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Magnesium Content in the Two Forms of *Proteus vulgaris*

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the Two Forms of *Proteus vulgaris*

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Abstract

Magnesium Content in the Two Forms of *Proteus vulgaris*

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In this study the long form or swarming forms of *Proteus vulgaris* were found to have twice the magnesium per gram of cell weight than the short or nonswarming forms of *Proteus vulgaris*. This assay was accomplished by use of atomic absorption spectroscopy.

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Last but not least, I dedicate this manuscript to all those who said I would never survive in college, and to those who have never had anything dedicated to them.

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Introduction

The swarming phenomenon of the genus Proteus was first described by Hauser (11) in 1885. Proteus was the Greek god of the oceans who assumed many shapes to escape questioning (10). This bacterium was named for the morphological changes of *Proteus mirabilis* and *Proteus vulgaris*. The genus Proteus includes five species, but of these only *P. vulgaris* and *P. mirabilis* have the swarming ability.

The swarming phenomenon of Proteus has at least three stages; the formation of swarm cells, the movement of swarm cells across the surface of the solid medium, and the division of the swarm cells back to short forms (32). The formation of the swarm cells is accomplished by the significant alteration of the cell morphology, and has been described by many investigators (6, 8, 10, 13, 14, 15, 17, 18, 19, 20, 21, 23, 26). Normally, the short rods are approximately 0.6 um in diameter and 2-4 um in length, while the long forms are approximately 0.7 um in diameter and 20-80 um in length. These swarm cells are believed to be products of an altered cellular division. The swarm cells contain several nuclei but have no septa (13, 17). This increase in length is accompanied by a sharp increase

in the number of flagella per unit surface area (13). When Proteus is exposed to an antiswarming agent such as the chelating agent ethylenediaminetetraacetic (EDTA), there appears to be an inhibition of both flagella activity and cellular division (3). A reversion to and/or continuation of swarming has been noted with the addition of magnesium sulphate or calcium chloride to the medium (30). This effect of magnesium in cell division has been observed by other authors working with several bacteria (1, 12, 24, 27, 28, 29). The lack of magnesium produces a cell curiously similar to the swarm cell of Proteus.

It is the purpose of this study to assay the concentration of magnesium in the two forms of Proteus. This has been accomplished by the use of atomic absorption spectroscopy.

Review of Literature

Speculation as to the cause of swarming in Proteus has resulted in the presentation of several theories seeking to explain this phenomenon. Moltke (21) and Russ-Munzer (23) believed the phenomenon to be a deliberate action due to starvation, or to positive chemotaxis. The theory of negative chemotaxis was proposed by Lominski and Lendrum (20) and was supported by Klieneberger-Nobel (19). This theory postulates a build-up of metabolites in the medium, the metabolites reach a critical concentration within the stationary growth site and causes the formation of filamentous or swarm cells. Formation of concentric rings of growth was regarded as supporting evidence for this theory. The rings were postulated as being formed due to a gradient of an increasing concentration of waste products.

The theory of positive chemotaxis and that of negative chemotaxis were dealt a serious blow by Williams and co-workers (31). Williams used extensive test for positive chemotaxis using procedures devised by Adler (2, 3, 4, 5) in studying *Escherichia coli*. Several approaches were used to test for the more widely held

negative chemotaxis. These included; the capillary method of Doetsch and Seymour (7), and the "cells in the well assay" or "agent in the plug assay" of Tso and Adler (25). The data from both negative chemotaxis indicated that no gradient was being detected by the Proteus. The positive chemotaxis test were inconclusive, though showing no evidence of positive chemotaxis taking place.

Hoeniger (13) found that when Proteus differentiated from the short form to the long swarm cells, they obtained definite cellular units. These units consist of a nuclei and adjacent cytoplasm. There is apparently no septa between successive units. This has been interpreted as an interrupted or an inhibited cell division. But it must be noted that the swarm cells are in every other way viable cells.

In a later paper Hoeniger (14) dealt with the morphology of Proteus treated with penicillin, lithium chloride, ultra-violet light and gamma radiation respectively. Data from this research refuted earlier work by Hughes (15) who found the morphology of normal swarmers indistinguishable from that induced by penicillin and other toxic agents. Hoeniger found abnormal looking

nuclei and malformed cell wall in the penicillin induced swarmers. When the induced swarmers were transferred to fresh, complete medium there appears to be a recovery to a normal sequential formation of both swarm cells and short forms.

When various amino acids were added to a well-defined minimal medium Jones and Parks (17) found that no particular amino acid was required for swarming. In a later paper (18) it was noted that the long forms have more flagella per unit surface of the organism, and they possess no free amino acid pool. In this paper it was postulated that the long forms develop due to an inhibition of cell division. They suggested that the increase in flagella and the formation of the long forms are not independent, but rather are interdependent events.

Webb noted, in his paper of 1953 (29), the effect of various concentrations of magnesium. In earlier studies by Webb (27, 28) it was shown that *Clostridium welchii*, grown in a magnesium-free media developed an abnormally filamentous cell. This finding was supported by the work of Hewitt (12), and of Shankar and Bard (24) who worked with *Bacillus cereus* and *Aerobacter aerogenes* respectively. Other metal ions, with the possible exception of divalent manganese, cannot overcome this adverse effect of

magnesium deficiency on the cell division processes. The formation of filaments in a media lacking magnesium is enhanced by adding certain divalent metal ions such as nickel, cadmium, manganese, zinc, and cobalt (1). This may result from competitive ion antagonism between the different ions and the residual magnesium present in the medium (22).

Weiser and co-workers (30) found that by adding magnesium sulphate ($MgSO_4$) to a culture of Proteus containing the antiswarm agent ethylenediaminetetra-acetic acid (EDTA), the antiswarm effect of the EDTA could be reversed. The antiswarm agent's mode of inhibition is unknown but is believed to inhibit growth and flagella action by chelation of the magnesium.

Materials and Methods

The organism *Proteus vulgaris* used in this study were obtained from Dr. Francis A. Montaldi. All cultures were maintained on slants of nutrient agar and stored at 4°C.

Standard nutrient broth and nutrient agar were used for growth media. The media was autoclaved, and the plates containing the solid media was dried overnight at 27°C before use.

Long forms were collected by the method described by Jones and Parks (17) from the edge of an actively swarming colony. The harvested cells were then suspended in 0.5M saline and fractionated with mortar and pestle. The cells were then centrifuged at 2,500 g for two hours (18). The pellet was removed and resuspended in distilled water and centrifuged at 10,000 g for ten minutes.

To obtain short forms for analysis nutrient broth cultures were incubated for twelve hours at 27°C. These organisms were then harvested by centrifugation at 2,500 g (18) for two hours and resuspended in saline. They were then subjected to the same treatment as the long form.

The relative purity of the material was insured by direct microscopic examination of each preparation. Samples of the short form were found to be free of any long forms. Testing for contaminants in the samples of long forms was more difficult, due primarily to the abnormal size and the mechanical forces applied in removing the bacterium from the agar surface, i.e. the breakage of cells. With proper technique and discrimination it became possible to obtain virtually pure samples.

Samples were then dried for forty-eight hours at 27°C and weighed. The samples were hydrolyzed by 6N HNO_3 at 90°C for two weeks, evaporated to 2 ml., and finally volumetrically diluted to 5.0 ml. The magnesium content of the samples was determined by atomic absorption spectroscopy.

Results

The assay for magnesium content in the two forms of *Proteus vulgaris* yielded some interesting results. These results obtained by atomic absorption spectroscopy showed a significant increase in the percent of magnesium contained in the long form or swarming cells of Proteus. This increase is shown to be a ratio of 2:1 (See Tables 1 and 2).

Sample Weight	Conc. Mg. ppm	% Mg
0.0005 g	0.475	0.480
0.0007 g	0.700	0.500
0.0230 g	12.8	0.560
0.0203 g	11.7	0.580
0.0186 g	10.7	0.580

Magnesium Content Long Forms

Table 1

Sample Weight	Conc. Mg. ppm	% Mg
0.0062 g	2.48	0.200
0.0072 g	2.65	0.185
0.0164 g	3.75	0.115
0.0194 g	9.60	0.247
0.294 g	14.63	0.249

Magnesium Content Short Forms

Table 2

Discussion

The dramatic rise in magnesium found in the swarming form of Proteus seems to give credence, to the idea that this is an aberrant form of the bacterium. This could correlate with Hoeniger's idea of an interrupted cell division. With an increase in metabolic activity that accompanies cell division, one would expect an increase in enzymes and cofactors. The findings of Jones and Parks (18) dealing with the lack of a free amino acid pool coupled with an increase in the size of the swarm cells and the number of flagella also lends weight to the idea that the two are interdependent. These increases would necessitate a relative increase in the amount of magnesium.

In a very recent article (16) involving *Salmonella typhimurium*, it was reported that metal ions alone are not chemoeffectors. But when the ions are complexed with citrate, a chemotaxis response appeared. There appears, in light of the previous work with Proteus and other bacteria, to be a correlation between chemotaxis and magnesium dependence. In testing for the presence of positive chemotaxis no researcher has yet tested Proteus for an affinity to metal ions, or a metal ion

complex. If chemotaxis is the triggering factor in the elongation of Proteus, it would appear to be a function of positive chemotaxis to magnesium.

With the information that is now at hand, I must consider the possibility that positive chemotaxis and the increase in magnesium content are somehow linked. The idea that positive chemotaxis was responsible for the formation of swarm cells and the subsequent swarming phenomenon never was totally disproved by Williams and co-workers. This idea, while not by any means conclusive does account for many of the observations that were previously stated.

It has been known for sometime that magnesium, in addition to its use as a cofactor in metabolism, has a functional purpose in the outer membrane of bacteria. The magnesium in the outer membrane acts to stabilize the anionic groups that compose the membranes. The mechanism that accomplishes this is not entirely clear at this time.

A speculative approach to the swarming of Proteus has been made by several authors. I am now considering a mechanism involving a system of priorities within Proteus. First, under normal circumstances (i.e. sufficient nutrient, etc.) the cell remains in the short form, but

as nutrients are depleted a priority system is triggered and the organism shifts it's metabolism to accommodate the new environment. If this is true the magnesium may well be removed from the outer membrane and drawn into the cell to fulfill a more pressing requirement. It has been shown in other bacteria that the cell membrane that is deprived of the stabilizing effect of magnesium, undergoes an abnormal growth cycle. In the case of Proteus the magnesium concentration may be either a triggering mechanism or a chemotaxis response or both.

In future studies an atomic absorption spectroscopy of all separate components of the cell envelope and the cytoplasm of both forms of Proteus may yield interesting results. This assay should be expanded to include not only magnesium but also copper, manganese, iron, calcium, nickel, cadmium, and zinc. If my idea is correct one might expect to see a shift in metal ions from the outer membrane to the interior of the cell.

Literature Cited

1. Abelson, P. H., and E. Aldous. 1950. Ion antagonisms in microorganism: Interference of normal magnesium metabolism by nickel, cobalt, cadmium, zinc, and manganese. J. Bact. 60:401-413.
2. Adler, J. 1966. Chemotaxis in bacteria. Science 153:708-716.
3. Adler, J. 1973. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. J. Gen. Microbiol. 74:77-91.
4. Adler, J. 1975. Chemotaxis in Annu. Rev. Biochem. 44:341-356.
5. Adler, J., and B. Templeton. 1967. The effect of environmental conditions on the motility of *Escherichia coli*. J. Gen. Microbiol. 46:175-184.
6. Bisset, K. A. 1973. The motion of the swarm in *Proteus mirabilis*. J. Med. Microbiol. 6:33-36.
7. Doetsch, R. N. and F. W. K. Seymour. 1970. Negative chemotaxis in bacteria. Life Sci. 9:1029-1037.
8. Fleming, A., A. Voureka, I. R. H. Kramer, and W. H. Hughes. 1950. The morphology and motility of *Proteus vulgaris* and other organisms cultured in the presence of penicillin. J. Gen Microbiol. 4:257-267.

9. Fuscoe, F. J. 1973. The role of extracellular slime secretion in the swarming of Proteus. J. Med. Lab. Technol. 30:373-380.

10. Harvey, P. 1940. The Oxford companion to classical literature. Oxford University Press, Oxford.

11. Hauser, G. 1885. Uber faulnissbacterien und deren bezienungen zur septicamie. F. G. W. Vogel, leipzig.

12. Hewitt, L. F. 1951. Cell structure of *Corynebacterium diphtheriae*. J. Gen. Microbiol. 5:278-292.

13. Hoeniger, J. F. M. 1964. Cellular changes accompanying the swarming of *Proteus mirabilis*. I. Observations of living cultures. Can. J. Microbiol. 10:1-9.

14. Hoeniger, J. F. M. 1966. Cellular changes accompanying the swarming of stained organisms. Can. J. Microbiol. 12:113-122.

15. Hughes, W. H. 1957. A reconsideration of the swarming of *Proteus vulgaris*. J. Gen. Microbiol. 17:49-58.

16. Ingolia, T. D., and D. E. Koshland, Jr. 1979. Response to a metal ion citrate complex in bacterial sensing. J. Bacteriol. 140:798-804.

17. Jones, H. E., and R. W. A. Park. 1967. The short forms and long forms of Proteus. J. Gen Microbiol. 47:359-367.

18. Jones, H. E. and R. W. A. Park. 1967. The influence of medium composition on the growth and swarming of Proteus. J. Gen. Microbiol. 47:369-378.

19. Klieneberger-Nobel, E. 1947. Morphological appearance of various stages in *B. proteus* and coli. J. Hyg. 45:410-412.

20. Lominski, I., and A. C. Lendrum. 1947. The mechanism of swarming of Proteus. J. Pathol. Bacteriol. 59:688-691.

21. Moltke, O. 1927. Contributions to the characterization and systematic classification of Bac. *Proteus vulgaris* (Hauser). Levin and Munksgaard, Copenhagen.

22. Neyland, M., P. Dunkel, and A. L. Schade. 1952. The uptake of cobalt by *Proteus vulgaris*. J. Gen Microbiol. 7:409-416.

23. Russ-Munzer, A. 1935. Das schwarmphanomem bei *Bacillus proteus*. Zbl. Bakt. A. Abt. Orig. 133:214-223.

24. Shankar, K., and R. C. Bard. 1952. The effect of metallic ions on the growth and morphology of *Clostridium perfringens*. J. Bacteriol. 63:279-290.

25. Tso, W., and J. Adler. 1974. Negative chemotaxis in *Escherichia coli*. J. Bacteriol. 18:560-576.

26. Vander Molen, G. E., and F. D. Williams. 1976. Observations of the swarming of *Proteus mirabilis* with scanning electron microscopy. Can. J. Microbiol. 23:107-112.

27. Webb, M. 1948. The influence of magnesium on cell division. I. The growth of *Clostridium welchii* in complex media deficiency in magnesium. J. Gen. Microbiol. 2:275-286.

28. Webb, M. 1949. The influence of magnesium on cell division. II. The effects of magnesium on the growth and cell division of various bacteriological species in complex media. J. Gen. Microbiol. 3:410-416.

29. Webb, M. 1953. Effects of magnesium on cellular division in bacteria. Science. 118:607-611.

30. Weiser, R., J. C. Wimpenny, A. W. Asscher, D. B. Moffat, and M. Sussman. 1971. Inhibition by Ethylenediaminetetraacetic acid (EDTA) of the swarming *Proteus mirabilis*. Zentralbl. Bakteriol. Parasitenkl. Infektionskr. Hyg. Abt. I Orig. Reiche A. 218:434-436.

31. Williams, F. D., D. M. Anderson, P. S. Hoffman, R. H. Schwarzhoff, and S. Leonard. 1976. Evidence against the involvement of chemotaxis in swarming of *Proteus mirabilis*. J. Bacteriol. 127:237-248.

32. Williams, F. D., and R. H. Schwarzhoff. 1978. Nature of the swarming phenomenon in Proteus. Ann. Rev. Microbiol. 32:101-122.

Vita

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Upon completion of his undergraduate program, Michael accepted an assistantship for graduate study at Appalachian State University, and began work on the Masters of Science degree in August 1977.