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TOXIC EFFECTS OF A SYNTHETIC LAS DETERGENT ON THE COLONIAL GREEN ALGA, *PLEODORINA CALIFORNICA*

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Abstract

The sensitivity of *Pleodorina californica* cells to a synthetic LAS detergent was studied.

Retardation of cell division, cell death and a decrease in content of chlorophyll occurred in colonies exposed to a low concentration of LAS. In affected cells, lamellae of chloroplasts were sometimes swollen and large vacuoles appeared in the cytoplasm, yet the oxygen production by chlorophyll continued to occur and R cells were still capable of reproduction. At higher concentrations, a majority of colonies died off, cell organelles being disintegrated.

LAS affected *Pleodorina* cells at lower concentrations in comparison with soap, although the two detergents caused cell death within a few minutes at high concentrations.

INTRODUCTION

In a previous paper (1), it was reported that, in mice, synthetic linear alkylbenzene sulfonate detergents (LAS) did not produce any severe damages to a variety of tissues, except for local dermatitis and adhesions between some organs.

Recently, the eutrophication by complex phosphate "detergency builders" has attracted increasing attention. Thus, synthetic detergents are still open to discussion as an important factor involved in water pollution. However, thorough studies on toxic effects of synthetic detergents on aquatic organisms have not yet been carried out.

The present experiments were conducted to observe the effects of a LAS on *Pleodorina* cells.

Pleodorina californica used in the current studies is a colonial green alga. In the colonies, cells may differentiate into two different types, i. e.

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reproducing cells (R) and somatic cells (S), so that the direct influence of LAS on the two kinds of cells as well as the fate of surviving cells could be followed.

MATERIALS AND METHODS

Pleodorina californica Shaw was cultured in the Volvox medium (pH 7.5) added with thiamine hydrochloride (0.03 μM) and sodium acetate (0.012 M). The methods of culture and some biological features of *Pleodorina* were described previously (2).

A liquid detergent (Lion Yushi Co.) which is said to be a 1.5×10^{-3} v/v aqueous solution of LAS was diluted with the culture medium and autoclaved. Powder soap (Minasama Sekken Co.) was also dissolved in the culture medium.

An aliquot from suspension of colonies was transferred to a 500 ml-Erlenmeyer flask containing 300 ml of the culture medium and incubated aseptically over 7 or 8 days. Colonies were separated and classified into 4 stages by filtration through a 150-mesh and a 200-mesh sieves, as described previously (2). They were then transferred to several 500 ml-flasks likewise containing 300 ml LAS medium.

Growth rate of the colonies was determined by a hematocrit (4,000 r. p. m. x 40 min) as packed cell volumes (ml pcv/ml medium). Chlorophyll contents and ratios of chlorophyll a to b were determined by the method of Ogawa and Shibata (3). Production of oxygen was measured by using a Bioxygraph (Kyusui Kagaku Co.) with a Galvani electrode. The vessel of reaction mixture was filled with nitrogen and rate of oxygen production was measured at 25°C at 6,000-lux illumination.

After exposure to LAS medium ($1.5/4 \times 10^{-4}$ v/v) for 20 minutes, damaged but half living *Pleodorina* colonies were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer for 120 min at room temperature and postfixed with 1% O_sO_4 in 0.1 M cacodylate buffer (5°C, 90 min). Block staining with uranyl acetate (0.5%) was performed before dehydration through graded ethanol and propylene oxide. Stained dehydrated colonies were then embedded in epoxy-resin. In order to ascertain surviving cells, some LAS treated colonies were randomly sampled, washed and cultured in a fresh medium.

RESULTS AND DISCUSSION

(I) Concentration of LAS lethal to *Pleodorina*

Colonies at 4 stage of development were exposed to different concentrations of LAS. The growth rates of the colonies are shown in Fig. 1. The initial total volume of colonies was always adjusted to $7\text{-}10 \times 10^{-4}$ ml packed cell volume per ml culture medium, since cell damage caused by the detergent

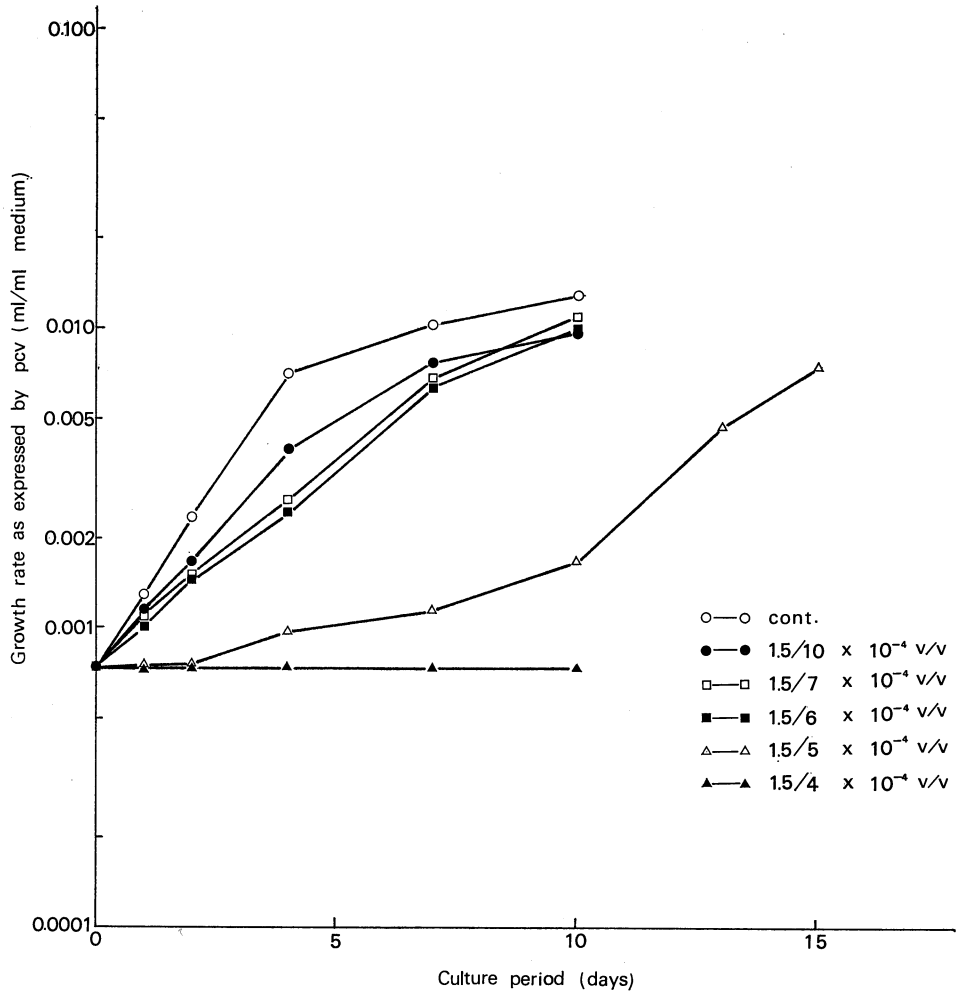


Fig. 1. Semilogarithmic plot of growth as expressed by packed cell volume (ml/ml medium). Time 0 is start of exposure to LAS.

varied considerably in degree according to the initial total volume of colonies (*vide, infra*). The growth rate, i. e. the rate of formation of daughter colonies, was lowered with concentration of LAS in the medium. The reduction in growth rate was brought about by both a delay of cell division and cell death in the affected colonies.

All cells, both R and S, died within a day in a 1.5/4 x 10^{-4} v/v LAS solution. When incubated for 1 or 2 days longer, chlorophyll was invariably

photobleached. In 1.5/5 x 10^{-4} v/v LAS, a part of R cells survived and later showed the ability of reproduction, but a majority of S cells died. In newly formed daughter colonies derived from surviving R cells were normal in both shape and S/R ratio (Fig. 2-a, b). In the same medium, granddaughter colonies grew fairly well (Fig. 1). The concentrations lower than 1.5/6 x 10^{-4} v/v exerted hardly any effect. It should be noted that very slight differences in effect on *Pleodorina* cells. Similar phenomena were also observed with soap (*vide, infra*). So far as I am aware, differences in effect between slightly different concentrations of detergents have only been reported on flagellar movements in some bacteria (4). The present findings show that the lethal concentration of LAS is 1.5/4 x 10^{-4} v/v (37.5 ppm). *Pleodorina californica* appears to be a suitable material for the determination of the threshold concentration of toxic agents.

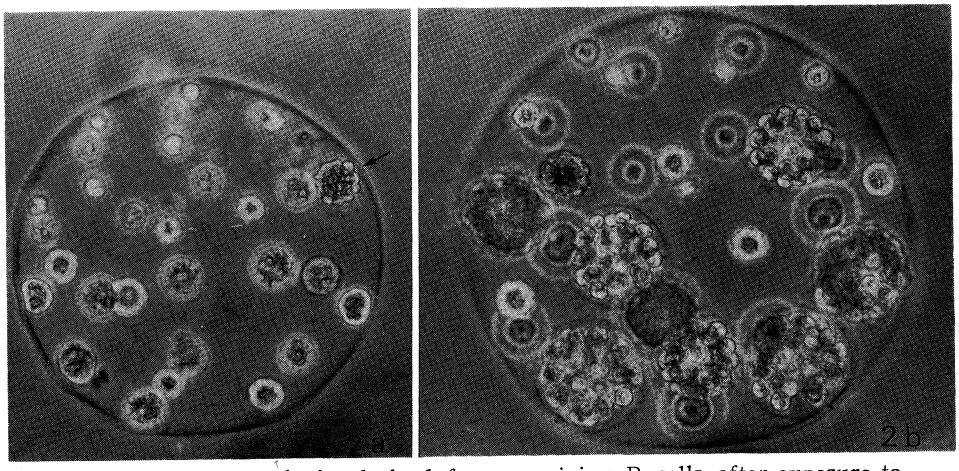


Fig. 2. Daughter colonies derived from surviving R cells after exposure to 1.5/5 x 10^{-4} v/v LAS. (a) 32-cell stage (S : R=12:20) Only one daughter colony is visible. All the other cells are not alive. (b) 32-cell stage (S:R=12:20) with 9 daughter colonies and 11 dead R cells.

(II) Time course of lethal damage

Colonies incubated in 1.5/4 x 10^{-4} v/v LAS (lethal dose) medium for 5, 10, 15, 20, 30, 60, 90 or 180 minutes were washed with sterilized water and cultured in a fresh medium without LAS to observe daughter colony formation. Surviving R cells invariably formed daughter colonies. As shown in Fig. 3, the initial total volume of colonies exerted some influence on the survival time and the survival rate of R cells. If the initial medium contained less than 0.001 ml pcv/ml of colonies, all cells died out within 4-5 hours after incubation.

By contrast, in incubations of 0.002 ml pcv/ml or more of colonies, increasing numbers of R cells survived.

These findings appear to suggest that LAS was biodegraded dy *Pleodorina* cells. Times needed for the death of 50% of cells were 10 min for 0.0011 ml pcv, 16 min for 0.0013 ml pcv, 19.5 min for 0.0026 ml pcv and 22 min for 0.0052 ml pcv.

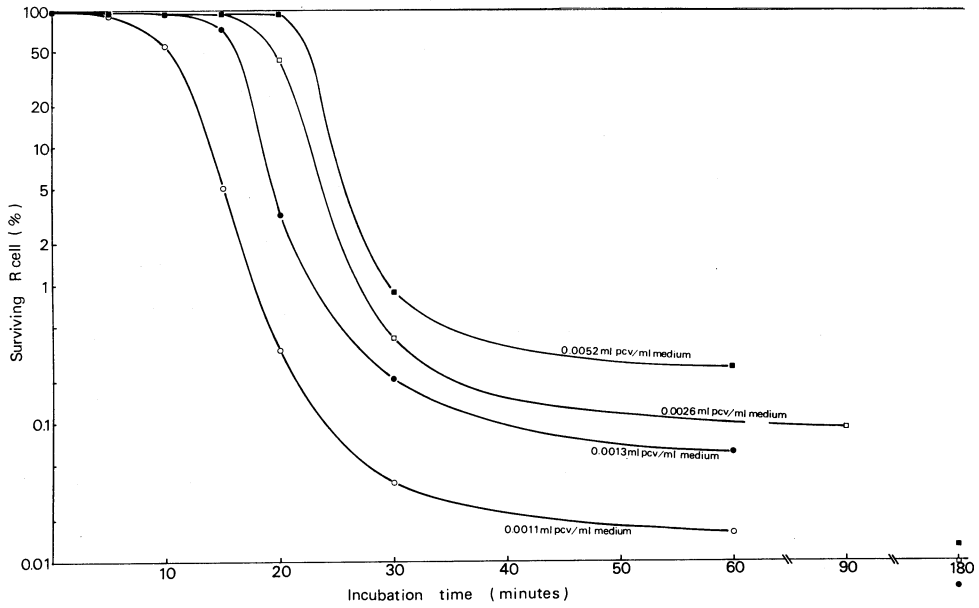


Fig. 3. Surviving R cells at different intervals after exposure to $1.5/4 \times 10^{-4}$ v/v LAS. Survival is much affected by initial total volume of colonies.

(Ⅲ) Electron microscopy

For electron microscopic examinations, 0.004 ml pcv/ml of colonies were exposed to $1.5/4 \times 10^{-4}$ v/v LAS medium for 20 minutes.

A normal R cell at stage 4 is shown in Fig. 4-a and a normal S cell is in Fig. 4-b. In this stage, each cell was enclosed by double sheaths, one was the maternal sheath and the other was of its own sheath (Fig. 4-c). Within the cells, a number of pyrenoids were observed and lamellated chloroplast occupied the greater part of the cytoplasm. The other organelles in these cells were similar in profile to those in other green algae so far studied. S cells had a large cup-shaped eye-spot (2-3 μ), appearing reddish orange in color under a light microscope. Electron microscopic studies revealed that some R cells also had a small eye-spot ($<1.5\ \mu$) (Fig. 4-d). This type of eye-spot was

never found by a light microscope.

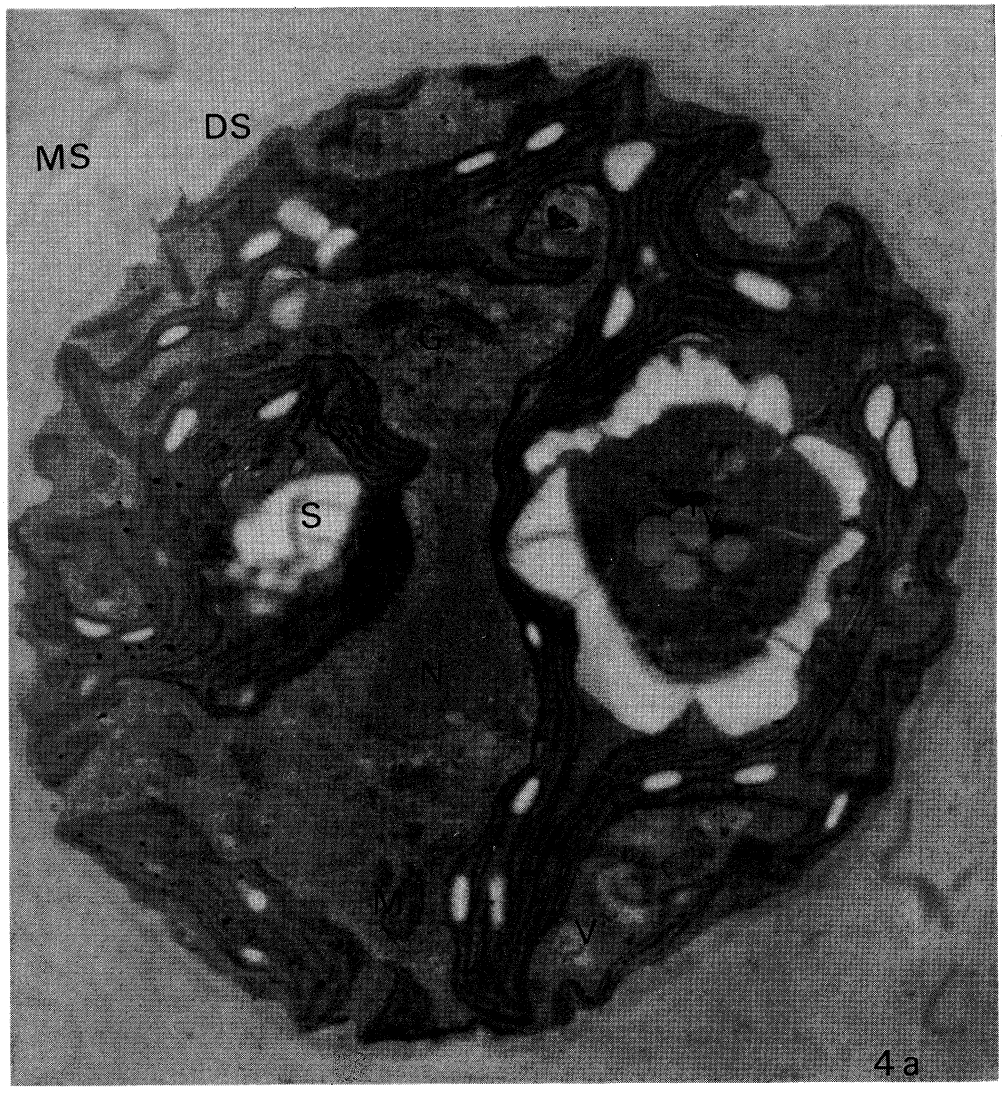
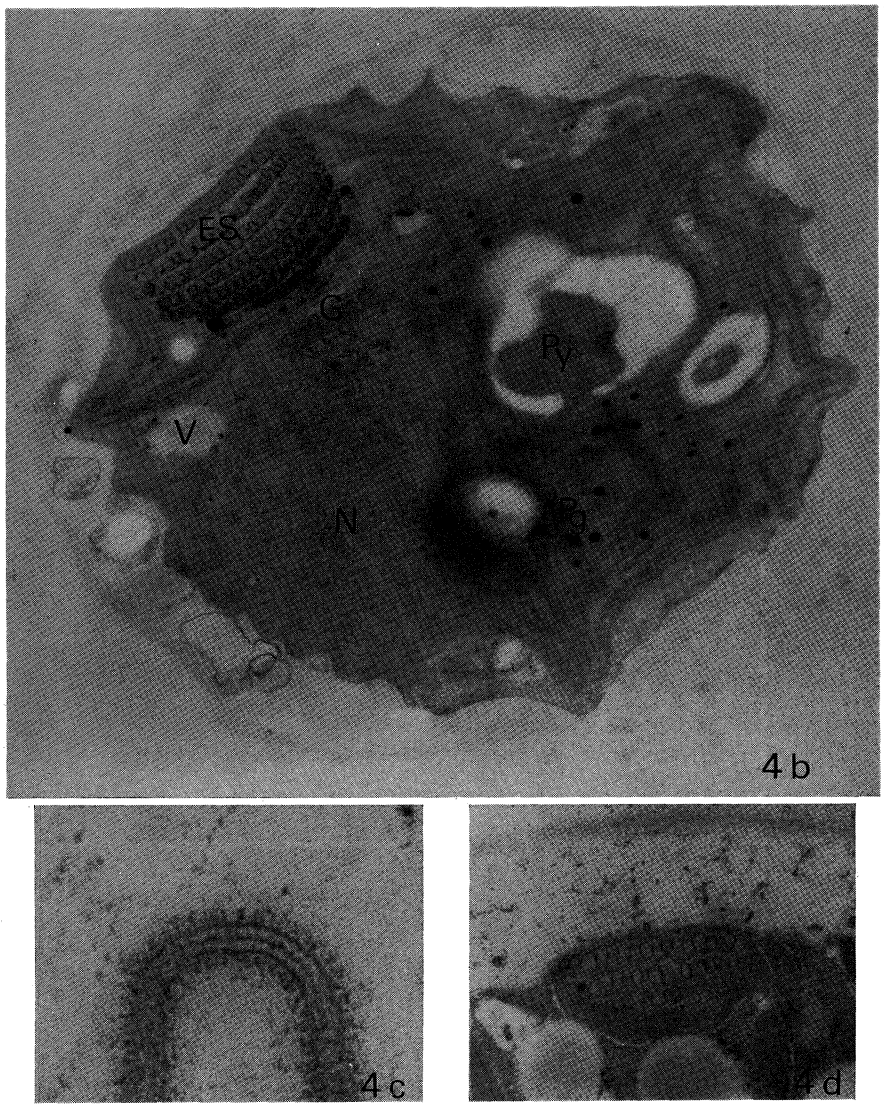
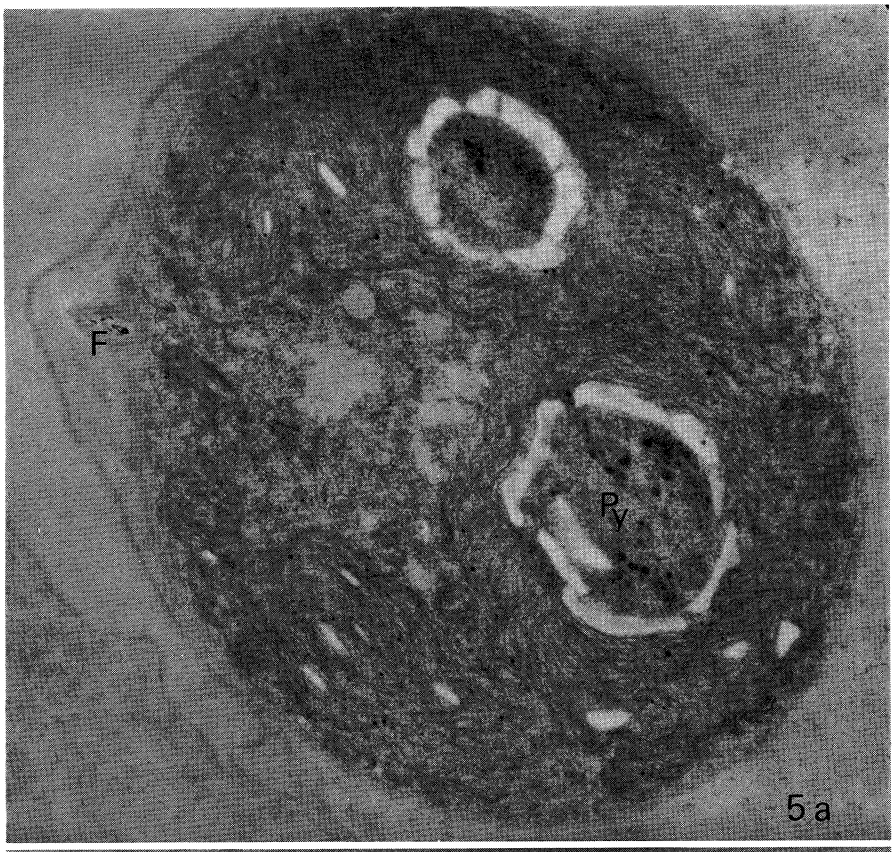


Fig. 4. Electron micrographs of normal *Pleodorina californica* cells. (a) R cell. Py: pyrenoid, S: starch, N: nucleus, M: mitochondrion, V: vacuole, G: Golgi complex, Chl: chloroplast, Pg: plastoglobule, M. S.: sheath of mother colony, D. S.: sheath of daughter colony. (b) S cell. ES: eye-spot. (c) sheath of mother colony. (d) small eye-spot of R cell. (a), ×9,500; (b), ×16,000; (c), ×90,000; (d). ×16,000



In severely damaged R cells (dead cells), lamellated chloroplasts were loosely arranged and swollen (Fig. 5-a). The nuclei and organellae, such as mitochondria and Golgi bodies could no longer be identified definitely. Most of the dead S cells still exhibited some mitochondria but were without eye-spot. The eye spots had probably been shed off from these cells (Fig. 5-b).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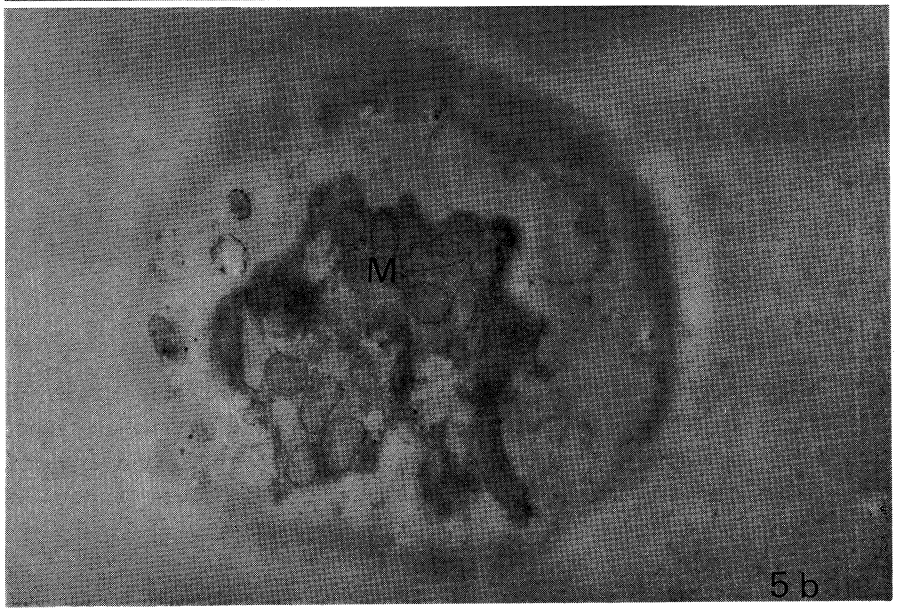
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Fig. 5. Electron micrographs of damaged cells. (a) R cell, (b) S cell. (a) ×8,700 ; (b) ×11,000

In slightly damaged cells, large vacuoles and a little swollen lamellae of chloroplasts were seen (Fig. 6-a, b). These cells might recover to normal later.

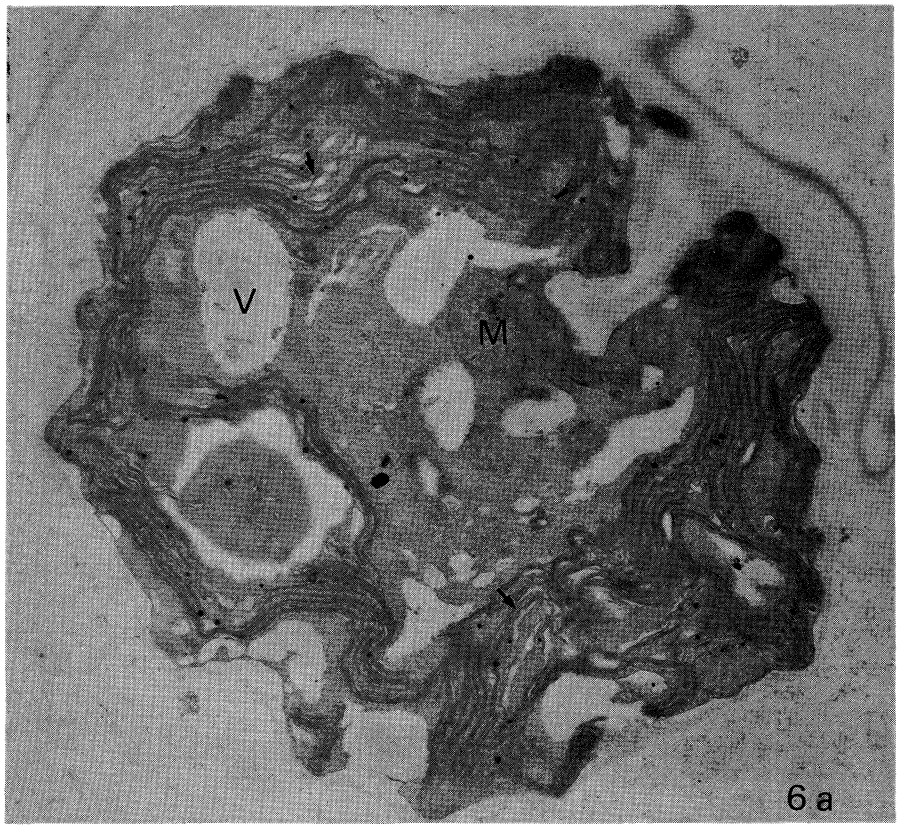

Fig. 6. Slightly damaged R cell (a) and S cell (b). Large vacuoles are visible. Arrows indicate swollen lamellated chloroplast. (a) ×8,700 ; (b) ×14,500

(IV) Chlorophyll contents and production of oxygen

Table 1 shows chlorophyll contents in colonies incubated for 7 days in a medium containing varying concentrations of LAS. In LAS medium, chlorophyll decreased in amount with LAS concentrations. However, ratios of chlorophyll a to b exhibited no appreciable differences between the cells exposed to LAS and the control cells.

Oxygen production was expressed as μ moles per ml packed cell volume

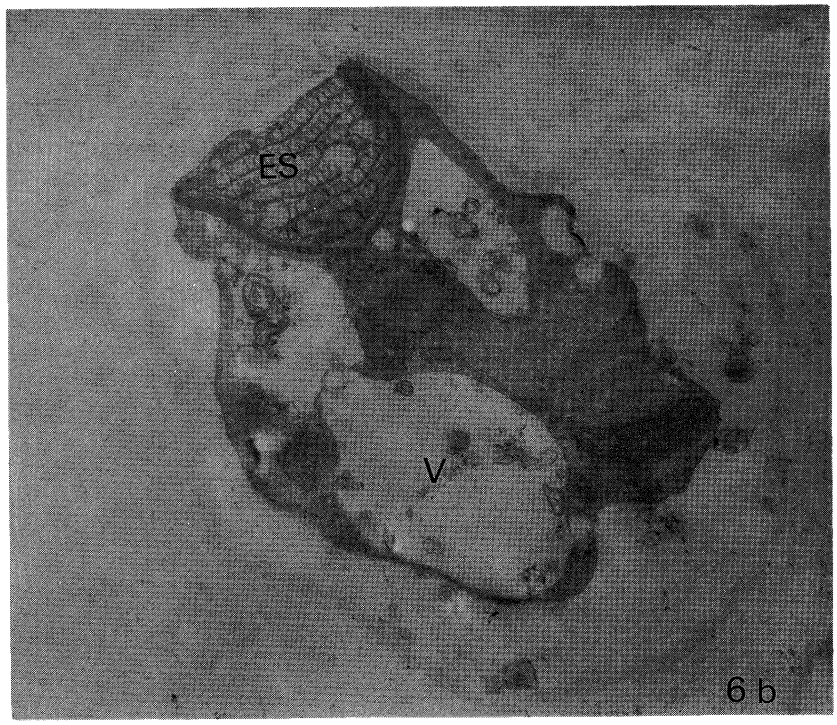

TABLE 1. Chlorophyll concentrations in LAS-treated *Pleodorina* (after incubation for 7 days)

LAS conc.	μg chl./ml pcv	chl. a/b
—	24.7	2.2
1.5/10 x 10^{-4}v/v	17.1	2.3
1.5/7 x 10^{-4}v/v	13.4	2.3
1.5/5 x 10^{-4}v/v	11.6	3.3
1.5/4 x 10^{-4}v/v	0	—

TABLE 2. Effects of LAS on production of oxygen in *Pleodorina* (after incubation for 7 days)

LAS conc.	μ moles O_2/min.	
	per ml pcv	per μg chl.
—	298.8	12.1
1.5/10 x 10^{-4}v/v	239.9	14.0
1.5/7 x 10^{-4}v/v	194.9	14.5
1.5/5 x 10^{-4}v/v	184.9	15.9

per min and per μg chlorophyll per min (Table 2). Values per ml pcv decreased with concentration of LAS, whereas those per μg chlorophyll appeared somewhat larger in LAS cells than in the control cells. These findings seem to suggest that chlorophyll failed to function effectively, especially in the control colonies, since the total volume of chlorophyll was too large. LAS caused a reduction in chlorophyll and consequently a decrease in O_2 production per pcv.

(V) Effects of soap on *Pleodorina* cells

At room temperature, some precipitation took place from a saline solution of powder soap. At higher concentrations, these deposits (metalic soap) frequently adhered to the surface of the colony sheath (Fig. 7). Colonies placed

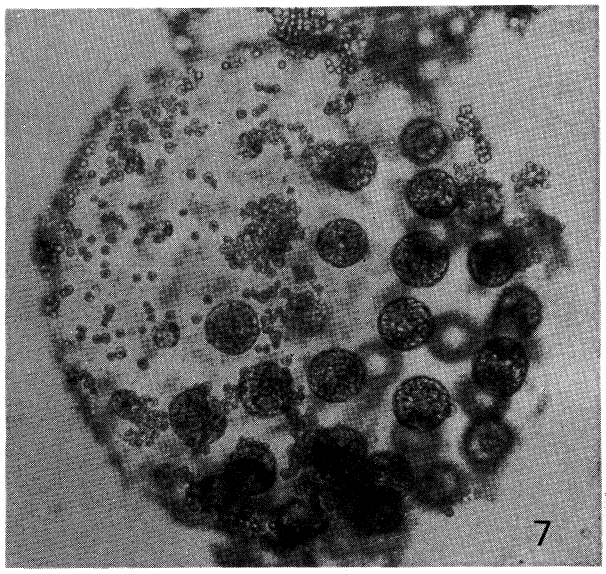

Fig. 7. Soap-treated colony with deposits clinging to the surface.

in media containing soap at high concentrations ceased to swim within a minute, although flagella continued to beat for a few minutes longer. Within about 10 minutes, all the colonies died out. Table 3 shows percentages of daughter colony formation from the colonies exposed to soap solution at different concentrations. It appears that damages to cells were not much related to the initial total volumes of colonies. At concentrations below $1.5/4 \times 10^{-3}$ w/w, there were no marked differences in survival between the control and soap-treated cells.

TABLE 3. Effects of soap on formation of daughter colonies

Soap conc.	Colony volume ml pcv / ml medium	Undivided colony (%)	Daughter colony formation by divided mother colony (%)
——	0.001	5.3	96.6*2
		94.7*1	
1.5/10 x 10^{-3}w/w	0.001	6.4	95.4*2
		94.9	
1.5/5 x 10^{-3}w/w	0.001	6.8	95.2*2
		69.2	
1.5/4 x 10^{-3}w/w	0.001	11.8	93.3*2
		71.4	
1.5/3 x 10^{-3}w/w	0.001	40.5	38.5*3
		16.0	
	0.002	31.3	34.2*3
		47.7	
1.5/2.5 x 10^{-3}w/w	0.001	27.6	42.9*3
		20.7	
	0.002	24.4	32.5*3
		30.8	
1.5/2 x 10^{-3}w/w	0.001	died	0
	0.002	died	0
	0.003	died	0
	0.004	65.2	44.4*3
		19.6	

*1 percentage of living R cells in undivided colonies
*2 after 4 days
*3 after 9 days

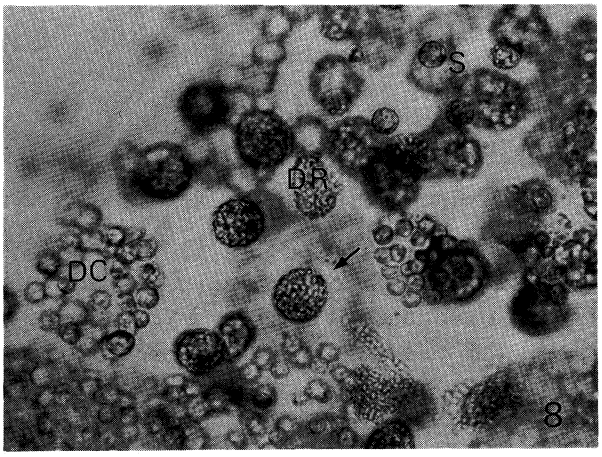

Fig. 8. R cells which have lost ability of reproduction. A large eye-spot is observed (arrow). S : living S cell of normal size, DC : newly formed daughter colony, DR : dead R cell.

Living R cells in undivided mother colonies were markedly injured at concentrations of soap over $1.5/3 \times 10^{-3}$ w/w. The R cells having a large eye-spot were not able to reproduce after they were transferred to a fresh medium without soap (Fig. 8), suggesting that the R cells had transformed into S cells. The concentration of $1.5/2 \times 10^{-3}$ w/w brought about overall cell death in 0.003 ml pcv/ml colonies, although many R cells survived in 0.004 ml pcv colonies. It is evident that soap solutions also did severe damage to *Pleodorina* R cells.

Acknowledgment

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