Molybdenum Toxicity: Abnormal Cellular Division of Teratogenic Appearance in *Euglena gracilis*

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Abnormal Euglena cells, observed by chance and described in the literature (ALEXANDER 1931, GODJICS 1934, GROSS and JAHN 1962, LEEDALE et al. 1965, WILLIAMS 1962) as freaks of nature or monsters, have been considered the result of abnormal cellular division of unknown cause, and, to the knowledge of the author, no abnormal Euglena cells of this type have been produced experimentally prior to this report. However, it has been observed by the author that molybdenum alters cellular division and induces clustered, dividing cells with three to nine cells attached to each other at one end, some appearing as the "rigid forms" described by ALEXANDER (1931), some showing the attached ends swimming with seemingly normally developed flagella, and some separating completely and swimming away as individuals. The purpose of this report is to show that such abnormal forms are produced experimentally in the presence of certain concentrations of molybdenum in the growth medium.

METHODS AND RESULTS

Euglena cells were grown axenically in continuous cool white fluorescent light at 300 ft. candles between 24 and 26°C in the acid 17 medium of Hutner (HUTNER et al. 1950, WOLKEN 1961) containing 5.68×10^{-5} M (5.44 ppm) molybdenum. They divided by normal longitudinal binary fission and had a normal growth rate with a generation time of about one day. Accidentally, the required molybdenum concentration, added to the growth medium as ammonium molybdate (Mo_7), was increased 20 fold. The culture medium became photosensitive, acquired a blue color, and abnormal clusters of three to nine actively moving cells attached to each other at one end appeared. The molybdenum extracted from the growth medium containing the abnormal cells was assayed spectrophotometrically as a thiocyanate complex (SANDELL 1959) and was found to be 1.13×10^{-3} M (108 ppm). Increase in molybdenum concentration (1×10^{-3}, 5×10^{-3}, and 1×10^{-2} M or 96, 480, and 960 ppm, respectively) by the addition of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$,

Bulletin of Environmental Contamination & Toxicology,
Vol. 9, No. 6, © 1973 by Springer-Verlag New York Inc.

MoO_3, or $Na_2MoO_4 \cdot 2H_2O$ to the culture medium was demonstrated to cause the abnormal cellular divisions that became apparent on the second day of growth.

The abnormally clustered and dividing cells were produced in cultures of the green photosynthesizing Euglena gracilis (Z), the green E. gracilis var. bacillaris-C, and a streptomycin-bleached, yellow, non-photosynthesizing mutant of E. gracilis, of which examples are shown in Figures 1 and 2.

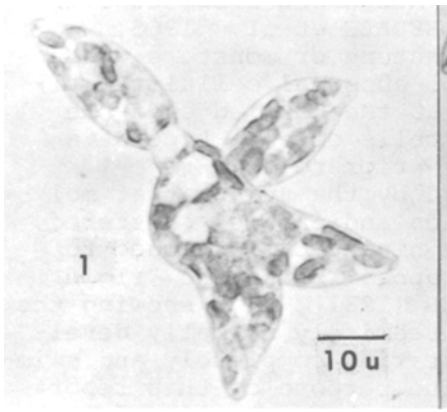

Fig. 1. Green photosynthesizing Euglena gracilis (Z) or E. gracilis var. bacillaris-C dividing abnormally in a culture medium containing 10^{-3} M molybdenum.

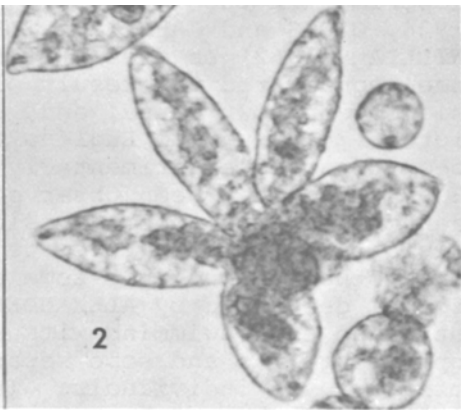

Fig. 2. Streptomycin-bleached, yellow, non-photosynthesizing mutant of E. gracilis dividing abnormally in a culture medium containing 10^{-3} M molybdenum.

For each of the above described types of Euglena, four series of cultures, one with normal (5.68×10^{-5} M) and three with high (1×10^{-3}, 5×10^{-3}, 1×10^{-2} M) molybdenum concentrations, were prepared. An excess (20X) or an omission of each of the other components of the growth medium was then used as a variable.* Only in those cultures with high molybdenum concentrations did the blue color and abnormal forms appear. Changes in concentration of the other components affected only the growth rate. No abnormal cells were observed below 1×10^{-3} M (96 ppm) molybdenum. Above 1×10^{-2} M (960 ppm) molybdenum, the cultures would not grow.

* KH_2PO_4, $MgSO_4 \cdot 7H_2O$, L-glutamic acid, DL-malic acid, $CaCO_3$, $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$, Thiamine HCl, Cyanocobalamin, Ethylenediaminetetraacetic acid, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $CuSO_4 \cdot 5H_2O$, H_3BO_3, KI.

The abnormal cells produced with high molybdenum were observed in cultures grown in continuous light at 300 ft. candles and in cultures synchronized by light cycling (9 hrs. light and 3 hrs. darkness). They were also present when the cultures were grown at constant temperatures varying in increments of 5°C/day between 20 and 35°C. The abnormal cells accounted for 2 to 5% and at times up to 10% of the total number of cells present in a culture. Controls with the above described variations in light and temperature and normal molybdenum did not produce abnormal cells.

DISCUSSION

ALEXANDER (1931) mentioned the occurrence of multiple monsters in cultures of E. gracilis (Krebs) to be independent of illumination and associated with hydrogen ion concentration. GOJDICS (1934) observed an anucleated anomalous individual of E. deses and saw two similar freaks with several parts fused to a common posterior mass. GROSS and JAHN (1962) reported multinuclear monstrous cells in E. gracilis var. bacillaris and ascribed them to cytoplasmatic alterations due to abnormal elevation in temperature. WILLIAMS (1962) described branched and multiple forms in E. gracilis (Pringsheim, Z strain) and suggested that their development was encouraged by a high concentration of calcium ions. In old cultures of E. spirogyra, LEEDALE et al. (1965) found two-headed monsters undivided at the tail end and various cellular deformities, which were associated with an overdose of iron and manganese.

The present findings show an involvement of molybdenum in the mechanism of cellular division with production of monsters in Euglena. Molybdenum may be considered toxic and may be an inhibitor of cellular fission with resultant nuclear and chromosomal polyploidy and abnormal mitoses. It is hypothesized that a similar toxicity of molybdenum may activate the uncontrolled growth patterns observed in some neoplastic growths.

ACKNOWLEDGMENTS

The cultures of Euglena gracilis (Z), E. gracilis var. bacillaris-C, and streptomycin-bleached E. gracilis were kindly furnished by Dr. J. A. Gross. He established the bacillaris-C-clone, obtained the Z strain from Dr. J. J. Wolken, and obtained the streptomycin-bleached strain from that established by Dr. J. B. Loefer.

I wish to acknowledge the competent assistance of Mrs. Carol Lacy in the isolation and preparation of the cultures. I am grateful to Dr. W. B. Gross for the photographic reproductions.

ABSTRACT

Molybdenum (10^{-3}M) induced the formation of abnormal multiple nuclear and cellular (teratogenic in appearance) divisions of Euglena gracilis-Z, E. gracilis var. bacillaris-C and streptomycin-bleached E. gracilis.

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