The Mechanism of Lysis of *Prymnesium parvum* by Weak Electrolytes

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SUMMARY

Organisms of the phytoflagellate *Prymnesium parvum* Carter undergo swelling and lysis in the presence of ammonia or acetic acid. The lytic activity was pH-dependent and increased as the concentration of undissociated weak electrolyte in the suspension medium was increased. The kinetics of swelling were followed with the aid of an electronic particle counter and by microscopic examination. In the presence of ammonia or acetic acid, the rate of swelling as well as the final volume of the *Prymnesium* organisms were a function of external osmotic pressure and of temperature. It is suggested that the swelling and lysis are osmotic in nature and depend on the intracellular accumulation of weak electrolytes by a 'pump' driven by differences between environmental and intracellular pH values. The similarities between this phenomenon and concentration of weak electrolytes in mammalian erythrocytes and other cells is discussed. Differences in the morphology and lysis of *Prymnesium* by ammonia and acetic acid were found which suggest the existence of intracellular compartments maintained at different pH values.

INTRODUCTION

Prymnesium parvum Carter, a phytoflagellate of the Chrysomonadinae has become the object of much study because of its ability to form toxic material which kills fish (Otterstroem & Steeman-Nielsen, 1939; Reich & Aschner, 1947; Shilo & Aschner, 1953; McLaughlin, 1956; Shilo & Rosenberger, 1960; Yariv & Hestrin, 1961). Reich & Aschner (1947) observed the extreme sensitivity of *Prymnesium* to ammonium sulphate and focused attention on the use of this salt to control the phytoflagellate in fishponds. In the present paper the effect of environmental conditions on the lytic activity of weak electrolytes on *Prymnesium* has been studied and the sequence of morphological changes which take place during lysis has been examined. Recent advances in cell-sizing by use of an electronic particle counter made it possible to follow the kinetics of swelling and lysis of *Prymnesium* populations by penetrating weak electrolytes. On the basis of these results a mechanism which concentrates weak electrolytes in the interior of *Prymnesium* cells and which may lead to osmotic lysis has been suggested.

METHODS

Prymnesium parvum cultures. Two strains of *P. parvum* grown in axenic culture were used. (1) A strain isolated from brackish water fishponds in Israel by K. Reich, (Dept. of Zoology, Hebrew University, Jerusalem; Reich & Kahn, 1954). (2) A strain isolated from supralittoral pools in Scotland by M. R. Droop (Marine Station, Millport, Great Britain; Droop, 1954). These organisms will be referred to as *Prymnesium*. *Dunaliella salina* Teodor. and *Syracosphaera carterae* from the stock collection of the Department of Botany, Hebrew University of Jerusalem were kindly given us by Dr I. Friedman.

Media and conditions of growth. Throughout this work a modification of a medium developed by Dr M. R. Droop (personal communication) for growth of marine phytoflagellates was used. It was composed of two equal parts, (A) sea water 25 % (v/v) and (B) (% w/v): liver extract (Difco), 0.02; glucose, 0.02; Bacto-tryptone (Difco), 0.02; NaCl, 0.75; MgCl₂·6H₂O, 0.5; KCl, 0.08; CaSO₄ (anhydrous) 0.1; in distilled water; pH 8. In some experiments the osmotic pressure of this medium was changed by altering the concentration of sea water (1.5–50 %, v/v) and of sodium chloride (0.1–3 %, w/v). Sterilization of media was performed by autoclaving at 120° for 20 min. Cultures were grown in Erlenmeyer flasks of 250 ml. capacity containing 100 ml. liquid medium in continuous 'fluorescent white' daylight, 220–260 foot candles at 26°–28°. Under these conditions populations of 3 to 5 × 10⁶ organisms/ml. were reached within 4–6 days.

Measurements of organism volume. For the measurement of the volumes of *Prymnesium* organisms, the Coulter Particle Counter, Model A (Coulter Electronics, Chicago, Illinois, U.S.A.) with an orifice of 100 μ was used. This instrument provides a means to determine size distribution of cell populations and has already proved extremely useful in the counting and sizing of erythrocytes (Mattern, Brackett & Olson, 1957), in the estimation of the volumes of cells in tissue cultures (Kuchler & Merchant, 1958; Brecher, Schneiderman & Williams, 1956) and of bacteria (Kubitschek, 1958; Lark & Lark, 1960).

The instrument applies principles of electrical gating. The cells are suspended in an electrically conductive medium and forced to flow through a small aperture having an immersed electrode on each side. As each particle passes through the aperture it replaces its own volume of electrolyte, momentarily changing the resistance value between the electrodes. This change produces a voltage pulse of short duration having a magnitude proportional to particle volume and the resultant series of pulses are electronically amplified, scaled and counted.

Samples of *Prymnesium* cultures for counting and sizing were diluted in the growth medium and carefully filtered to give low background values. Population densities of 40,000–60,000 organisms/ml. were used, since at this concentration the coincidence effect of more than one organism passing through the orifice at the same time was comparatively small (5–7.5 %).

Lysis and swelling in *Prymnesium* populations under various experimental conditions were estimated by measurement of the size distribution of organisms in populations at different times. Organism size distribution in a single population followed a typical normal distribution curve (Fig. 1). The threshold value corresponding to 50 % of the population (corresponding to the maximum of the Gaussian

distribution curve) was a convenient measure of the size characteristics of the population and has been used in the expression of our results. This measure has been given in 'threshold units' of the counter which, under standard conditions, are directly proportional to volume. This relationship was established experimentally with erythrocytes of various mammalian species (Fig. 2). The erythrocytes were suspended in the Prymnesium growth medium isotonic for these cells and the

Fig. 1

Fig. 2

Fig. 1. Size distribution of Prymnesium organisms as measured in the Coulter electronic particle counter. Organisms of a 2-day culture were suspended in Prymnesium growth medium, counted and sized at 30°. Readings were taken at the following settings of the Coulter counter: gain switch, 8; gain trim, 7; aperture current setting, 5.

Fig. 2. The relation between mean cell volume of various mammalian erythrocytes and 'threshold readings' in the Coulter counter. Erythrocytes, 20,000–30,000/0.5 ml. were suspended in Prymnesium growth medium. Haematocrit values were estimated at 20° and readings in the Coulter particle counter were made at 3°, 20°, and 30°. Threshold readings at 50% of the distribution curve of the population were made and the Counter was set as shown in Fig. 1. Erythrocytes: ● = human, ▲ = rabbit, ■ = mouse.

measurements made under standard temperature conditions. In Fig. 2, threshold values of 50% of the population of erythrocytes were plotted against the volume of these cells as measured by a micro-haematocrit method (Strumia, Sample & Hart, 1954). The results obtained, like those described by Peacock, Williams & Mengoli (1960) and by Grant, Britton & Kurtz (1960), showed that a linear relationship existed between the threshold values and the volume measurement arrived at by the haematocrit method.

For the determination of the lytic activity of weak electrolytes an additional method was used, based on the estimation of the minimal effective dose (MED) of various agents capable of inducing lysis in *Prymnesium* populations. The test was carried out in test tubes containing 4.5 ml. of *Prymnesium* culture. The pH value of the medium was adjusted by adding dilute NaOH or HCl and 0.5 ml. of glycine + NaOH buffer (Sørensen; pH 6.0–9.3) or of sodium acetate + acetic acid buffer (Michaelis; pH 5.0–6.5). The cultures were kept at standard temperatures in continuous white daylight and microscopic observations made at different times. The MED value expresses the minimal concentration of weak electrolytes which induces total lysis of the *Prymnesium* population within 24 hr.

Fig. 3. The effect of pH value on lysis of *Prymnesium* by ammonia and acetic acid. Organisms from a 4-day culture were suspended in 4.5 ml. of *Prymnesium* growth medium; the pH value was adjusted as described under Methods; 0.5 ml. vols. of different amounts of ammonium sulphate or of sodium acetate + acetic acid buffer were added. The minimal effective dose (MED) was determined after 24 hr. at 25°.

RESULTS

Effect of pH value on the lysis of Prymnesium by ammonia and by acetic acid

Observations with the microscope have shown that the nature of the lethal activity of ammonium sulphate on *Prymnesium* discovered by Reich & Aschner (1947) was a lysis which followed swelling of the organisms (Shilo & Shilo, 1961). It was further shown that the lytic activity of ammonia was dependent on the pH value of the milieu (Shilo & Shilo, 1953, 1955). In the present work various weak acids (acetic, citric, oxalic, and tartaric acids) were tested for their lytic activity on *Prymnesium*. Only acetic acid was found to have marked lytic activity on *Prymnesium* and, as with ammonia, this activity was pH-dependent. Figure 3 summarizes results of the effect of the pH value (in the range pH 5.0–9.0) on the lytic activities

of ammonium sulphate and of acetic acid. It can be seen that the lytic activity of ammonia increased with increasing pH value while the activity of acetic acid was greater the smaller the pH value. From these findings it was concluded that the undissociated acetic acid molecule rather than the acetate ion was the active compound in the lysis of Prymnesium, a condition like that suggested for lysis of Prymnesium by ammonia (Shilo & Shilo, 1953, 1961).

Direct measurements of the kinetics of swelling of Prymnesium induced by

Fig. 4

Fig. 4. Effect of pH value of medium on the swelling of Prymnesium induced by ammonia. Organisms of a 4-day culture were suspended in Prymnesium growth medium which was divided into samples adjusted to different pH values and kept at 30°. Cell volumes were determined at different times by the Coulter particle counter, set as described in Fig. 1. The results are given in relative volume units; untreated organisms = 1. Solid symbols: suspensions containing ammonium sulphate 0.1% (w/v). Open symbols: cell suspensions, at corresponding pH values without ammonium sulphate. ↓ = change of pH from pH 9.0 to 6.5.

Fig. 5

Fig. 5. Effect of pH value on swelling of Prymnesium by acetic acid. Organisms of a 4-day culture were suspended in Prymnesium growth medium. The suspension was divided into samples adjusted to different pH values and kept at 30°. Acetic acid + sodium acetate buffer of appropriate pH value was added to a final concentration of 0.01 M. Solid symbols = suspensions containing acetic acid + sodium acetate (0.01 M). Open symbols = suspensions at corresponding pH values without acetic acid.

ammonia at various pH values were made; a typical experiment is summarized in Fig. 4. It was found that with increasing pH value of the suspension medium from pH 6.5 to 9.0 the rate of swelling increased and the final volumes of the swollen organisms were greater the greater the pH value. Upon change from pH 9.0 to 6.5,

the volume of the organisms already swollen by contact with ammonium sulphate at pH 9 quickly returned to a volume near that of untreated organisms. In Fig. 5 the effect of the pH value of the suspension medium on the swelling of *Prymnesium* organisms in contact with acetic acid is shown. Spheres of polystyrene (Dow Chemical Company, Midland, Michigan) 8–16 μ diam., spores of the gastromycete *Rhizopogon luteolus* or pollen of grassweed (Coulter Electronics, Chicago) suspended in the *Prymnesium* growth medium served as controls in these experiments. When measured in the Coulter particle counter none of these particles showed any increase in volume after addition of ammonia or acetic acid at pH and temperature conditions optimal for the swelling of *Prymnesium*.

If lysis of *Prymnesium* depends on differences between external and intracellular pH values, as suggested by Jacobs (1940) for mammalian erythrocytes, no lysis should occur with ammonia or acetic acid in conditions where the pH value of the medium approaches that of the interior of the *Prymnesium* organism. This should give a method for measuring the internal pH value of the organism as a whole, or of different internal regions, if such exist. Indirect measurements of internal pH values, based on interior accumulation of weak electrolytes, have been made in yeasts with acetic acid (Conway & Downey, 1950), in *Valonia* with H_2S (Osterhout, 1925) and by following the uptake of ammonia in *Valonia* (Cooper & Osterhout, 1930), in *Nitella* (Irwin, 1925*a*) and in *Halicystis* (Blinks, 1933). To determine the internal pH value of *Prymnesium*, 'the point of no lysis' was approached from the alkaline side with ammonia and from the acid side with acetic acid. With both compounds no lysis occurred in the region between pH 6.5 and 7.0 (Table 1) thus suggesting that the internal pH value of *Prymnesium* is in this pH range.

Table 1. *Lysis of Prymnesium by ammonia and acetate at different pH values*

Organisms grown for 4 days in *Prymnesium* growth medium were used. The pH value of the suspending medium was adjusted as described under Methods. Lysis within 4 hr. after addition of weak base or weak acid at 25° was observed microscopically in the phase contrast microscope (Reichert Zetopan). Degree of lysis was recorded as: + + +, 100%; + +, 50%; +, 25%; –, 0%.

	pH value of suspension medium					
	5.5	6.0	6.5	7.0	7.5	8.0
	Degree of lysis					
Acetic acid + acetate 0.01 M	+ + +	+ +	+	–	–	–
Ammonium sulphate 0.01 M	–	–	–	+	+ +	+ + +

The effect of age of Prymnesium on sensitivity to swelling by ammonia

The effect of the age of *Prymnesium* organisms on their sensitivity to swelling by ammonia is shown in Fig. 6. Organisms from young cultures (2–4 days) in the early logarithmic phase of growth were compared with organisms taken after 13 and 30 days of incubation. On contact with ammonia the young organisms swelled much more rapidly than the organisms from the older cultures. The young organisms

reached their final volume within 1–5 min., swelling from $250 \mu^3$ to about $500\text{--}600 \mu^3$, while the older organisms continued to swell slowly up to 60 min. and reached smaller final volumes.

*The effect of temperature on lysis of *Prymnesium* by ammonia*

The effect of temperature in the range of $0^\circ\text{--}30^\circ$ on the lysis of *Prymnesium* by ammonia was tested; the results are given in Fig. 7. The rate of swelling as well as the final volumes of the organisms increased with increasing temperature. These results are in agreement with our earlier findings on the correlation between temperature and lysis by ammonia as expressed by the minimal effective dose of ammonium sulphate in cultures of *Prymnesium* and the temperature dependence of its algicidal activity against *Prymnesium* blooms in fishponds (Shilo & Shilo, 1953).

Fig. 6

Fig. 7

Fig. 6. The effect of age of *Prymnesium* organisms on the kinetics of swelling by ammonia. Organisms were suspended in *Prymnesium* growth medium at pH 9.0. Readings were taken at 30° in the Coulter particle counter set as described in Fig. 1. Volume is given in relative units; untreated organisms = 1. Solid symbols: suspensions containing ammonium sulphate 0.1 % (w/v). Open symbols: suspensions of corresponding age without ammonium sulphate.

Fig. 7. The effect of temperature on the course of swelling of *Prymnesium* organisms induced by ammonia. Organisms of a 4-day culture were suspended in *Prymnesium* growth medium and kept at different temperatures at pH 9.0. Readings were taken in Coulter particle counter (set at gain switch, 7; gain trim, 3; aperture current setting, 4). Volumes as relative units; untreated organisms = 1. Solid symbols: suspensions containing ammonium sulphate 0.1 % (w/v). Open symbols: suspensions of corresponding temperatures without ammonium sulphate.

*The effect of osmotic pressure of the milieu on lysis of *Prymnesium* by ammonia and acetic acid*

Different weak electrolytes were found to act alike in causing lysis of *Prymnesium*, this effect resembled the ammonia-induced lysis of human erythrocytes described by Jacobs (1940). Attempts were made to see whether an osmotic mechanism underlies the lysis of *Prymnesium* induced by weak electrolytes. The lytic activity of ammonia and acetic acid on *Prymnesium* at different osmotic pressures of the suspension medium was tested. Figure 8 summarizes results testing different salt concentrations on the lytic activity of ammonium sulphate. It can be seen that the minimal amount of ammonia which caused lysis was a function of the external osmotic pressure. For the marine strain of *Prymnesium* and for the strain isolated from brackish fishponds, the lytic activity of ammonia salts was found to decline

Fig. 8

Fig. 9

Fig. 8. Effect of osmotic pressure of the suspending medium on sensitivity of *Prymnesium* to lysis by ammonium sulphate. Organisms of 4-day cultures of the two *Prymnesium* strains were suspended in Droop medium of different salinities by altering sea water and sodium chloride content. A relative salinity of 2.0 was obtained by using concentrated sea water. Minimal effective dose (MED) was determined at pH 8.8 at 25° as described under Methods. ● = *Prymnesium* isolated from sea water (Droop's strain); ○ = *Prymnesium* isolated from brackish water fishponds (Reich's strain).

Fig. 9. The effect of sucrose on the course of swelling of *Prymnesium* organisms by ammonia. Organisms of 2-day cultures were suspended in *Prymnesium* growth medium and kept at 30°, pH 9.0, with different amounts of sucrose. Volume was estimated in the Coulter counter, set at gain switch, 7; gain trim, 8; aperture current setting, 4. Solid symbols = suspensions containing 0.1% (w/v) ammonium sulphate. Open symbols = suspensions of corresponding sucrose concentration without ammonium sulphate. ↓ = addition of sucrose (0.4 M).

with increasing osmotic pressure of the suspension medium. Change of external pressure by addition of sucrose (0.1–0.4M) to the suspension medium caused a marked delay in swelling and lysis of *Prymnesium* by ammonia or acetic acid. Figure 9 shows the effect of different sucrose concentrations on the kinetics of swelling of *Prymnesium* organisms by ammonia as measured in the Coulter counter.

Fig. 10. Stages of swelling and lysis of *Prymnesium* induced by ammonia. The organisms were grown in *Prymnesium* growth medium for 4 days. The stages of swelling and lysis of a typical *Prymnesium* organism in contact with 0.1% (w/v) ammonium sulphate at pH 9.0 at 25° were drawn with the aid of a camera lucida under the phase contrast microscope (Reichert Zetopan). Enlargement $\times 1250$.

Sucrose at 0.4M almost completely inhibited swelling of the organisms; with lower concentrations of sucrose partial suppression of swelling was observed. Addition of sucrose to 0.4M to organisms already swollen by ammonia to their maximal size in absence of sucrose led to immediate shrinkage of the organisms until the volume attained by *Prymnesium* organisms in similar sucrose concentrations was reached.

*The morphological changes in swelling and lysis of Pymnesium
induced by ammonia and by acetic acid*

The sequence of morphological changes leading to lysis by weak electrolytes was observed under the phase-contrast microscope. Pymnesium organisms swell considerably when put into ammonia or acetic acid at appropriate pH values and temperatures, and they are very suited for the study of visual changes of internal structure. Furthermore, the rupture of the organism causes the liberation of internal organelles into the medium and these can be observed under various experimental conditions (Shilo, 1961).

The morphological changes leading to lysis of Pymnesium by ammonia are shown in camera lucida drawings (Fig. 10) and in photomicrographs (Pl. 1). They show the sequence of events in single organisms undergoing swelling and lysis. When ammonia was added at appropriate pH values and temperatures the organisms began to swell because of the enlargement of two lateral vacuoles, thus causing the usually elongated organism to take a swollen ball-like form. At this stage the flagella broke off, the contractile vacuole at the anterior part of the organism stopped its rhythmical beating and the elongated chromatophores were pushed to the anterior part of the organism. The organism continued to swell. This was seen in the phase microscope by noting the increase in distance between the granules in the envelope. Upon rupture of the envelope the two lateral vacuoles became spherical and continued to swell until they burst. With lysis induced by acetic acid the sequence of morphological changes seen in the phase contrast microscope was different from that seen during lysis by ammonia. In lysis with acetic acid, like lysis in hypotonic conditions (Pl. 1), the organisms swelled because of increase in volume of the large posterior vacuole and finally lysed. The lateral vacuoles which were the site of swelling in ammonia-induced lysis did not become visible at all during the process of lysis with acetic acid.

DISCUSSION

The pH-dependence of lysis of Pymnesium by weak electrolytes may be explained by the fact that the organism is permeable to the undissociated electrolyte but not to the ion. This is in agreement with results on the penetration of weak electrolytes found with other biological systems (Irwin, 1925*a, b*; Osterhout, 1925; Cooper & Osterhout, 1930; Collander, Turpeinen & Fabritius, 1931; Jacobs & Parpart, 1938; Conway & Downey, 1950; MacMillan, 1956).

The degree of concentration of weak electrolyte inside the organism is suggested to be dependent upon the difference existing between the interior and exterior pH values. These relationships were expressed for mammalian erythrocytes by Jacobs (1940), based on the Hasselbach-Henderson equation for the pH values of aqueous solutions of weak electrolytes, as follows:

$$C = \frac{1 + 10^{pH_o - pK}}{1 + 10^{pH_i - pK}} \cdot C_o$$

where C = total concentrations of all forms of NH_3 ;

o = outside the erythrocyte; i = inside the erythrocyte.

This equation allowed the calculation of the total concentration of all forms of the weak electrolyte within the cell at different pH values, different strengths of electrolyte, and different electrolytes.

The results with Prymnesium showed that there was a general similarity between the behaviour of erythrocytes and the phytoflagellate as to the dependence of the lytic activity of ammonia on the pH value of the medium (Shilo & Shilo, 1955, 1961). Table 2 shows that the experimental values for the lytic activity of ammonia at different external pH values, as expressed by the minimal effective dose (MED) of the weak electrolyte, were near the values calculated by the equation proposed by Jacobs.

Table 2. *The lytic activity of ammonium sulphate on Prymnesium at different pH values*

pH value	Concentration of total intracellular ammonia calculated concentration factor C_i/C_o *	Lytic activity of ammonium sulphate. Experimental value†
7.0	1	1
8.0	10	9
9.0	64	56

* Intracellular concentration factor C_i/C_o was calculated on the basis of the equation of Jacobs (1940) assuming internal pH 7.0 for Prymnesium.

† The experimental value of lytic activity is expressed as a function of the reciprocal of minimal effective dose of ammonium sulphate on Prymnesium. For comparison, the minimal effective dose of $(\text{NH}_4)_2\text{SO}_4$ (38 mg. %) at external pH 7.0 has been taken as 1.

The findings of the present work suggest that an osmotic mechanism underlies the lytic activity of weak electrolytes upon Prymnesium. The weak electrolytes accumulate in the organism until the internal osmotic pressure is greater than the pressure which the envelope of the organism can resist. The osmotic nature of the lysis of Prymnesium induced by weak electrolytes is further suggested by its dependence on the osmotic pressure of the suspending medium. As might be expected with an osmotic mechanism of lysis, the organisms were more sensitive to lysis by weak electrolyte the smaller the osmotic pressure of the suspension medium. That acetic acid and ammonia lyse Prymnesium is consonant with the idea that lysis is due to entry of weak electrolyte which causes an osmotic imbalance, rather than that these compounds have some other toxic effect.

The morphological changes of Prymnesium organisms, during lysis caused by a weak acid or a weak base, showed that the increase in size of different regions of the organism was responsible for the swelling which leads to rupture. These findings might be explained by a difference in the pH value of different regions. It would follow that the lateral vacuoles responsible for lysis by ammonia must have a pH value smaller than that of the posterior vacuole responsible for lysis by acetic acid. These vacuoles of Prymnesium seem to behave like different compartments each surrounded by a semipermeable membrane.

The drastic increase of lytic activity of ammonia towards Prymnesium with increase in temperature (range 0° – 30°) indicates that the factors which influence the concentration of the weak electrolyte inside the organism must be much more temperature-sensitive than would be expected on the basis of penetration by

diffusion alone. Irwin (1925*b*) showed that penetration of the weak electrolyte brilliant cresyl blue in undissociated form into *Nitella* had a high-temperature coefficient.

The close agreement of our experimental findings with the theoretical predictions of Jacobs makes it likely that a concentration mechanism dependent on pH differences is operative. However, it may perhaps be supposed that the functioning of a temperature-sensitive metabolic process can affect the rate of swelling and final volume of an organism. Such a process which might affect the lysis of *Prymnesium* by a weak electrolyte might be that of an internal pH stabilization of different intracellular regions.

Ammonia has found wide application as an effective agent in controlling mass development of *Prymnesium* in brackish-water fishponds, thus preventing extensive fish mortality from the toxin produced by *Prymnesium*. On the basis of the proposed osmotic mechanism better understanding of the reasons which underlie the algicidal activity of ammonia in fishponds may come from the proposed osmotic mechanism. Resistance of *Prymnesium* to the lytic activity of ammonia salts often found in ponds was shown to be mainly due to small pH values, low temperatures, or the relatively high salinity of the pond water. The results described may also explain the outstanding sensitivity of *Prymnesium* to lysis by ammonia or acetic acid in comparison with other micro-organisms. *Prymnesium* until recently was the only known nucleated organism which in relatively low concentrations of these weak electrolytes undergoes lysis. *Prymnesium parvum* has a normal habitat of high salinity, i.e. it can develop in salt concentrations of 6%, but can also thrive in a milieu containing only 0.1% as in brackish fishponds. This is about the lowest degree of salinity in which this phytoflagellate is able to survive. Under such conditions even a slight increase in the internal osmotic pressure as may be caused by accumulation of ammonia or acetic acid at certain pH values and temperatures could cause osmotic imbalance and lead to lysis.

Other micro-organisms such as the flagellate *Dunaliella salina* Teodor and *Syracosphaera carterae* and also certain luminous marine bacteria were found to swell and lyse when brought in contact with ammonia under conditions of high external pH and when the osmotic pressure of the surrounding medium approached the lower limit for viability of these organisms. These findings indicate that lysis of microbial cells by weak electrolytes may, in fact, be a widespread and general phenomenon, provided the cells are already under conditions of severe osmotic stress.

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EXPLANATION OF PLATE 1

Photomicrographs of the swelling and lysis of typical single *Prymnesium* organisms followed under the phase contrast (Reichert Zetopan) oil immersion. $\times 1250$. Cells were suspended in *Prymnesium* growth medium containing (a) 0.1% (w/v) ammonium sulphate at pH 8.5 at 25°; (b) 0.02 M-acetate at pH 5.0 at 25°; (c) cells suspended in distilled water.