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GROSS EFFECTS OF GROWTH INHIBITING LEVELS OF TRITIUM OXIDE ON CHLORELLA PYRENOIDOSA

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GROSS EFFECTS OF GROWTH INHIBITING LEVELS OF TRITIUM OXIDE ON CHLORELLA PYRENOIDOSA

By

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Biology Section

November 20, 1953

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ABSTRACT

Growth and cell division rates and average size of cells were determined for Chlorella growing in the presence and absence of tritium oxide.

Then plate assays were made to determine the number of viable cells. Assays of selected colonies were also made to determine whether irradiation of Chlorella produced genetically changed cells which differed in growth rate from that of unirradiated cells.

Results showed that the rate of cell division is inhibited to a greater extent than the rate of cell growth. Supporting evidence for this conclusion was found in an increase in the average size of Chlorella after exposure to tritium oxide.

Approximately 70 per cent of the cells present after 3 days of growth in the presence of 20 mc/ml of tritium oxide were unable to form colonies on inorganic nutrient agar. The majority of these cells were not dead on plating but died gradually after they were plated. Neither the viability of Chlorella, nor the growth rate, in per cent of control, could be increased by supplying the cells with organic nutrients. It is concluded, therefore, that the processes of photosynthesis and biosynthesis of the substances present in yeast extract and bacto-peptone are not selectively damaged in Chlorella by tritium oxide.

An appreciable number of genetically changed Chlorella were found among cells surviving treatment with tritium oxide. Twenty-five per cent of the colonies of a random sector grew more slowly than the slowest growing control selection. One of the selections proved to be a biochemical mutant. All others grew at the same rate, or slower, in organic nutrient solution as in inorganic nutrient solution.

Genetic changes observed included those which yielded rough, lightly pigmented and clumping selections.

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GROSS EFFECTS OF GROWTH INHIBITING LEVELS OF TRITIUM OXIDE ON CHLORELLA PYRENOIDOSA

INTRODUCTION

A previous paper, Porter and Knauss[1], reported on the growth of Chlorella pyrenoidosa in the presence of P^{32}, S^{35}, Sr^{90}-Y^{90} and H^3 and in subculture in non-radioactive nutrient solution. Some data on the average size of control and irradiated cells were also given.

This paper presents the results of continued studies with tritium oxide. These studies included 1) determinations of the mass, numbers and size of cells growing in the presence and absence of radioactivity, 2) determinations of mass, numbers and size of control and irradiated cells in subculture in organic and inorganic nutrient solutions, 3) determinations of numbers of cells plated and colonies developing on organic and inorganic nutrient agar plates, and 4) determinations of growth rates of Chlorella selections in inorganic and organic nutrient solutions. These selections were isolated from platings on organic nutrient agar of cells grown in the presence and absence of tritium oxide.

METHODS

Growth in the Presence of Tritium Oxide

Procedures and techniques were the same as reported previously, Porter and Knauss[1], except that algae were carried on agar slants enriched with glucose instead of sucrose, and Myer's nutrient solution[2] was usually adjusted to pH 6.0 instead of 5.5.

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Growth on Subculture

Inoculum for subculture was prepared by centrifuging cells from the radioactive solution at approximately 10,000 rpm in a Servall centrifuge immediately after 3 days of growth in the radioactive solutions. The cells were then suspended in fresh Myer's nutrient solution and stored in the refrigerator until subcultured.

Algae were subcultured in inorganic nutrient solution under conditions described previously, Porter and Knauss[1]. Algae were subcultured in organic nutrient solution under the same conditions of light intensity, temperature and rate of air flow as used when the cells were grown in inorganic nutrient solution. Carbon dioxide, other than that present in air, was not provided for these cells. The organic nutrient solution contained Myer's salt solution (adjusted to pH 6.0), 1% glucose, 0.1% bacto-peptone and 0.05% yeast extract. Sterile conditions were maintained in all experiments in which cells were subcultured in organic nutrient solution.

Counts and Size Measurements of Cells

Cell counts were made by the standard technique[1] for counting white blood cells. The algal suspension was diluted with WBC pipette, placed in a haemocytometer and counted at 100 fold magnification. Each sample was pipetted in duplicate and counting results were averaged.

The size of the cells, in a wet mount, was determined with a calibrated filar micrometer eyepiece and the oil immersion lens. Diameters of 50-100 cells were measured and then averaged.

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1. Spencer Bright-Line Haemocytometer booklet.

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Plating Experiments

The entire plating procedure was carried out under aseptic conditions. Cell suspensions were diluted to approximately 800,000 cells/ml before counts were made. The suspension was further diluted to 100-2000 cells/ml for plating.

Two per cent of agar was added to inorganic and organic media of the same composition as used in the liquid assays. Tubes of media were than autoclaved, cooled to 53° - 58° C and poured into 100 mm petri dishes. The media were allowed to cool to near the solidification point, and then one ml of the inoculum was added. The cells were distributed evenly in the agar by gently rotating and tipping the plate. After solidification of the agar, the plates were inverted and incubated at room temperature in light of 50-100 f. c. Colonies developed in 10-14 days and were counted with a Quebec Colony counter.

Isolates from Plates

Chlorella selections were isolated from organic nutrient agar plates and then transferred to organic nutrient agar slants. Growth rates of experimental selections in organic and inorganic nutrient solutions were compared with those of control selections.

RESULTS

Effect on Rate of Growth

Growth of *Chlorella* in the presence of tritium oxide in inorganic nutrient solution was followed in several experiments. Masses of cells, in per cent of controls, are plotted against time in Figure 1 for cells growing in different concentrations of tritium oxide.

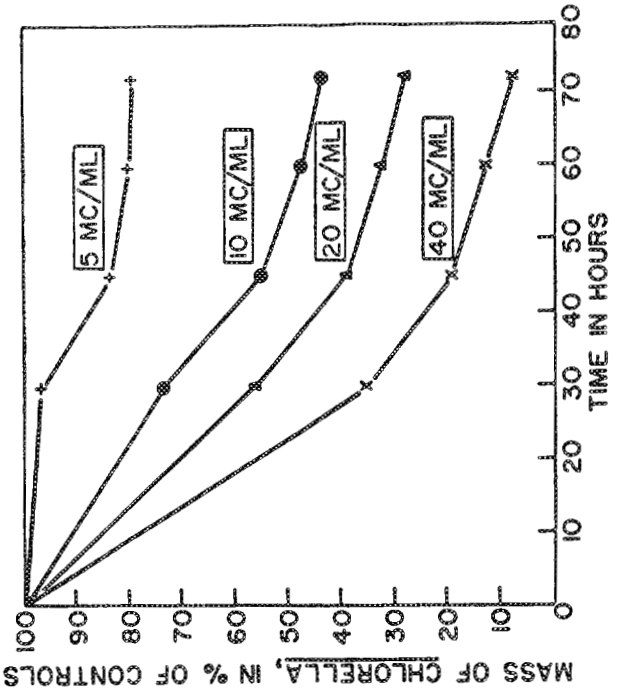

Figure 2

Growth of H_2^3O exposed Chlorella cells on subculture in non-radioactive inorganic media. Each point is the average of values obtained on duplicate tubes in each of 2-10 experiments.

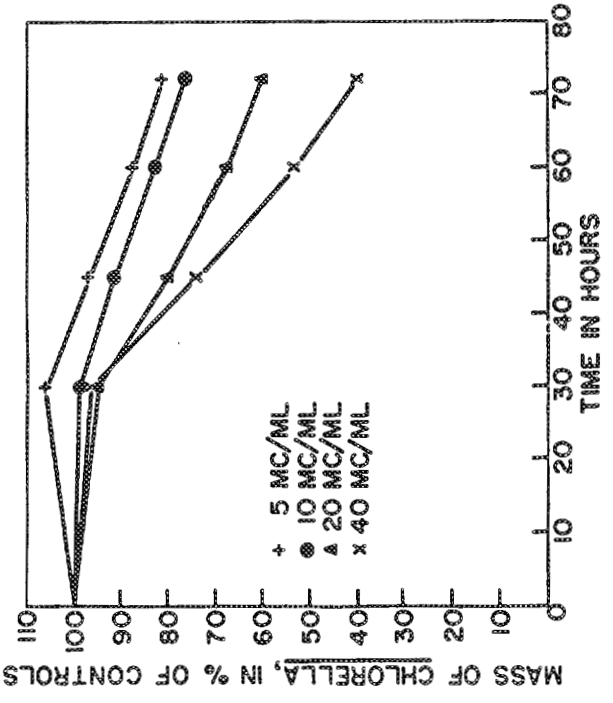

Figure 1

Growth of Chlorella in the presence of H_2^3O. Each point is the average of values obtained on duplicate tubes in each of 2-4 experiments.

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The growth of Chlorella on subculture in inorganic and organic nutrient solution, and the growth on repeated subculture in inorganic nutrient solution were determined. Results are plotted in Figures 2-4.

It is evident from Figures 1-4 that the mass of cells formed per unit of time in the presence of tritium oxide and on subculture is an inverse function of the amount of irradiation of the cells. It is also evident that cells grown in the presence of 5 mc/ml of H_2^3O grew as rapidly as control cells after one subculture whereas two subcultures of the cells grown in the presence of 15 mc/ml of H_2^3O were necessary before these cells returned to the same growth rate as controls[2].

Effect on Rate of Cell Division

The rate of cell division, in per cent of control cells, of Chlorella growing in the presence of tritium oxide in inorganic nutrient solution was followed through daily cell counts. Results are plotted in Figure 5.

A comparison was made between the cell numbers and mass, in per cent of controls, of cells grown 3 days in inorganic nutrient solution in the presence of different concentrations of tritium oxide. Results are plotted in Figure 6.

The rate of cell division during subculture in inorganic and organic nutrient solution was also followed. Typical results are shown in Figure 7.

It is evident, Figures 5-7, that the number of Chlorella cells formed in the presence of tritium oxide and on subculture in organic and

2. The symbol H_2^3O is used for convenience. However, inasmuch as the ratio of H^3 to H^1 was an infinitely small value in our experiments, H^3 actually existed as H^1H^3O in our solutions.

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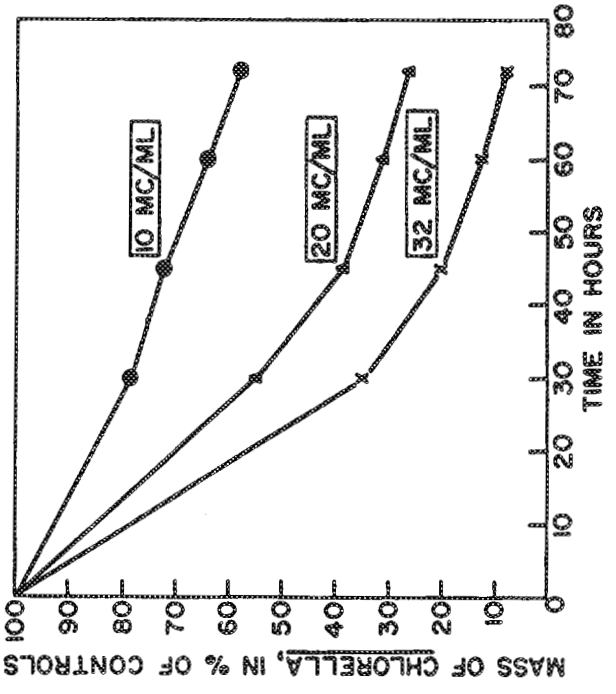

Figure 3

Growth of H_2^3O exposed Chlorella cells in non-radioactive organic media. Each point is the average of values obtained on duplicate tubes in a typical experiment.

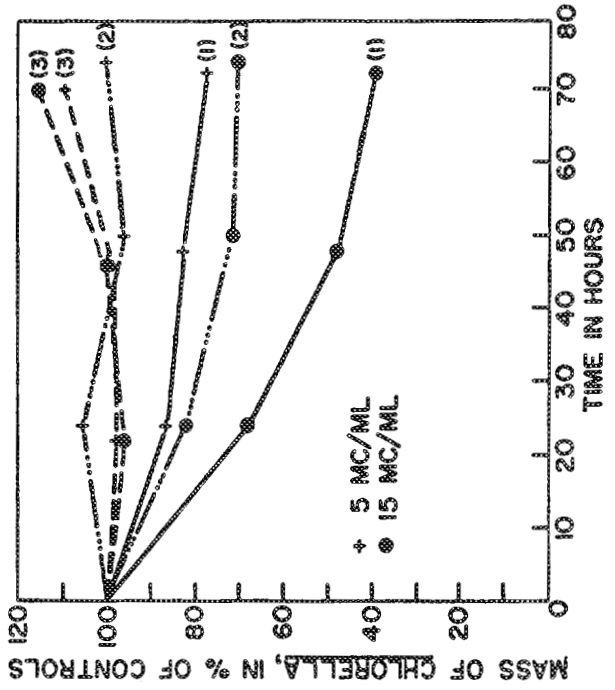

Figure 4

Growth of H_2^3O exposed Chlorella cells on consecutive subculture in non-radioactive inorganic media.

(1) First subculture
(2) Second subculture.
(3) Third subculture

Each point is the average of values obtained on triplicate tubes in a single experiment.

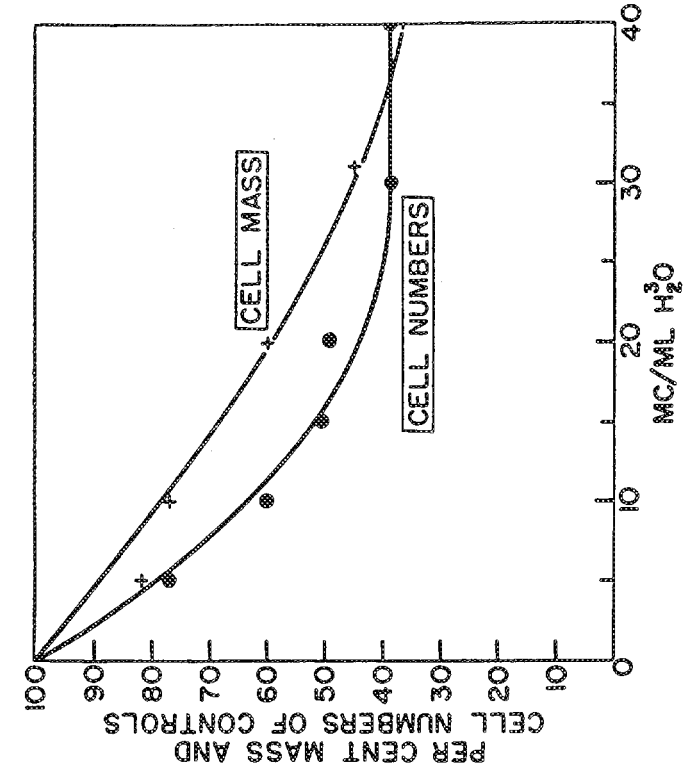

Figure 6

Cell numbers and mass of Chlorella after 3 days growth in the presence of H_2^3O. Each point is the average of values obtained on duplicate tubes in each of 2-10 experiments.

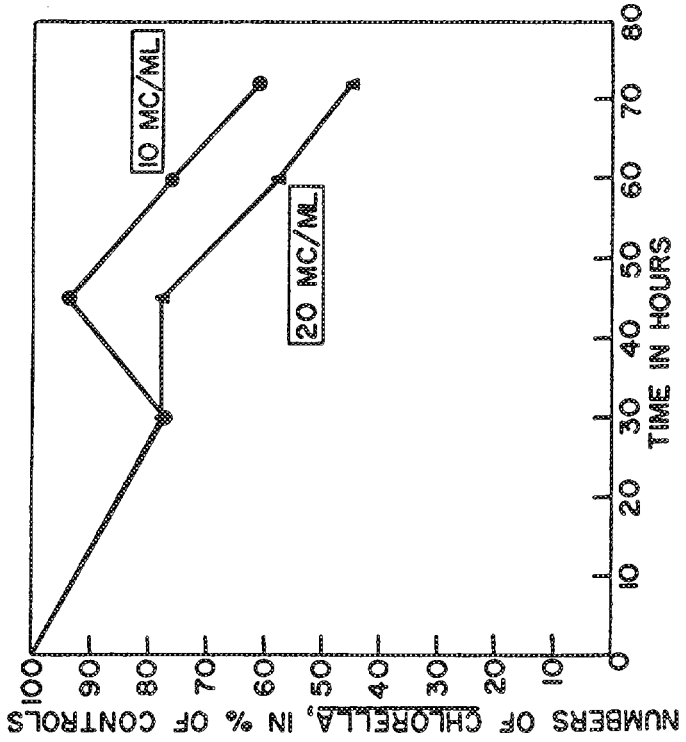

Figure 5

Number of Chlorella cells as a function of time of growth in the presence of H_2^3O. Each point is the average of values on duplicate tubes in a typical experiment.

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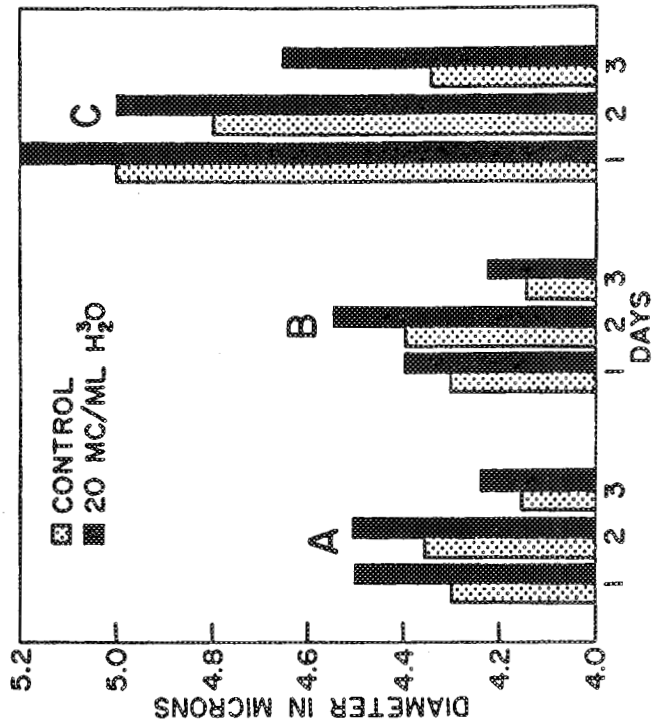

Figure 8

Cell size of Chlorella grown in the presence of H_2^3O and in subculture.

A - Grown in the presence of H_2^3O

B - Grown on subculture in inorganic media

C - Grown on subculture in organic media

The diameters of the cells at time 0 averaged 4.1 μ. Each point is the average of 50-100 determinations on samples taken from duplicate tubes in a typical experiment.

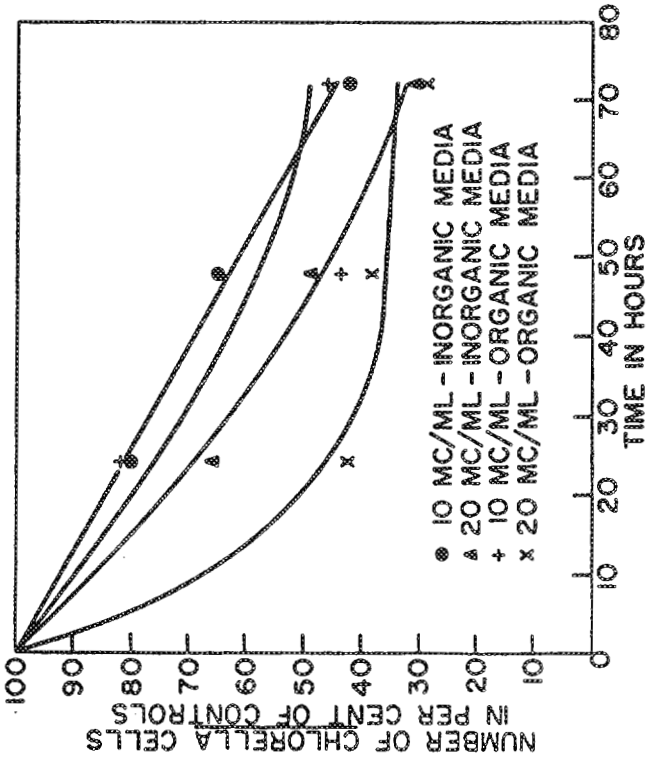

Figure 7

Number of cells on subculture of H_2^3O exposed Chlorella in organic and inorganic media. Each point is the average of values obtained on duplicate tubes in a typical experiment.

inorganic nutrient solution is an inverse function of the dose of radiation received by the cells. Further, the number of cells formed in the presence of tritium oxide is less, in per cent of controls, than the quantity of mass formed.

Effect on Size of Cells

Data on the size of Chlorella cells growing in the presence of tritium oxide in inorganic nutrient solution and in subculture in organic and inorganic nutrient solutions are given in Figure 8. A small but consistent difference in average size will be noted.

Effect on Viability of Cells

Counts were made of the number of cells plated and the number of colonies developing for irradiated and unirradiated cells. Table 1 shows that an average of 73 per cent of the irradiated cells, control cells set at 100 per cent, were unable to form colonies on inorganic nutrient agar. Further, it can be deduced from Figures 2, 3 and 7 that the cells which were unable to form colonies died gradually after they were plated.

Production of Mutations

The number of colonies, in per cent of cells plated, of irradiated and unirradiated cells growing on organic and inorganic nutrient agar plates are given in Table 1. The difference, in per cent of control cells, between the number of colonies developing on organic and inorganic media might be considered to be the average per cent of biochemical mutants (requiring glucose, yeast extract or bacto-peptone) found in these studies. However, statistical analysis did not show the difference to be significant.

Isolates have been made from random sectors of organic nutrient agar plates of colonies of unirradiated and irradiated cells. Forty of the latter and ten of the former selections were grown 3 days in inorganic

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TABLE 1

Viability of Chlorella Cells after Growth for 3 Days in the Presence of 20 mc/ml H_2^3O in Inorganic Nutrient Solution

Organic Medium			Inorganic Medium		
% Cells Plated That Formed Colonies			% Cells Plated That Formed Colonies		
Control	20 mc/ml	% of Control Cells	Control	20 mc/ml	% of Control Cells
89	34	39	83	25	30
21	7	33			
35	14	40	26	7	27
76	20	26	61	15	25
86	26	30	46	12	26
Avg.[1] 61 ± 39	20 ± 13	34 ± 7	54 ± 38	15 ± 12	27 ± 3

1. Average ± standard error at 95% confidence level.

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nutrient solution. Figure 9 shows that 10 selections (25 per cent) from surviving irradiated cells grew more slowly than the slowest growing unirradiated isolate. Nineteen of the remaining 30 selections (47 per cent) grew more slowly than the average rate of growth of all of the unirradiated selections.

The 10 slowest growing selections were then grown three days in organic and inorganic nutrient solution. One selection was definitely a biochemical mutant. It synthesized 8 times as much weight in organic nutrient solution as it did in inorganic solution. The other selections grew at the same rate, or at a slower rate, in relation to controls, in organic solution as they did in inorganic solution.

A large number of colonies, differing in shape, size and color from normal colonies, were selected from plates of irradiated cells. Figure 10 shows the growth of ten of these selections, in per cent of controls, in inorganic nutrient solution. It will be noted that 3 of these selections grew at a rate significantly greater than that of controls.

DISCUSSION

It appears from the results reported in this paper that cell division of Chlorella is inhibited somewhat more than cell growth (mass) by ionizing particles from tritium oxide. This may mean that a primary action of the ionizing particles from tritium oxide is on some phase of the process of cell division or it may mean that the action is on some phase of the growth process that is also essential for cell division. In support of these deductions is the fact that the average size of irradiated cells is somewhat larger than that of unirradiated cells.

The action of the ionizing radiations requires time for the expression of the effect, and this expression continues for periods of time which are dependent upon the dose of radiation received. During this time cells

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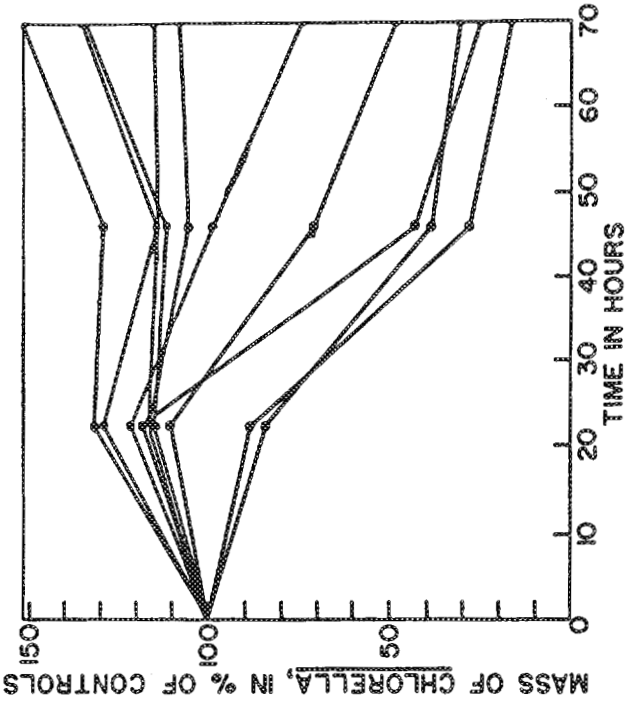

Figure 10

Growth of 10 selections of irradiated Chlorella cells in inorganic media. Each point is the average of values obtained on duplicate tubes in a single experiment.

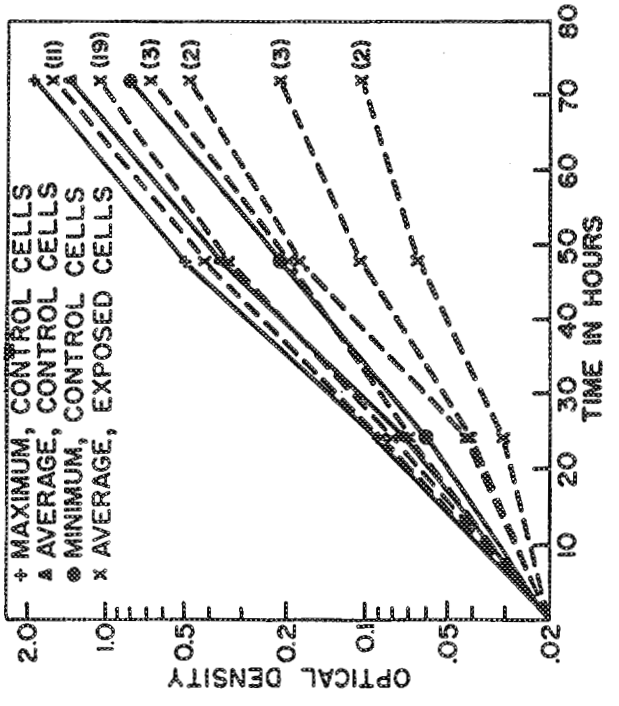

Figure 9

Growth rates in inorganic media of Chlorella cells isolated after growing in the presence of 20 mc/ml H_2^3O. Each numerical value to the right of the figure represents the number of selections averaged to yield each line. Each selection was grown in duplicate.

gradually cease to grow and divide. The number of such cells failing to grow and divide cannot be decreased appreciably by culturing the cells in a medium containing glucose, bacto-peptone and yeast extract. Hence, the action of the ionizing radiations is not on the cellular processes of photosynthesis or the synthesis of the substances present in yeast extract and bacto-peptone, or if it is in part on these processes, the action is also on other processes which are essential for cell division and growth. The results are in general agreement with those of Redford and Myers[3] who found that ultraviolet light affects photosynthesis far less than glucose respiration in Chlorella.

The action of ionizing radiations from tritium oxide on Chlorella might be on enzyme systems involved in basic cellular processes such as energy transfer (oxidations and reductions). It has recently been reported, Anderson[4], that pepsin is gradually inactivated during a period of enzyme activity after exposure to ionizing radiations. Conceivably the the same effect could be exerted upon cellular enzymes with a gradual slowing of the growth and division rates of cells followed by death of many of the cells. The action might also be directly on the enzymes involved in cell division. Finally the action might be on genes controlling the synthesis of large molecules (enzymes) within the cell. Changes in the genes might result in a failure of the cell to synthesize these necessary components. Death of the cell would ensue.

The action of ionizing radiations on Chlorella resulted in an appreciable frequency of genetic changes. Thus 25 per cent of a randomly selected group of colonies grew more slowly after many cell generations than the slowest growing unirradiated selection.

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The genetic changes produced in Chlorella by ionizing radiations are principally those which result in a slowing of the growth rate. Surprisingly, the general level of cellular metabolism, in relation to controls, was very nearly the same in organic and inorganic nutrient solution. These results suggest that tritium radiation generally acts on the genetic system of Chlorella in such a way as to reduce the enzyme content of one or more cellular processes rather than to completely block a reaction.

Genetic changes observed among Chlorella cells surviving irradiation from tritium oxide were similar to those reported by Davis[5]. Some rough selections were obtained; other selections had a much yellower color than control selections. It appears, however, that many more slow growing selections arose in the presence of tritium oxide than Davis found after exposure of Chlorella to ultraviolet light. We also obtained some selections which clumped and sedimented.

LITERATURE CITED

1. Porter, J. W., and H. J. Knauss, "Inhibition of growth of Chlorella pyrenoidosa by beta-emitting radioisotopes," Plant Physiol. (In press). (1953)
2. Myers, J., Final Report to Office of Naval Research, p. 23 (1950).
3. Redford, L., and J. Myers, "Some effects of ultraviolet radiations on the metabolism of Chlorella," J. Cell. and Comp. Physiol. 38, 217-244 (1951).
4. Anderson, R. S., Abstracts of papers presented before the Radiation Research Society, Iowa City, June 22-24, 1953.
5. Davis, E. A., "Photosynthetic Chlorella mutants," Amer. Jour. Bot. 39, 535-539 (1952).

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