A STUDY OF THE EFFECT OF CERTAIN CARCINOGENIC CHEMICALS UPON THE GROWTH AND STRUCTURE OF SELECTED PROTOZOA

By

Dorcas Jane Anderson

Contributions of the Graduate School
Indiana State Teachers College
Number 396

Submitted in Partial Fulfillment of the Requirements for the Master of Science Degree in Education

1939
The thesis of Dorcas Jane Anderson, contribution of the Graduate School, Indiana State Teachers College, Number 396, under the title *A Study of the Effect of Certain Carcinogenic Chemicals upon the Growth and Structure of Selected Protozoans* is hereby approved as counting toward the completion of the Master's degree in the amount of 8 hour's credit.

Committee on thesis:

[Signatures]

Date of Acceptance *Sept. 2, 1939*
TABLE OF CONTENTS

CHAPTER                                      PAGE

I. INTRODUCTION                               1
   The problem                                 1
   Statement of the problem                    1
   Validation of the study                     1
   Definitions of terms used                   2
   Carcinogenic chemicals                      2
   Growth                                      2
   Structure                                   2
   Protozoa                                    2
   Review of previous related studies          3
   Method of procedure                         10
   Sources of data                             13
   Statement of organization of thesis         13

II. EFFECTS OF DIFFERENT CONCENTRATIONS OF
    CERTAIN CARCINOGENIC CHEMICALS             14
    Series I                                   14
    Series IA                                  15
    Series II                                  15
    Series IIA                                 15
    Series III                                 16
    Series IIIIA                               16
    Series IV                                  16
    Series V                                   17
<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series VI</td>
<td>18</td>
</tr>
<tr>
<td>Series VII</td>
<td>18</td>
</tr>
<tr>
<td>Series VIII</td>
<td>19</td>
</tr>
<tr>
<td>Series IX</td>
<td>19</td>
</tr>
<tr>
<td>Series X</td>
<td>20</td>
</tr>
<tr>
<td>Series XI</td>
<td>20</td>
</tr>
<tr>
<td>Series XII</td>
<td>20</td>
</tr>
<tr>
<td>Series XIII</td>
<td>21</td>
</tr>
<tr>
<td>III. SUMMARY AND CONCLUSIONS</td>
<td>22</td>
</tr>
<tr>
<td>Summary</td>
<td>22</td>
</tr>
<tr>
<td>Conclusions</td>
<td>23</td>
</tr>
</tbody>
</table>

**BIBLIOGRAPHY**  
Periodical articles and reprints 25
# TABLE OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>14a</td>
</tr>
<tr>
<td>Figure 2</td>
<td>15a</td>
</tr>
<tr>
<td>Figure 3</td>
<td>16a</td>
</tr>
<tr>
<td>Figure 4</td>
<td>17a</td>
</tr>
<tr>
<td>Figure 5</td>
<td>17b</td>
</tr>
<tr>
<td>Figure 6</td>
<td>18a</td>
</tr>
<tr>
<td>Figure 7</td>
<td>18b</td>
</tr>
<tr>
<td>Figure 8</td>
<td>19a</td>
</tr>
<tr>
<td>Figure 9</td>
<td>19b</td>
</tr>
<tr>
<td>Figure 10</td>
<td>20a</td>
</tr>
<tr>
<td>Figure 11</td>
<td>20b</td>
</tr>
<tr>
<td>Figure 12</td>
<td>20c</td>
</tr>
</tbody>
</table>
CHAPTER I

About two decades ago, a new field of research opened up to biologists when workers perfected the technique of growing certain strains of protozoa in bacteria-free cultures. It was thus made possible to determine the physiological effects of any introduced substance or condition without danger of modification by uncontrolled elements in the environment. Most of the work done in this field so far has been on perfecting the techniques of growing bacteria-free cultures of protozoa and in increasing the number of strains of protozoa so grown. Some few studies have been made on the effect of various chemicals on the growth of such strains of protozoa.

I. THE PROBLEM

Statement of the problem. It was the purpose of this study (1) to compare the accelerating or inhibitory effects of certain carcinogenic chemicals upon the growth of selected protozoans grown in bacteria-free cultures; (2) to study in isolation cultures any changes of structure in the protozoans brought about by the chemicals.

Validation of the study. In the field of science it is necessary to lay many small bricks before a pathway
to a goal can be formed. An attempt was made in this investigation to lay one such brick in order to add to the accumulation of knowledge concerning the protozoa, and, incidentally, perhaps, concerning the cause of cancer.

From an educational standpoint, this study may be validated by pointing to the growing interest in bacteria-free cultures of protozoa and the increasing tendency to include such work in the courses of high school biology. Naturally, a knowledge of the techniques and expected results of such cultures is necessary to the teacher.

II. DEFINITIONS OF TERMS USED

Carcinogenic chemicals. Carcinogenic chemicals are those chemicals which will produce cancer in an experimental animal. They may be either rubbed on the skin or injected. Those used in this investigation are: 2-amino-5-azotoluene, methylcholanthrene, 1:3:5-triphenylbenzene, 1:2:5:6-dibenzanthracene. These are all coal tar products or derivatives.

Growth. In this investigation "growth" was taken to mean an increase in the total number of protozoans present and not an increase in the size of any individual protozoan.

Structure. By the term "structure" was meant the form and the arrangement of the contents of the individual protozoan.

Protozoa. "Protozoa" is a general term covering all one-celled animals. The term "protozoan" refers to one
individual of the group "protozoa," or to one particular species of that group.

III. REVIEW OF PREVIOUS RELATED STUDIES

There are two general groups of investigations concerning the pure culture of protozoa. In one group bacteria-free cultures are maintained, the protozoa being grown in a peptone or similar medium. In the other group, the protozoa are washed free of bacteria and transferred to media containing known species of dead or living yeasts, bacteria, or other microorganisms.

The work on bacteria-free cultures includes the classical research of Lwoff who succeeded in isolating Glaucoma piriformis in a peptone medium.¹ Oehler² reported the isolation of bacteria-free strains of Colpoda steinii and Colpoda cucullus in 1924. In 1929, Butterfield³ established pure cultures probably of Colpidium campylum in peptone, and in 1933, Hetherington produced bacteria-free cultures of Colpidium campylum, Glaucoma scintillans, and Loxocephalus

² Ibid., p. 264.
³ Ibid., p. 264.
granulosus in yeast extract and peptone glucose solutions. Elliott in 1933 isolated Colpidium striatum in bacteria-free cultures, and Loefer reported the isolation of Paramecium bursaria a year later, both workers using a tryptone medium.

Using dead microorganisms as food, Oehler was the first to obtain pure cultures of ciliates (ciliated protozoans) in 1919. Glaser and Coria in 1930 reported success in freeing protozoans from associated bacteria and later established Paramecium caudatum in one species of dead bacteria and yeast. Although the cultures of Glaser and Coria are free from living bacteria, the fact that they contain dead bacteria or yeast makes a technical distinction necessary between their work and that of investigators using only a peptone or allied medium.

Nine species of ciliates have been successfully grown in bacteria-free cultures at the present time, and three other species have been grown in cultures with dead microorganisms as food. Also, mixed cultures of protozoans and only one or two other known living microorganisms (bacteria, yeast, algae) have been grown by various investigators.

The next work along this line taken up by investigators was the effect of the hydrogen ion concentration on the growth of the protozoans in bacteria-free cultures. Among the first

---


6 Johnson, op. cit., p. 264.

7 Ibid., p. 264-5.
to work on this phase were Pruthi and Darby who correlated the pH of the medium with a definite sequence of forms and found the range for Colpidium striatum to be about 4.0 to 8.6 with a narrower range in a medium containing sodium acetate.\(^8\) Elliott noted a bimaximal growth curve with ciliates, the entire range being from a pH of 4.0 to 8.6, in 1933.\(^9\) The same author in 1935 showed a difference in media made a difference in the bimaximal pH growth curves, the two maxima appearing in Bacto-peptone, but not in Bacto-proteose-peptone, Bacto protone, or Bacto veal. Moreover, he reported that the maximal growth of Colpidium always occurred on the alkaline side of the range, while that of Glaucoma was on the more acid side of the range.\(^{10}\) In 1931, Jahn reported a wide pH range of 3.9 to 7.5 for Euglena gracilis in a solution of casein decomposition products.\(^{11}\) Loefler found Chilomonas paramecium to have a range of pH 4.1 to 8.4 and two growth optima 4.9 and 7.0, while Chlorogonium euchlorum increases progressively from a pH of 4.8 to an optimum of 7.4.\(^{12}\) In 1937

---

8 Elliott, op. cit., p. 54.

9 Ibid., p. 55.


Johnson\textsuperscript{13} worked out the pH range of \textit{Glaucoma ficaria} and \textit{Glaucoma piriformis} finding the former to be pH 4.9 to 9.5 and the latter pH 4.0 to 8.9. The pH optimum for \textit{Glaucoma ficaria} is 5.1 to 6.0.

Some work has been done concerning the effect of various chemicals in bacteria-free cultures of certain protozoans. Loefer and Elliott have been particularly active in this type of investigation. In 1935 Elliott reported the effects of organic acids and protein derivatives on the growth of \textit{Colpidium}. Of five peptones tested, those containing a high percentage of amino acid supported growth of the protozoan best. Sodium salts of the five lower acids were completely toxic to the organisms while glycollate, pyruvate, and tartrate showed moderate degrees of toxicity. He found ethyl alcohol and glycerine were inert to \textit{Colpidium campylum}, the former being slightly toxic to \textit{Colpidium striatum}.\textsuperscript{14}

Loefer and Hall agree with this last statement. This is evidenced in their report published in 1936, in which they describe growing various strains of protozoans in bacteria-free cultures, adding different concentrations of ethyl alcohol. They found no evidence indicating acceleration of growth by the alcohol except in two species of \textit{Euglena}.\textsuperscript{15}

\textsuperscript{13} Johnson, \textit{op. cit.}, p. 275.
\textsuperscript{14} Elliott, \textit{op. cit.}, p. 493.
\textsuperscript{15} J. B. Loefer and R. P. Hall, "Effect of Ethyl Alcohol on Growth of Eight Protozoan Species in Bacteria-free Cultures," \textit{Archiv für Protozientkunde}, 87:129, 1936.
In 1935 an investigation by Hall and Elliott demonstrated that the growth of Colpidium is accelerated by the addition of a series of single amino acids and asparagin by 20% to 50% over the plain gelatin controls. Although Colpidium is able to make use of such a series, none of the amino acids alone is sufficient to promote growth. No incomplete proteins used by these investigators promoted growth.\(^\text{16}\)

In the same year Loefer conducted an investigation into the growth-promoting properties of a series of proteins on Chlorogonium (euchlorum and elongatum) and Chilomonas paramecium. Bacto-tryptone, Bacto-yeast extract, and Bacto-beef blood serum gave quick, prolific growth of the protozoa. After a longer period, growth was also good in Proteose-peptone and Bacto-gelatin, while casein, Bacto-veal, and Bacto-peptone supported moderate growth. It was found that Chilomonas is unable to utilize as a source of nitrogen any of the amino acids tested, but Chlorogonium increases its growth rate with certain amino acids, especially aspartic acid.\(^\text{17}\)

A similar study the following year was by Loefer on a series of "peptone" media in promoting growth of Paramecium bursaria. Proteose-peptone, Bacto-tryptone, Bacto-veal, and Seidenpepton all increased growth in the order named. Of the carbohydrates tested in a parallel experiment, dextrose,


mannose, maltose, dextrin, and melezitose proved best while levulose, galactose, mannitol, lactose, sucrose, soluble starch, and salacin had little effect, and arabinose, xylose, rhamnose, and inulin were unfavorable.  

Elliott in 1935 ran a series of tests on bacteria-free cultures of Colpidium campylum and Colpidium striatum with fifteen different carbohydrates. Growth of C. striatum was accelerated by eleven of the fifteen, and that of C. campylum by only six of the fifteen. The pH of the medium was found to be related to the accelerating effect of the carbohydrate, maximum growth taking place below a pH of 7.0. The addition of insulin to sugar media caused a decrease in the growth rate of both species, while no effect was found in sugar-free medium.

Loefer in 1935 ran a series of experiments with carbohydrates and organic acids on Chlorogonium euchlorum, Chlorogonium elongatum, and Chilomonas paramecium. Of eleven carbohydrates tested, levulose, galactose, maltose, and lactose produced the greatest acceleration of growth on C. euchlorum. The results were the same for C. elongatum except that lactose instead of being one of the best accelerators was one of the poorest. With Chilomonas, growth was best with dextrin, and

---


there was no acceleration at all with rhamnose or starch. Growth of Chilomonas was accelerated by salts of the following fatty acids in descending order: acetic, butyric, valeric, propionic, and isobutyric. The results were the same for Chlorogonium except that propionate did not accelerate growth with either species.20

Hall found in 1937 that certain concentrations of manganese chloride produced an accelerative effect on the growth of Euglena anabaena, the acceleration being more marked in an inorganic medium. Cultures of Astasia sp and Colpidium campylum did not support an acceleration in growth. Hall assumed the effect on the Euglena was due to some relationship between manganese and the metabolism of chlorophyll-bearing flagellates.21

The last work is that of Elliott in 1938 on the relation of certain plant hormones to the rate of growth of protozoa. He found that all three of the plant hormones he selected stimulated the growth of Euglena gracilis, while none favorably affected the growth of Khawkinea halli, or Colpidium striatum. This seems to support the theory that the accelerating effects of phytohormones are dependant upon the presence of chlorophyll, for Euglena is the only one of the three containing chlorophyll.22


IV. METHOD OF PROCEDURE

The method of procedure included the use of bacteriological technique in the maintenance of bacteria-free cultures. The three strains of protozoa used, Colpidium striatum, Colpidium campylum, and Glaucoma ficaria were supplied by Dr. R. P. Hall of New York University, where the original cultures have been maintained bacteria-free for several years. Stock cultures of the protozoa were grown in test tubes in a tryptone medium at room temperature. Transfers to fresh media were made two days before being used in an experiment.

A tryptone base was used for all experiments. The medium included ten grams of Bacto-tryptone and two grams of potassium phosphate (dibasic) per liter of distilled water. The desired concentration of the experimental chemical was added, and the material tubed in 9 c.c. amounts. The pH of the media was adjusted to 6.8 by means of a LaMotte Hydrogen Ion Tester, Model 3B. The tubes were autoclaved and the pH of one tube of each series was again determined, all tubes or series not falling in the range of pH 6.4-7.2 being discarded. The protozoans were diluted in 100 c.c. of sterile distilled water, and the flask was rotated before each transfer in order to distribute the protozoans equally. From this flask, by means of sterile graduated pipettes, exactly one cubic centimeter of protozoans was transferred to each tube. One extra-
tube was made for each species and immediately the organisms in it were killed with Bouin's fixing solution (75 parts picric acid, 25 parts formalin, 5 parts acetic acid) to determine the number of protozoans originally inoculated. Controls for each series containing everything but the experimental chemical were also made. The cultures were allowed to grow for seventy-two hours at room temperature and then were killed by Bouin's fixing solution. The initial and final counts of the protozoans were made by a Sedgwick-Rafter counting chamber and a Whipple micrometer.

The greatest difficulty experienced by the investigator lay in getting the carcinogenic chemicals in non-toxic, aqueous solutions. Based on the findings of Loefer and Hall\(^{23}\) in 1936 that ethyl alcohol had no accelerative effects on the three species used and only slight inhibitory effects in low concentrations, it was decided to dissolve 1:3:5-triphenylbenzene and 2-amino-5-azotoluene, both very slightly soluble in alcohol, in 95% ethyl alcohol. The concentration of alcohol in the tubes of organisms did not exceed 1% in any case.

Methylcholanthrene and 1:2:5:6-dibenzanthracene are not even slightly soluble in alcohol and can be dissolved only in fat solvents, all of which are non-aqueous or toxic to animal life, or both. From a report of LeRoy, Kandel,  

\(^{23}\) J. B. Loefer and R. P. Hall, op. cit., p. 128.
and Brunschwig, a description of Boyland's method for making a colloidal suspension of these chemicals was found. This method as used follows: .025 gm. of the hydrocarbon was dissolved in 8.3 cc. of pure acetone. One volume of this was poured slowly with continuous, vigorous stirring into ten volumes of warm (45°C) .5% gelatine in distilled water. The acetone was removed by vacuum distillation and the solution examined under the microscope. If crystals in large aggregates were present, the solution was discarded. The solutions used were added to the media in the usual manner.

Isolation cultures of individual protozoans were also prepared. The lethal point of 2-amino-5-azotoluene was discovered by running a series of concentrations between the last point of growth and the point of no growth. Three or four protozoans were isolated by means of a sterile micro-pipette and transferred to .2 c.c. drop of media in a depression slide containing a concentration of the hydrocarbon just under the lethal point. They were observed at intervals with a bi-focal microscope until their deaths occurred.

The results of these experiments were organized and charted and conclusions were drawn from the findings.

V. SOURCES OF DATA

Practically all the data for this report were obtained by laboratory experimentation. Additional sources included reprints of articles and theses by workers in the field of bacteria-free cultures of protozoa, scientific magazines, and scientific journals.

VI. STATEMENT OF ORGANIZATION OF THESIS

There are three chapters in this thesis. The first one deals with the definitions of the terms used, the literature concerning the subject, and the method of procedure. The second chapter is a report of the experiments, each experiment being written as a separate series and the results charted. The conclusions drawn from the results of the experiments and a summary of the work done constitute the last chapter. A bibliography concludes the work.
CHAPTER II

EFFECTS OF DIFFERENT CONCENTRATIONS OF CERTAIN CARCINOGENIC CHEMICALS

Series I. In the first attempt to discover the effect of carcinogenic chemicals, 2-amino-5-azotoluene was added to the tryptone base and the solution inoculated with Colpidium striatum. The concentrations of the chemical added were as follows: 100 mgm. of 2-amino-5-azotoluene in 25 c.c. of 95% ethyl alcohol, 50 mgm. in 25 c.c., and 10 mgm. in 25 c.c. Also 5 mgm. in 25 c.c., 1 mgm. in 25 c.c., and .1 mgm. in 25 c.c. One part of each of these solutions was added to ninety-nine parts of the tryptone media. The final concentrations were: .004%, .002%, .0004%, .0002%, .00004%, .000004%.

The pH of each solution was adjusted to 6.8, since the optimal pH is between 6.4 and 7.2, and the solutions tubed in 9 c.c. amounts, autoclaved, and the pH of each concentration again determined to make certain it was still in the optimal growth pH range. The tubes were inoculated with Colpidium striatum and allowed to incubate at room temperature for seventy-two hours. The initial concentration was 47 organisms per cubic centimeter. The cultures were fixed and the final concentration determined. The results are expressed graphically in Figure 1 as \( x/x_0 \) (ratio between final and initial concentrations of organisms).
Oolpidium striatum, growth in relation to varying concentrations of 2-amino-5-azotoluene, X/X₀ (ratio of final to initial concentration of organism).
Greatest growth occurred in the least concentration, that of .000004%, and in this concentration there was an increase above the control. In the next concentration, that of .00004%, the growth was cut to half that of the control, and a concentration of .002% proved lethal to the organisms.

**Series IA.** In order to determine the exact lethal point of 2-amino-5-azotoluene for *C. striatum*, a series of concentrations between .002%, the point at which all organisms had been destroyed, and .0004%, the last point of growth, was run. The same procedure was followed as in Series I. The concentrations were: .0018%, .0016%, .0014%, .0012%, .0001%, .0008%, .0006%. The lethal point was found to be .0012%.

**Series II.** The same procedure was used in determining the effect of 2-amino-5-azotoluene on *Colpidium campyllum*. Six different concentrations were made as before and the tubes inoculated. The initial count was 29, and the pH of the tubes was between 6.4-7.2. The final results are expressed graphically in Figure 2 as \( \frac{x}{x_0} \).

The results are the same as with *C. striatum*. Again, the greatest growth, exceeding the control, was obtained in the concentration .000004%. No growth occurred in .002%.

**Series IIIA.** The same concentrations as in Series IA
*Figure 2*

*Colpidium campylum,* growth in relation to varying concentrations of 2-amino-5-azotoluene, $X/X_0$. 

[Chart showing growth in relation to varying concentrations of 2-amino-5-azotoluene.]
were used in determining the lethal point of *C. campylum*. The lethal point in this case was found to be .00012%.

**Series III.** *Glaucoma piriformis* was used in inoculating the third series for 2-amino-5-azotoluene. The same concentrations as in the first two series were used, and the optimal pH growth range for *Glaucoma piriformis* is also 6.4-7.2. The initial count was 73. The final results are expressed graphically in Figure 3 as $x/x_0$.

The results were somewhat different for *G. piriformis* for the rate of growth fell below the control in all concentrations. Again, however, the concentration of .002% was lethal to the organisms.

**Series IIIA.** The same concentrations of 2-amino-5-azotoluene as used in IIA were inoculated with *Glaucoma piriformis* to determine the lethal point of the chemical for this particular protozoan. In this case, the lethal point was .00014%.

**Series IV.** The second carcinogenic chemical, 1:3:5-triphenylbenzene, was tested on the three strains of protozoa. The concentrations of the chemical used were: .3 gm., .2 gm., .1 gm., .05 gm., .01 gm., .002 gm.,
FIGURE 3

Glaucoma piriformis, growth in relation to varying concentrations of 2-aminon-5-azotoluene, $X/X_0$. 

RATIO

0.004 0.002 0.0004 0.0002 0.00004
and .0002 gm., each in one hundred cubic centimeters of ethyl alcohol. One part of these concentrations was added to ninety-nine parts of the tryptone base, making final concentrations of .003%, .002%, .001%, .0005%, .0001%, .00002%, and .000002%. This was tubed, pH determined, and the tubes inoculated with Colpidium striatum in the usual manner. The initial count was 135. Final results are shown graphically in Figure 4 as $x/x_0$.

A bimaximal growth curve is noted with this chemical and C. striatum, the first optimum being at the lowest concentration, .000002%, and the second optimum at .001%. None of the concentrations stimulated growth above the control, nor did any of them prove lethal to the protozoans.

**Series V.** The same concentrations of 1:3:5-try-phenylbenzene were used in a similar series on Colpidium campylum. The initial count in this case was 190. Final results are shown as $x/x_0$ in Figure 5.

Again, a bimaximal curve is noted, with the first optimum the lowest concentration as before, but with the second optimum the highest concentration. Only once, in the concentration .000002%, did the final count exceed the control, but the increase was so small (902-958) that it can not be said to indicate anything. None of the concentrations was lethal.
**FIGURE 4**

*Colpidium striatum*, growth in relation to varying concentrations of 1:3:5-triphenylbenzene, $x/X_0$. 
Colpidium campyllum, growth in relation to varying concentrations of 1:3:5-triphenylbenzene, \( X/X_0 \).
Series VI. With *Glaucoma piriformis*, the same concentrations of 1:3:5-triphenylbenzene were used as in Series IV and V. The initial count was 191. Final results are shown as $x/x_0$ in Figure 6.

The growth curve was extremely irregular. In solutions of .003% and .001% the growth exceeded the control slightly, and in .0005% the growth equalled the control. None of the concentrations proved lethal.

Series VII. Methylcholanthrene, a stronger carcinogenic chemical than the preceding ones, was used in tests on the same three strains of protozoa. It was necessary to emulsify methylcholanthrene in the manner described in Chapter I, page 12, in order to obtain an aqueous solution of it. The concentrations of the chemical used were: .001%, .00075%, .0005%, .00025%, .000125%. This was tubed, pH determined, and the tubes inoculated with *Colpidium striatum* in the usual manner. The initial count was 103. Final results are shown graphically in Figure 7 as $x/x_0$.

Greatest amount of growth is noted with .0005% concentration. None of the concentrations was lethal. A double control, one with and one without gelatin, was run to determine the effect of the gelatin. The results showed an increase of growth of about 43% in the tryptone broth alone, indicating a distinct inhibitory effect of the gelatin.
**FIGURE 6**

*Glaucoma piriformis*, growth in relation to varying concentrations of 1:3:5-triphenylbenzene, X/Xo.
**FIGURE 7**

**Colpidium striatum**, growth in relation to varying concentrations of methylcholanthrene, X/X₀.
of which there was about 3% in the tubes.

Series VIII. Five concentrations of methylcholanthrene made as in Series VII were inoculated with Colpidium campylum. The initial count was 41. The final results are expressed graphically in Figure 8 as $x/x_0$.

The results are the same as with C. striatum, the proportions of the two curves being the same. Again the greatest growth occurred in concentrations of 0.0005% and none of the concentrations was lethal. Controls of C. campylum supported the previous evidence that gelatin inhibits the growth of the protozoans. The percentage of gain in the plain tryptone tube was 39%.

Series IX. Glaucoma piriformis was used to inoculate the third series of methylcholanthrene. Five concentrations were made as before, and the test carried out as usual. The initial count was 38. Final results are expressed graphically in Figure 9 as $x/x_0$.

Growth of Glaucoma followed the trend of the other two protozoans save for a greater proportionate growth in 0.00125%. Again 0.0005% led the growth and none of the concentrations was lethal. Here, too, gelatin proved inhibitory to growth.
FIGURE 8

Colpidium campylum, growth in relation to varying concentrations of methylcholanthrene, $X/X_0$. 
FIGURE 9

Glaucoma piriformis, growth in relation to varying concentrations of methylcholanthrene, X/xo.
Series X. Using the same concentrations as in methylcholanthrene, a group of three experiments was worked with 1:2:5:6-dibenzanthracene, one of the most powerful carcinogenic chemicals known. The chemical was emulsified with the gelatin as previously described. This group was worked in conjunction with the previous group; therefore, the initial counts are the same. In this case, the initial count of Colpidium striatum is 103. The final results are shown in Figure 10 as x/x₀.

As in methylcholanthrene, the greatest growth occurred in the concentration of 0.0005%. None of the concentrations was lethal. In all three strains a drop in growth was noted at 0.00075%.

Series XI. Colpidium campylum was used to inoculate the next series of 1:2:5:6-dibenzanthracene. The initial count was 41, and the final results are indicated in Figure 11 as x/x₀.

The results were practically the same as in Series X with 0.0005% the high point of growth and none of the concentrations lethal.

Series XII. A third group was tested with 1:2:5:6-dibenzanthracene using Glaucoma piriformis as the organism tested. The initial count was 38. Figure 12 expresses the final results graphically as x/x₀.

The results were the same as for the other two strains except for a less hearty growth in general of the
Colpidium striatum, growth in relation to varying concentrations of 1:2:5:6-dibenzanthracene, $X/X_0$. 

**FIGURE 10**
Colpidium campylum, growth in relation to varying concentrations of 1:2:5:6-dibenzanthracene, X/Xo.
Glaucoma piriformis, growth in relation to varying concentrations of 1:2:5:6-dibenzanthracene, X/Xo.
Series XIII. With 2-amino-5-azotoluene, a series of isolation cultures was worked out to determine what effect the chemical had upon the structure of the organisms. Using the method described in Chapter I, pages 12-13, two to twelve protozoa of each of the three series were transferred to depression slides in sterile Petri dishes.

A medium containing .0012% of 2-amino-5-azotoluene was used. The organisms were observed at intervals of ten minutes. At first the organisms seemed normal but at the second observation they were spinning and hurrying about in an abnormal manner. At the next observation, they were rounded, swollen to a larger size, and trichocysts were apparent. Shortly after, the cytoplasm could be seen cozing out of the individual, and soon, the organism was entirely broken up and appeared as a black piece of debris.

With a concentration of .0006%, the same results were observed, the only difference being that it took about twenty more minutes for the organisms to die.
Summary. A group of series of experiments were performed on *Colpidium striatum*, *Colpidium campylum*, and *Glaucoma piriformis* with certain carcinogenic chemicals. Growth of the protozoa was compared in different concentrations of the chemicals.

With 2-amino-5-azotoluene, a definite increase of growth over the control is noted in a concentration of .000004% with *C. striatum* and *C. campylum*. With all the protozoans, a concentration of .002% proved lethal. *Glaucoma piriformis* did not grow so well as the other two species.

The use of 1:3:5:triphenylbenzene gave erratic results. Bi-maximal growth curves were obtained with each species, but with no consistency as to the place of the maxima. None of the concentrations was lethal. Growth of *C. striatum* never exceeded the control, but with the other two, growth in some of the concentrations exceeded the control.

Results with methylcholanthrene showed the greatest increase in growth at .0005%. Growth in the chemical exceeded the control in all concentrations save .000125% with *C. striatum*, and .00025% and .000125% with *C. campylum* and *G. piriformis*. None of the concentrations was lethal.
In the same concentrations as was methylcholanthrene, 1:2:5:6-dibenzanthracene gave very similar results. Greatest growth was again at .0005% for all three strains. None of the concentrations was lethal and growth exceeded the control in all cases but with .000125% inoculated with G. piriformis.

Isolation cultures with 2-amino-5-azotoluene showed the organisms were killed by excessive swelling, causing them to burst.

Conclusions. 2-amino-5-azotoluene, methylcholanthrene, and 1:2:5:6-dibenzanthracene caused a definite increase of growth in the three species of protozoans. Best growth occurred in the strongest carcinogenic chemical used, 1:2:5:6-dibenzanthracene, medium growth in the next strongest, methylcholanthrene, and least growth in the weakest, 2-amino-5-azotoluene. This would indicate that the stimulus to the protozoans is attributable to the carcinogenesis of the chemicals.

Some investigators doubt that 1:3:5-triphenylbenzene has carcinogenic properties. The vague and unpredictable results obtained with 1:3:5-triphenylbenzene in this test tend to support this theory.

The observations made in the isolation cultures

---

indicate that the protozoans react to 2-amino-5-azotoluene in much the same manner as they do to any unfavorable environment. Lack of time prevented similar work with the other carcinogenic chemicals.

Further investigation with weaker concentrations of 2-amino-5-azotoluene, and with both stronger and weaker concentrations of methylcholanthrene and 1:2:5:6-dibenzanthracene would prove valuable. A method of emulsifying the last two chemicals without the use of the gelatin, which retarded the growth of the ciliates, would lead to more accurate results.
BIBLIOGRAPHY

PERIODICAL ARTICLES AND REPRINTS


"Cause of Cancer," Science ns, 64:sup. 12-14, April 5, 1929.


Hall, R. P., "Food Requirements and Other Factors Influencing Favorable Growth of Protozoa in Pure Cultures," Unpublished report of a survey, Biological Laboratory, University College, New York University, 1939.


Jahn, Theodore L., "Studies on the Physiology of the Euglenoid Flagellates. III. The Effect of Hydrogen Ion Concentration on the Growth of


Loefer, John E., "The Trophic Nature of Chlorogonium and Chilomonas," Biological Bulletin, Vol. LXVI,


"Research in Cancer," *Science*, 62:sup. 10-12, August 8, 1925.
