SCIENCE DILIMAN
Received November 4, 1991

The Effects of Different Light Qualities on Gamma Ray-Treated *Scenedesmus*

Adoracion T. Arañez
Institute of Biology
College of Science

ABSTRACT

Unialgal culture of Scenedesmus quadricauda *(Turp.) Breb. suspended in distilled water was treated with 0 Gy (control), 40 Gy, 80 Gy, and 120 Gy gamma rays from a to ^{60}Co source at Philippine Nuclear Research Institute, placed in one-liter flasks, enough Bold basal medium added to give a transmittance of 97% determined by using a spectrophotometer, kept in the dark for 24 hrs., then placed in vials and distributed equally in three growth chambers with white light, red light, and blue light. The lighting was continuous with algae subjected to the same light intensity, and the temperature maintained at 30 ± 1^{o} C. A sterile regimen was followed.*

For the first two weeks, the growth rates of algae under white light were highest, followed by those under red light, while those grown under blue light had the lowest growth rate. After the second week, differences in growth rates were not significant anymore. During the first week, generally low doses of gamma radiation had stimulating effects on growth rates while high doses had inhibitory effects on growth. The abnormal Scenedesmus *observed were enlarged cells of normal shape in coenobia of two cells and four cells, enlarged cells that were not forming coenobia, coenobia composed of enlarged cells of abnormal shape, coenobia composed of enlarged cells of different sizes, cells with kidney-shape chloroplast, coenobia with cells that were not in alignment as in the normal ones, and cells that were colorless. Percentage of coenobia with enlarged cells determined one week after the treatment showed that higher doses generally produced more enlarged cells than lower doses. Treated algae grown in red light and blue light which showed lesser growth rates than those under white light had higher percentage of enlarged cells. Enlarged cells of normal shape were successfully propagated for more than ten generations.*

INTRODUCTION

Scenedesmus is a microalga that is a good source of protein. It has a protein content of 50 to 56% based on its dry weight. Unlike other plants which have proteins deficient in some essential amino acids, *Scenedesmus* contain all the amino acids required in human nutrition (1).

Conventional sources of proteins are very costly and many cannot afford to include this in their diet in a sufficient amount that is needed by the human body. Incorporating unconventional sources of proteins to human diet or supplementing the human diet with dried *Scenedesmus* in capsule form may help solve the protein requirements of some Filipinos. Feeding experiments in a hospital in Bangkok showed that incorporating 12 to 14 grams of dried *Scenedesmus* to human diet does not affect the uric acid content of the blood plasma (2).

Induced mutation and selection is one way of improving the properties and qualities of the green alga *Scenedesmus*. The yield can be improved by developing strains that are faster-growing, larger, and with a higher protein content.

Gamma radiation is widely known to produce effects on various organisms as alterations in mitosis, cytokinesis, and cell size as well as in individual cell structures such as the nucleus, chromosomes, and chloroplast (3). Studies on the progeny of irradiated *Oedogonium* cells showed different abnormalities in the population as giant cells, nuclear fragments, anucleate cells, abnormal cell plates as well as chromosome aberrations (4). Filamentous nonbranching blue-green algae treated with radiations produced branches as in *Nostoc* treated with ultraviolet radiation (5), *Anabaena* treated also with ultraviolet radiation (6), and *Nostoc* treated with gamma radiation (7). Other morphological changes produced in *Nostoc* treated with gamma radiation were increased length and width of cells, enlarged heterocysts, and chains of multiheterocysts (7).

Effects of radiation on algae can be modified by light quality. It was suggested that the radiosensitivity of microorganisms is genetically controlled and that it is also dependent upon environmental conditions before, during, and after irradiation (8). Inactivating effect of ultraviolet light on the blue-green alga *Gloeocapsa alpicola* was totally reversed by blue light immediately after irradiation (9). However, if the irradiated cells were placed in the dark before exposure to blue light, reversal became progressively less efficient and almost disappeared after 60–80 hours in the dark (9). Black, blue, and white light were able to photoreactivate the ultraviolet irradiated

Anacystis nidulans while green, yellow, and red lights were not capable of photoreactivation (11).

Light quality influenced the metabolic processes. In *Chlorella* cells grown in white light and then transferred to blue light, formation of DNA, RNA, protein, and total carbohydrates were inhibited to a marked extent (11). Blue light increased the synthesis of ribulose biphosphate carboxylase in green alga *Chlorogonium elongatum* (12). Formation of pigments was altered upon exposure to different light qualities. In *Anabaena cylindrica* the highest amount of C-phycocyanin was observed in red light, while blue light increased C-phycoerythrin (13). In *Anabaena variabilis,* highest amount of C-phycocyanin was found in yellow light and allophycocyanin was found in blue light (14).

Scenedesmus cells adapted to blue light showed a higher chlorophyll content, a higher ratio of chlorophyll to carotenoid and a much higher ratio of chlorophyll to cytochrome f than those adapted to red light (14). Blue-light-adapted *Scenedesmus* cells had smaller chlorophyll a to chlorophyll b ratio (14).

Effects of different light qualities on the frequency and the kind of mutant cells produced by gamma radiation may be useful in the propagation of better strains of *Scenedesmus.*

The objectives of this study are to determine the effects of different light qualities (white, red, and blue light) on growth rate of normal and gamma ray-treated *Scenedesmus* and the types and frequency of mutant cells produced by these treatments.

EXPERIMENTAL SECTION

The organism used in this study was a unialgal culture of *Scenedesmus quadricauda* (Turp.) Breb. in Bold basal medium (15) maintained in the Natural Sciences Research Institute of the University of the Philippines at Quezon City. The original stock was obtained from the Phycology laboratory of the Department of Botany, University of the Philippines at Los Baños, the identification of which was verified by Dr. Milagrosa R. Martinez.

Scenedesmus quadricauda was subcultured in Bold basal medium for one week under continuous white light of 300 lux. Around 200 ml was centrifuged at 4,000 rpm for 20 minutes. The sediment was suspended in 200 ml sterilized distilled water and shaken carefully to produce a homogenous mixture. Twenty ml of the algal suspension was transferred to each of eight 125 ml flask with cotton plug. Two flasks, set A and set B, were exposed to each of the following doses of gamma radiation: 0 Gy (control), 40 Gy, 80 Gy, and 120 Gy from ^{60}Co source at the Philippine Nuclear Research Institute (PNRI).

After the treatment, the algae in each of the eight 125 ml flask were transferred to a 1000 ml erlenmayer flask and enough Bold basal medium was added to give a transmittance of 97% determined by using a Bausch and Lomb Spectronic 20 spectrophotometer at wavelength 555. The algae were kept in darkness for 24 hrs. by covering each of the eight flasks with aluminum foil to prevent photoreactivation repair.

For the growth rate determination, 240 vials were prepared. To each of 30 vials was placed 10 ml aliquots from each of the eight 1000 ml flasks. Ten vials with 10 ml of algal suspension from each of the batch treated with varying doses of gamma radiation, were placed under white light; ten vials under blue light; and the other ten vials, under red light. The algal population was analyzed weekly using a spectrophotometer (16) at wavelength 555. The average of the percent transmittance of set A and set B was taken and recorded as percent transmittance. The algal cell concentration was expressed as optical density (O.D.) which was computed from the percent transmittance by using the formula of Seeley and Van Demark (17) given below:

$$\text{O.D.} = \log 100 - \log \text{galvanometric reading (transmittance)}$$

The rate of growth (K), expressed as number of cell doublings per week, was determined by using the modified formula of Posner and Sparrow (18) as follows:

$$K = \log_2 (N_f/N_i)$$

where N_i was the intial optical density or the optical density immediately preceeding the N_f and N_f was the optical density after one week.

The remaining algal suspensions were placed in vials and distributed equally in the growth chamber with white light, red light, and blue light. These were examined regularly for the determinations of kinds and frequency of morphological changes produced by the treatments.

The height of the rack in each growth chamber was adjusted to get a uniform light intensity of 13μ E $s^{-1}m^{-2}$ for each chamber of white light, red light, and blue light. The temperature was maintained at 30±1°C. Lighting was continuous. Data given in all of the tables were based on two treatments. There were two replicates per treatment. Statistical analyses of data were done using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT).

Mutants from the above treatments were isolated and cultured under continuous white light. Pure culture of enlarged cells was prepared by serial dilution technique and agar plate method using 1.5% bacto agar in Bold basal medium. A sterile regime was followed throughout the study.

RESULTS AND DISCUSSION

For the first two weeks, the growth rates of algae under white light were highest, followed by those under red light, while those grown under blue light had the lowest growth rate (Table 1). However, the difference in growth rates in week one of algae in white light and red light was not significant. The lowest rate observed in algae grown under blue light was significantly different at 1% level from those under white light and red light. On the second week, effect of light quality on growth rate was still significant but this time growth rates of algae in red light and blue light did not differ significantly. After the second week generally treated algae grown in red light and blue light had higher growth rates than those grown in white light. However, difference in growth rates were not significant anymore.

Hess and Tolbert (19) investigated growth rates of *Chlamydomonas reinhardtii* and *Chlorella pyrenoidosa* cells grown under white, red, and blue lights. Rates of growth under blue light and red light were initially found to be low, but increased over a period of five days until normal growth was reestablished. The reduced growth rates under red and blue lights that were recovered later may be an indication that the algae were able to adapt to red and blue lights after sometimes (19). In a study by Sokowa and Hase (20) on the effects of light on *Chlorella protothecoides*, blue light induced the most active formation of chlorophyll and red one, the least. However, blue light was found to be most effective in the suppression of the DNA replication. The rate of cell division is affected by the rate of DNA replication.

A decrease in growth rates, which is significant at 1% level, from week one to week 6 in algae grown under white light and red light was observed. There was also a tendency for algae grown under blue light to follow this trend although the difference in rates of growth was not significant. This decrease in growth rate may be due to accummulation of toxic waste products or reduction in nutrients or both accummulation of waste products and reduction of nutrients.

During the first week, generally low doses of gamma radiation had stimulating effects on growth rates while high doses had inhibitory effects on growth.

Stimulating effects on growth of low doses could be due to the effects of the treatment on growth regulators. Reduction in growth may be due to the effects of gamma radiation on enzymes or growth regulators or both enzymes and growth regulators. In higher plants, Ussuf et al. (21) inferred that irradiation interfered with the synthesis of enzymes and, at the same time, accelerated the degradation of existing enzymes involved in the formation of auxins which are involved on cell wall elongation; Ananthaswamy et al. (22) observed that ionizing radiation may affect auxin metabolism which eventually may affect the synthesis of specific enzymes and that retardation of growth may be due to hampered respiratory enzyme synthesis; Gordon (23) pointed out that the inhibition in auxin synthesis is one of the main possible causes of inhibition of growth by ionizing radiation; and Machaiah and Screenivasan (24) and Machaiah and Vakil (25) mentioned that ionizing radiation causes inactivation of growth regulators. Such harmful effects of ionizing radiation may lead to various forms of physical expression of damage among which are abnormal and retarded plant growth.

The abnormal *Scenedesmus* observed were enlarged cells of normal shape in coenobia of two cells and four cells, enlarged cells that were not forming coenobia, coenobia composed of enlarged cells of abnormal shape, coenobia composed of enlarged cells of different sizes, cells with kidney-shaped chloroplast, and coenobia with cells that were not in alignment as in the normal ones. Cells that were colorless were observed also. The abnormal *Scenedesmus* mentioned above were observed in treated algae grown under white light, red light, and blue light. In Figure 1 to Figure 9 are some of the algae grown under the white light; Figures 10 to 16 are some of those grown under red light; and Figures 17 to 23 are some algae grown under blue light. The abnormal cells observed were morphologically similar to those observed by Arañez and Antonio (26).

Percentage of coenobia with enlarged cells in *Scenedesmus* treated with different doses of gamma rays and exposed to different light qualities determined one week after the treatment is found in Table 2. Generally, the higher the dose, the more enlarged cells were observed. Treated algae grown in red light and blue light which showed lesser growth rates than those under white light had higher percentage of enlarged cells. Arañez and Soriano (27) and Soriano (28) in their work on higher plants, had the same observation that fast-growing plants had less mutations than slow-growing ones. Enlarged *Scenedesmus* of normal shape in coenobia of two cells, coenobia of four cells, and those not forming coenobia were successfully propagated for more than ten generations.

ACKNOWLEDGMENTS

The author wishes to extend her gratitude to the Natural Research Institute (NSRI) for the financial support and for housing the research project; Dr. Milagrosa Martinez for verifying the identity of the specimen; and Miss Teresita Taglinao and Dr. Bernadette Lourdes E. Arañez for taking the pictures.

REFERENCES

1. Soeder, C.J. and W. Pabs, 1970. Berichte de Deutschen Botanischen Gesellschaft 83:607. Thru Feldheim, W. 1975. Studies in the use of micro-alga in human food. *Pl. Res. Dev.* 1:98–103.
2. Feldheim, W. 1975. Studies in the use of micro-algae in human food. *Pl. Res. Dev.* 1:98–103.
3. Wells, C.V. and R.W. Hoshaw. 1980. Gamma irradiation effects on two species of the green alga *Sirogonium* with different chromosome types. *Environ. Exp. Bot.* 20:39–45.
4. Horsley, R.J., S.N. Benerjee, and M. Benerjee. 1967. Analysis of lethal responses in *Oedogonium cardiacum* irradiated at different cell stages. *Radiat. Bot.* 7:465–474.
5. Singh, R.N. and D.N. Tiwari. 1969. Induction of ultraviolet irradition of mutation in the blue-green algae, *Nostoc linckia* (Roth.) Born. et al. *Nature* 221:62–64.
6. Fernandez, T. and J. Thomas. 1978. Effects of high and low ultraviolet fluorescence on the filamentous blue-green alga *Anabaena* L31. *Environ. Exp. Bot.* 16(4):229–240.
7. Arañez, A.T. and T.E. Taglinao. 1989. The effects of gamma radiation on nitrogen-fixing blue-green alga *Nostoc linckia*. *Asian Jour. Pl. Sci.* 1:41–48.
8. Singh, H.N. 1968. Effect of acriflavine on ultraviolet resistant strains of blue-green alga *Anacystis nidulans*. *Radiat. Bot.* 8:355–361.

9. Williams, E., J. Lambert, P. O'Brien and J. Houghton. 1979. Evidence for dark repair of far ultraviolet light damage in the blue-green alga *Gloeocapsa alpicola*. *Photochem. Photobio.* 29(3):545–548.
10. Amila, D.V. 1979. Photoreactivation of ultraviolet irradiated blue-green alga *Anacystis nidulans* and Cyanophage ASI. *Arch. Virol.* 59(3):173–180.
11. Pirson, A. and W. Kowallik. 1964. Regulation of CO_2-fixation in *Chlorella* by light of varied wavelengths and intensities. *Photochem. Photobiol.* 3:489–497.
12. Roscher, E. and K. Zetsche. 1986. The effects of light quality and intensity on the synthesis of ribulose-1,5 biphosphate carboxylase and its messenger RNA species in the green alga *Chlorogonium elongatum*. *Planta* (Berl.) 167:586–587.
13. Czeczuga, B. 1987. The effect of light on the content of photosynthetically active pigments in plants: IV. Chromatic adaptation in blue-green algae, *Anabaena cylindrica* and *A. variabilis*. Phyton (Horn.) 26(1):19. In: *Biological Abstract* 83:58722.
14. Humbeck, K. and N.I. Bishop. 1986. Changes in photosystem II during biosynthesis of components and development of function in chloroplasts of a greening mutants of *Scenedesmus obliquus*. *Pl. Cell Physiol.* 27(7):1407–1418.
15. Starr, R.C. 1964. The culture collection of algae at Indiana University. *Am. J. Bot.* 51:1013–1044.
16. Arañez, A.T. 1987. The effects of ethyl methanesulfonate on the nitrogen-fixing blue-green alga *Nostoc linckia*. *Nat. Appl. Sci. Bull.* 39(1):117.
17. Seeley, H.W. and P.J. Van Demark. 1971. Microbes in action A laboratory manual of microbiology, 2nd ed. W.H. Freeman and Co., San Francisco, U.S.A.
18. Posner, H.B. and A.H. Sparrow. 1964. Survival of *Chlorella* and *Chlamydomonas* after acute and chronic gamma radiation. *Radiat. Bot.* 4:253–257.
19. Hess, J.L. and N.E. Tolbert. 1976. Changes in chlorophyll a/b ratio and products of $^{14}CO_2$ fixation by algae grown in red and blue light. *Plant Physiol.* 42:1123–1130.

20. Sokowa, Y. and E. Hase. 1967. Effect of light on the chorophyll formation in the "glucose-bleached" cells of *Chlorella protothecoides*. *Plant Cell Physiol.* 8:495–508.
21. Ussuf, K.K. and P.M. Nair. 1974. Effect of gamma irradiation on the indole acetic acid synthesizing system and its significance in sprout inhibition of potatoes. *Radiat. Bot.* 14:251–256.
22. Ananthaswamy, H.N., U.K. Vakil and A. Screenivasa. 1971. Biochemical and physiological changes in gamma-irradiated wheat during germination. *Radiat. Bot.* 11:1–12.
23. Gordon, S.A. 1957. The effects of ionizing radiation on plants. Biochemical and physiological aspects. *Quart. Rev. Bio* 32:3–14.
24. Machaiah, J.D. and A. Screenivasan. 1976. The effects of gamma irradiation on the biosynthesis of gibberellins in germinating wheat. *Environ. Exp. Bot.* 16:131–140.
25. Machaiah, J.D. and U.K. Vakil. 1979. The effects of gamma irradiation in the formation of alpha-amylase iso-enzyme in germinating wheat. *Environ. Exp. Bot.* 19:337–348.
26. Arañez, A.T. and B.A. Antonio. 1983. The effects of gamma radiation on *Scenedesmus quadricauda* (Turp.) Breb. *Nat. Appl. Sci. Bull.* 35(2):83–100.
27. Arañez, A.T. and J.D. Soriano. 1976. Responses of some rice varieties to alkylating agents. *Nat. Appl. Sci. Bull.* 28(2):41–141.
28. Soriano, J.D. 1971. The response of several rice varieties to fast neutrons. *Radiat. Bot.* 11:341–346.

EXPLANATION OF FIGURES

Scenedesmus Grown Under White Light

1–2. Untreated coenobia of *Scenedesmus*, x 1658
  1. Coenobium of two cells
  2. Coenobium of four cells

3–9. *Scenedesmus* from batches treated with gamma radiation
  3. Enlarged unicellular *Scenedesmus*
  4. Enlarged cell i coenobium of two cells
  5. Enlarged cells in coenobium of two cells
  6. Enlarged cells in coenobium of four cells
  7. Enlarged and abnormally shaped cells in 2-celled coenobium
  8. Two-celled coenobium with enlarged cells that differ in size

9. Enlarged cells with kidney-shaped chloroplast in 4-celled coenobium

Scenedesmus Grown Under Red Light

10–11. Untreated coenobia of *Scenedesmus*, x 1658

10. Coenobium of two cells
11. Coenobium of four cells

12–16. *Scenedesmus* from batches treated with gamma radiation

12. Enlarged unicellular *Scenedesmus* together with a normal-sized coenobium of two cells
13. Enlarged *Scenedesmus* in coenobium of two cells
14. Enlarged *Scenedesmus* of abnormal shape in coenobium of two cells
15. Enlarged *Scendesmus* in coenobium of two cells
16. Coenobium of four cells that are not in normal alignment

17–23 *Scenedesmus* Grown Under Blue Light, x 1658

17. Coenobium of two cells
18. Coenobium of four cells
19. Enlarged unicellular *Scenedesmus*
20. Enlarged *Scendesmus* i coenobium of two cells
21. Enlarged *Scenedesmus* is coenobium of four cells
22. Enlarged cells with kidney-shaped chloroplast in coenobium of four cells
23. Enlarged and abnormally shaped cells in two-celled coenobium

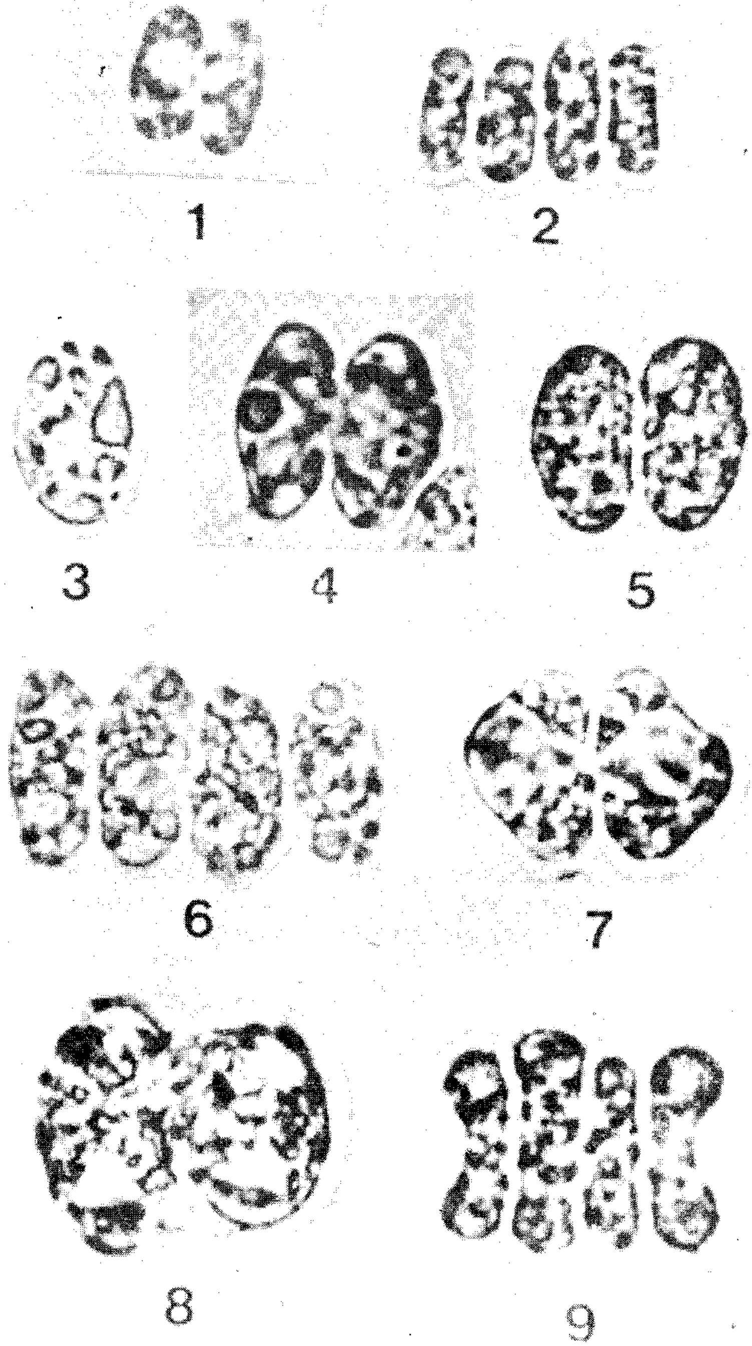
Table 1. Growth rate (ratye of doubling per week) of Scenedesmus treated with different doses of gamma rays and grown under different light qualities*

		Number of weeks after treatment					
Light quality	Dose (Gy)	1	2	3	4	5	6
White light	0	2.3	1.8	1.4	0.6	0.5	0.2
	40	2.8	2.0	0.7	0.6	0.4	0.2
	80	2.6	2.1	0.9	0.4	0.5	0.2
	120	2.1	1.8	1.4	0.7	0.5	0.2
Red Light	0	2.0	1.4	1.2	0.8	0.7	0.4
	40	2.2	1.3	1.0	0.9	0.5	0.3
	80	2.3	1.6	1.0	0.7	0.4	0.4
	120	2.0	1.3	0.9	1.0	0.7	0.4
Blue light	0	0.7	1.4	0.6	0.5	0.8	0.5
	40	1.2	1.2	1.0	0.6	0.7	0.4
	80	0.8	1.2	1.1	0.7	0.7	0.4
	120	0.8	0.9	0.6	0.8	0.8	0.7

*Average of two treatments with two replicates per treatment

Table 2. Percentage of coenobia with large cells in Scenedesmus treated with different doses of gamma radiation and grown under different light qualities one week after the treatment

	Light Qualities								
	White Light			Red Light			Blue Light		
Dose (Gy)	No. of cells	No. of large cells	% large cells	No. of cells	No. of large cells	% large cells	No. of cells	No. of large cells	% large cells
0	6200	0	0	2450	0	0	750	0	0
40	7100	500	7.0	3950	100	2.5	500	0	0
80	4900	150	3.1	3750	350	9.3	800	100	12.5
120	3000	100	3.3	2050	300	14.6	450	100	22.2

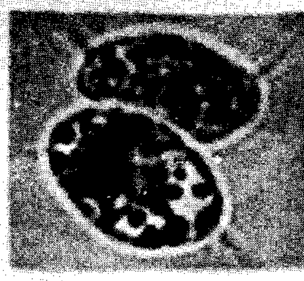


1
2
3
4
5
6
7
8
9

10

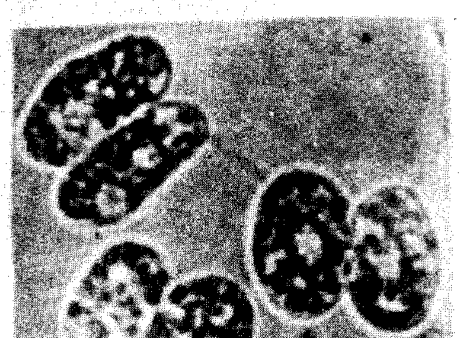
11

12

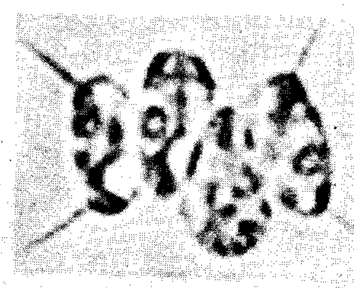


13

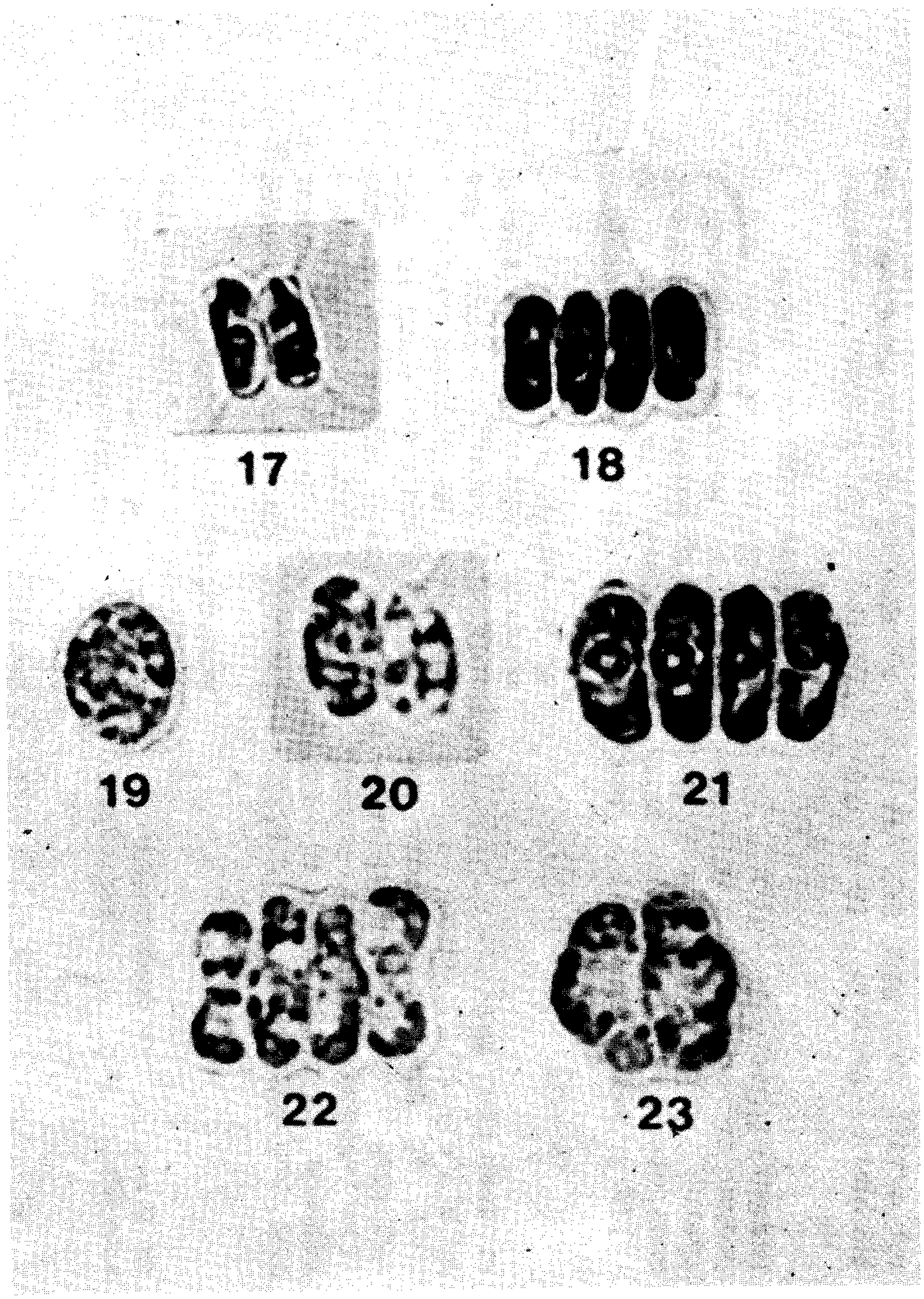
14



15



16


17
18
19
20
21
22
23