

NOTES ON THE LOSS AND REGENERATION OF THE PELLICLE IN *BLEPHARISMA UNDULANS*.

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The color which is so characteristic of *Blepharisma undulans* has been the object of study and interest of many protozoölogists. It is usually a pink purple but may vary from deep purple violet to light rose, or the animals may be perfectly colorless. It has been recently suggested by Dawson (1929) that color changes accompany the process of digestion. It is believed that a somewhat different light is thrown on this general question by the observations here recorded.

When a drop of M/10,000 strychnine sulphate is added to individuals of this species on a slide they soon cast off their pellicles, swim away and leave the empty capsules behind. The color, so characteristic of this ciliate, is seen to be restricted to the pellicle which retains its color, the "naked" organism, on the other hand, being colorless.

During the process of shedding the pellicle, the cilia are withdrawn inside it where they go on beating in coördination (Fig. 1). This retraction starts at the anterior end of the animal and proceeds as a wave backward. After rotating for a short time inside of the pellicle the naked animal escapes by gradually working its way out in an ameboid-like manner through either the region of the posterior contractile vacuole or the base of the gullet. If for any reason the naked organism is unable to escape from the pellicle, death invariably follows. Dividing individuals shed their pellicle as one. Pieces of the ciliate, cut with a microdissection needle, are likewise able to shed their pellicles. Conjugating pairs shed their pellicles as do non-conjugants but always escape through the gullet, either as separate individuals or as one, depending on the stage of conjugation and the degree of union.

During this process the conjugants frequently fuse giving rise to double monstrous forms.

The cast off pellicle is easily studied and presents a continuous

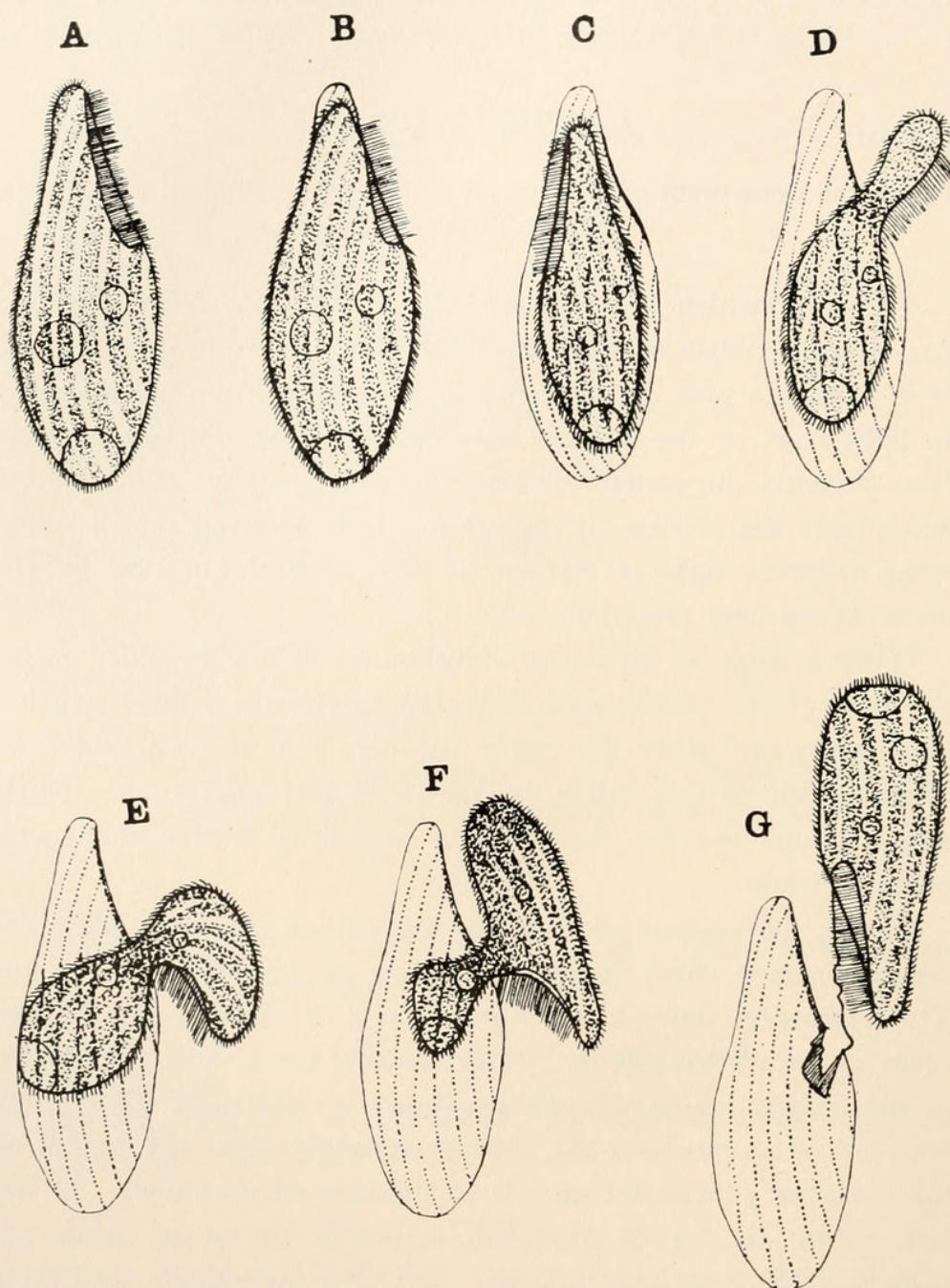


FIG. 1. Diagrams showing the method of shedding of the pellicle, the animal escaping by way of the gullet.

membrane-like structure with rows of small holes running lengthwise, through which the cilia had protruded, showing a definite arrangement of the cilia in from ten to eighteen rows. The

pellicle appears to be fastened more firmly at the base of the gullet than elsewhere and the empty membranous shell is usually dragged around by the animal for a short time by a strip of the pellicle attached to the base of the gullet.

The naked animal is much more flexible and is cut more easily with a microdissection needle than when the pellicle is intact. When cut, in this condition, the cut end instantly closes and the pieces behave like those with a pellicle.

The process of shedding may also be induced by a low concentration ($M/100$ to $M/10,000$) of morphine sulphate, codeine sulphate, cocaine hydrochloride and novocaine while it has not been found possible to produce it by the use of caffeine citrate, brucine, apomorphine, mercury succinate, picrotoxin, phenacetin, quinine hydrochloride, carbon tetrachloride, veronal, veratrine, nictotine, and saponin over a wide range of dilutions. Alcohol, ether and chloroform first decolorize and then dissolve the pellicle, both on the animal and when cast off. The chemicals that produce shedding do not seem to have any very obvious underlying property common to all, and little light is therefore thrown on the mechanism of the process. This fact is even more striking when the substances which produce shedding are compared with those which do not. The hydrogen ion concentration does not appear to play any great rôle in the process, as shedding can take place within the range of pH 5.6 to pH 8.2. Likewise, drastic alterations in the tonicity of the solutions by addition of salt or sugar do not cause shedding. This shedding is not merely due to a shrinking of the organism away from the pellicle, as caffeine produces such a shrinking without shedding of the pellicle taking place.

If *Blepharisma* is allowed to remain in a non-lethal concentration of the drugs which cause shedding, the pellicle after being lost is not regenerated. Individuals grown in $M/100$ morphine sulphate for 110 days did not form new pellicles although they grew, divided and moved perfectly normally. If removed to pure culture media as soon as the pellicle is shed the animals grow and divide, and after some time (1 to 12 days), regenerate new pellicles. Naked ex-conjugants may regenerate new pellicles in as short a time as 24 hours.

Regeneration is best obtained in culture media of wheat (average time for regeneration 9 days) and least successfully in hay infusion (average time 11 days, color pale). On a malted milk diet the pellicle is regenerated in about 10 days. The test for a new pellicle is the presence of color and the ability again to go through the shedding process.

Cultures with limited food supply are light pink in color while those with an excess of food are deeply colored. If starved, the organisms lose their color and pellicles in about 24 hours; the pellicle in this case does not appear to be shed but gradually becomes lighter in color and finally disappears (absorbed?). While the kind of food is likely an important factor in modifying the thickness of the pellicle the differences in color observed appear to depend to a marked degree on the thickness of the pellicle and this in turn on the amount of food available.

Further studies on the function and nature of the pellicle are in progress. It is a great pleasure to thank Professor M. H. Jacobs for many valuable suggestions.

SUMMARY.

1. It has been observed that when *Blepharisma undulans* is treated with strychnine sulphate, morphine sulphate and several other chemicals the pellicle is shed. The pellicle and the "naked" animal have been studied.
2. The pink color so characteristic of this animal is restricted to the pellicle.
3. The pellicle is not essential for life, division or motion and its character is dependent on the amount of food available.
4. Under favorable conditions the pellicle is easily regenerated.

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Nadler, J Ernest. 1929. "NOTES ON THE LOSS AND REGENERATION OF THE PELLICLE IN BLEPHARISMA UNDULANS." *The Biological bulletin* 56, 327-330.
<https://doi.org/10.2307/1537071>.

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