. The failure of the pellicle to re-

gain its original size even after the pellicle has been burst and the internal pressure released, warrants the conclusion that the pellicle is contractile in the living organism and that the solution caused the destruction of that elasticity. It is probable that the homologous elements in other Euglena lose their elasticity, but that conditions which are necessary to demonstrate that loss of elasticity are lacking in those Euglena. It would seem that a combination of a certain physiological state of the protoplast or associated portions, and a certain lack of elasticity of pellicle is necessary before the separation of pellicle from protoplast occurs.

This physiological condition may be correlated in some way with the sol-gel state of the cyst wall, since the separation of pellicle from protoplast occurs in resting cysts but not in thick-walled cysts. It may be that the thick cyst wall, when treated with the immersion fluid, fails to exert an osmotic pressure great enough to cause the reaction, yet the resting cyst and trophozoites are capable of such. Or, it may be that the fixative reaches the plasma membrane of the trophozoite and resting cyst, causing a change in the permeability of the membrane, but fails to reach the plasma membrane of the thick-walled cysts. If that is the situation, some of the osmotically active salts would pass outside the plasma membrane, where they exert their osmotic activity. This seems unlikely, since a loss of salt large enough to cause the phenomenon would be followed by a corresponding loss in cell sap and a definite shrinking of the protoplast, which does not occur.

Summary

1. The separation of the pellicle from the protoplast of *Euglena rubra* is described.

2. Evidence is presented that the pellicle in the living organism is an elastic structure.

3. A possible explanation of the separation phenomenon is given.

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