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# Effects of manganese on the growth of *Euglena anabaena, Astasia* sp. and *Colpidium campylum.*

By

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In view of the findings of BISHOP (1928) and HOPKINS (1930, 1931) that manganese is apparently essential to certain plants, and HOPKINS' observations that growth is accelerated by manganese, it seemed that similar observations on Protozoa might prove of interest. Accordingly, the writer has determined the effects of several concentrations of manganese chloride on growth of *Euglena anabaena* var. *minor*, *Astasia* sp. and *Colpidium campylum.*

## Material and methods.

Bacteria-free strains of these species were obtained from the following sources: *E. anabaena* var. *minor* (MAINX strain), from Professor E. G. PRINGSHEIM and Dr. F. MAINX; *Astasia* sp. (JAHN strain), from Dr. T. L. JAHN; *Colpidium campylum* (HETHERINGTON strain), from Dr. T. L. JAHN.

The following media were used: medium A, a 0.75 % solution of Difco tryptone in distilled water; medium EA, containing in each liter of solution 0.5 gm $NH_4NO_3$, 0.5 gm $KH_2PO_4$, 0.1 gm $MgSO_4 \cdot 7H_2O$, 0.1 gm NaCl, and a trace of $FeCl_3$. Medium A is comparable to the peptone medium used by HOTCHKISS (1923) and, in certain experiments, by WINSLOW and DOLLOF (1928) for similar investigations on bacteria. It is known to be adequate for growth of all three species listed above, and hence was used for experiments with each one. The fact

that tryptone solution will support growth of *Euglena anabaena* suggests that this peptone contains a minimal quantity of manganese if this element is actually essential to growth of the species. The results of a qualitative analysis of tryptone, available through the kindness of Dr. H. G. DUNHAM of the Difco Laboratories, failed, however, to show a detectable amount of manganese in this peptone. Hence, if any is present at all it must be present in very small traces. Medium EA, an inorganic solution known to be adequate for growth of *Chlorogonium euchlorum* (LOEFER, 1934; HALL and SCHOENBORN, MSS), was also used for *Euglena anabaena* in view of the supposed ability of this species to utilize inorganic nitrogen (DUSI, 1933). This medium was presumably free from manganese, as indicated by analyses of the chemicals used.

Each medium was adjusted to the desired $p_H$ and then tubed in 8.0 cc amounts. To each of the manganese tubes, 1.0 cc of a solution of manganese chloride was added, the concentration varying with the different sets of tubes as indicated below. Each of the control tubes received 1.0 cc of distilled water. The tubes were all sterilized in the autoclave and, approximately 24 hours later, inoculated from a stock flask. In each series, the flask contained approximately 75 cc of the appropriate control medium, and had been seeded from a stock tryptone culture of the species involved.

After inoculation, one tube of each set was used for determination of the initial $p_H$, and another for determining the initial concentration of organisms per cubic centimeter (initial count). The remaining tubes of each set were incubated as described below. After incubation, the final $p_H$ of one tube of each set was determined, and the remaining tubes were fixed for counting. Counts were made with the Whipple ocular micrometer and Sedgwick-Rafter counting cell, as described in detail elsewhere (HALL, JOHNSON and LOEFER, 1935), and $p_H$ determinations were made with a LaMotte roulette comparator.

## *Euglena anabaena.*

Series I. Medium A was used in this series at an initial $p_H$ of 6.7, no change in $p_H$ being noted after incubation for 8 days in darkness at room temperature. The concentrations of added manganese ranged from $10^{-9}$ to $10^{-5}$ molar, while medium A was used in the controls. The initial count was 112, and $x/x_0$ (ratio of final to initial concentration of flagellates per cubic centimeter) was 1.9 for the controls. In $10^{-7}$ molar solution of manganese there was an apparent

acceleration (Table 1), growth being 29 % greater than in the controls. In other concentrations of manganese, the number of flagellates was either less than in the controls or else not significantly greater.

Series II. The procedure was similar to that in series I, except that the initial $p_H$ was 7.3 and the initial count was 600. The cultures were incubated at room temperature near an east window for 6 days, and then counted. In the controls, $x/x_0$ was 19.3. Apparent accelerations (Table 1) were observed in the three lower concentrations of manganese, growth being from 10.3 to 19.6 % greater than in the controls. In $10^{-6}$ molar the increase was not significant, and in the higher concentrations growth was less than in the controls.

Series III was a duplicate of series II except that the cultures were placed at some distance from an east window and received less light during the period of incubation. In every case (Table 1) growth in the manganese cultures was greater than in the controls, which showed an $x/x_0$ of 11.1.

Series IV was similar to series II, except that the initial count was 1328 and the cultures were incubated in darkness for 12 days. The controls showed an $x/x_0$ of 1.8. An apparent increase (Table 1) was noted in the lowest concentration of manganese, but growth in the other concentrations was either less, or else not significantly greater than in the controls.

Table 1.

Percentage differences between $x/x_0$ (ratio of final to initial concentration of organisms per cubic centimeter) in manganese media and in the controls. Thus, —11% represents a case in which $x/x_0$ was 11% less than in the controls; 29 %, $x/x_0$ was 29 % greater than in the controls. Molar concentrations of manganese chloride are listed.

| Manganese concentration | percentage differences between $x/x_0$ in manganese media and in controls, different series | | | | | | |
|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII |
| $10^{-9}$ | —11.0 | 19.6 | 10.9 | 11.8 | 29.0 | —12.7 | —14.5 |
| $10^{-8}$ | 3.1 | 11.9 | 10.9 | 0.6 | 10.8 | —5.0 | —8.1 |
| $10^{-7}$ | 29.0 | 10.3 | 11.0 | —3.0 | 34.4 | —12.5 | —7.1 |
| $5\times10^{-7}$ | — | — | — | — | 101.4 | — | — |
| $10^{-6}$ | —41.2 | 2.1 | 11.1 | —12.4 | 19.7 | —8.6 | —30.6 |
| $2.5\times10^{-6}$ | — | — | — | — | 25.1 | — | — |
| $5\times10^{-6}$ | — | — | — | — | 20.1 | — | — |
| $10^{-5}$ | —43.5 | —6.2 | 22.6 | —11.2 | —3.4 | —5.6 | —28.2 |
| $5\times10^{-5}$ | — | — | — | — | —11.3 | — | — |
| $10^{-4}$ | — | —10.3 | 10.7 | —4.5 | —9.3 | — | — |

Series V. In this series medium EA, an inorganic solution, was used. In the procedure of inoculation, the peptone carried over from the original stock culture was reduced to less than 1:750,000. The initial and final $p_H$ were 6.6, and the initial count was 660. The cultures were incubated for 9 days under constant illumination and at a temperature of 28° C. In the controls, $x/x_0$ was 2.1. Acceleration was noted (Table 1) in manganese concentrations ranging from $10^{-9}$ to $5 \times 10^{-6}$, with the maximal effect appearing in $5 \times 10^{-7}$ molar solution. In the higher concentrations, growth was less than in the controls.

### *Astasia* sp. and *Colpidium campylum*.

Series VI. *Astasia* sp. was grown in medium A in the same concentrations of manganese as used in series IV. Initial and final $p_H$ were 6.7, and the initial count was 580. Cultures were incubated in darkness at room temperature for 5 days. In the manganese cultures (Table 1), growth was in every case less than that in the controls ($x/x_0$, 62.1).

Series VII. *Colpidium campylum* was grown in medium A with concentrations of manganese the same as in series VI. The initial $p_H$ was 6.7, and the initial count 68 ciliates per cubic centimeter. After incubation at room temperature for four days in darkness, $p_H$ changes were not greater than 0.2. As in the case of *Astasia*, growth (Table 1) was always less than in the controls ($x/x_0$, 1564).

## Discussion.

Several possible factors might be operative, either singly or in combination, in accelerative effects of manganese on the growth of protozoa. In the first place, growth might be stimulated specifically by manganese in *Euglena* and other chlorophyll-bearing types, in view of the apparent relationship between manganese and synthesis of chlorophyll (e. g., Bishop, 1928). In medium A (series I—IV), known to be adequate for growth of *Euglena anabaena* and possibly containing an extremely small quantity of manganese, comparatively little specific acceleration would be expected from added manganese. The experimental results are in agreement, since the increase in growth in the manganese-cultures was usually slight, growth being in only two cases more than 12 % greater than in the controls. Such relatively insignificant effects are possibly the result of an increase in manganese to a more nearly optimal concentration. In a manganese-free inorganic medium, on the other hand, a specific effect of added manganese should be more noticeable. This was the

case in medium EA (series V), a medium apparently inadequate for growth of *E. anabaena* through successive transfers (Hall and Schoenborn, MSS). The results in the inorganic medium are comparable to those of Hopkins (1930), although the acceleration in *E. anabaena* is less striking than that reported for *Chlorella.*

Another possibility is that manganese might act on protozoa generally through its effect on the oxidative mechanism. For plants, such a function of manganese has been suggested by Bishop (1928) and Hopkins (1930). Or, another possible effect might involve modification of the rate of absorption of foods. Such general effects might be responsible for increased growth of *E. anabaena* in a peptone medium known to be satisfactory even without added manganese. Since such non-specific effects would be expected in other protozoa as well as in *Euglena*, experiments were carried out with the colorless euglenoid flagellate, *Astasia* sp., and with the ciliate, *Colpidium campylum.* Neither of these species showed any increase in growth in the concentrations of manganese tested, and hence the results failed to indicate a non-specific accelerating effect of manganese chloride.

In bacteria, on the other hand, an accelerative action of cations has been reported by several workers (Sherman, Holm and Albers, 1922; Hotchkiss, 1923; Winslow and Dolloff, 1928; Winslow and Haywood, 1931). Winslow and Dolloff concluded that various metallic cations stimulate growth „when present in sufficiently low concentration and inhibit it when present in sufficiently high concentration, both the stimulating and the toxic concentrations varying widely for the different salts". These investigators have suggested further that „the cations exert a certain primary effect upon the bacterial cell . . . . which is perhaps qualitatively the same for all cations . . . . but quantitatively different for each cation".

While it is possible that the present failure to observe such non-specific accelerating effects on protozoa might be explained on the basis of unsatisfactory concentrations of manganese chloride, it should be pointed out that Winslow and Haywood (1931) found this salt to be accelerative for *E. coli* in concentrations ranging from $3 \times 10^{-5}$ to $4 \times 10^{-4}$ molar. Comparable effective concentrations were determined by Hotchkiss (1923) for $PbCl_2$ and $HgCl_2$. This range includes the higher concentrations used by the writer; except in one instance (series III, *Euglena*), no accelerating effect was apparent. It seems unlikely that the writer has failed to use concentrations low enough to detect non-specific effects of manganese,

since the dilutions were extended to $10^{-9}$ molar. Furthermore, the lower concentrations, which partially inhibited growth of *Astasia* and *Colpidium*, produced in most cases an apparent increase in growth of *Euglena*.

It thus appears that a substance undoubtedly beneficial to growth of *Euglena anabaena* is not only useless but even harmful to a related flagellate, *Astasia* sp., which shows only minor morphological differences. Such results call attention once more to the difficulties inherent in generalizations concerning reactions of protozoa to environmental factors since the species itself, as a controlling influence in physiological response, may sometimes be much more important than a particular environmental factor.

Very few investigations comparable to those on bacteria have been carried out with protozoa. PACE (1933), however, has determined for *Amoeba proteus* the effects on growth of different concentrations of various cations, the concentrations overlapping the upper part of the range covered in the present investigation. In most cases, PACE observed a bimodal type of growth curve with one peak at a relatively low salt concentration and the second at a higher concentration. Such a bimodal growth curve was observed by the writer in only one case and the differences responsible for the depression in the curve are not striking. Since PACE did not state clearly the amount of growth in control cultures, it is difficult to determine, from his published data, whether or not growth of *Amoeba* was actually accelerated by the salts which he tested.

## Summary.

An accelerative effect of certain concentrations of manganese chloride on growth of *Euglena anabaena* var. *minor* (MAINX strain) has been demonstrated. The effect was marked in an inorganic medium, but the addition of manganese to a peptone medium already adequate for growth produced less noticeable effects. Acceleration of growth by manganese was not observed in cultures of *Astasia* sp. (JAHN strain) and *Colpidium campylum* (HETHERINGTON strain). It is assumed that the effect on *Euglena* involves some specific relationship between manganese and the metabolism of chlorophyll-bearing flagellates. A bimodal relationship between growth and concentration of manganese chloride was not demonstrated.

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