PART I. THE EFFECT OF DESICCATED THYROID ON THE REPRODUCTION OF *PARAMECIUM CAUDATUM*

PART II. THE EFFECT OF DESICCATED THYROID ON THE RESPIRATION OF *PARAMECIUM CAUDATUM*

by

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PART I. THE EFFECT OF DESICCATED THYROID ON THE REPRODUCTION OF PARAMECIUM CAUDATUM

INTRODUCTION

Paramecium has for many years been a favorite form for studies of various kinds, and with justification since it is composed of a single cell, is of considerable size, is easily obtained and is readily maintained in vigorous condition in laboratory cultures. Many of the factors which are involved in the cultivation of Paramecium have been carefully studied by numerous workers with the result that there is much complete and concise data on their significance.

The influence of different products upon the growth and metabolism in the higher animals has been interpreted by many investigators as indicating a direct effect on cell multiplication. Considerable research has accordingly been made on protozoans as a means of experimenting directly with single cells.

The work of Jollos (1922) indicated the possibility of inducing hereditary changes in Paramecium by means of external agents. The use of solutions of endocrine glands, particularly thyroid glands, offered a favorable means of attacking the problem.

Subsequent studies have been made by different investigators on the effects of thyroid and its derivatives on Paramecium. Discrepancies in their findings have appeared, however, with the result that the conclusions drawn from these earlier experiments

offered little in regard to correlating them with other aspects of protozoan life, such as respiration and metabolism.

It thus became desirable to conduct experiments with desiccated thyroid in an effort to understand more fully its action on the reproduction of Paramecium, and to find what possible influence this multiplication would have on subsequent studies of the metabolic activities of the animal.

REVIEW OF LITERATURE

The investigations of Maupas (1888) were perhaps the most extensive and carefully conducted of the earlier attempts to understand the reproductive activities of certain infusorians. Due to the variability of methods employed, however, his results could not be duplicated or his conclusions verified.

The first to undertake a careful study of the growth of Paramecium under known conditions with controlled factors was Calkins (1902). Temperature and other physical factors were either controlled or known, but he was working with an unknown variable as far as food was concerned. He concluded, nevertheless, that "the division-rate is taken as the measure of vitality, for it represents the rate of metabolism, growth, and reproduction... fluctuations mark out clearly the periods of vigor and depression."

The first attempt on experiments with thyroid extracts on protozoans was made by Nowikoff (1908). He observed that para-

media treated with thyroid appeared to divide more rapidly than those kept on a hay infusion medium. He concluded that the thyroid extract "exerts an intensive influence" on the organisms.

The next person to investigate this problem was Shumway (1914). His study was very extensive and he reached the conclusion from his experiments that "the effect of the thyroid is to increase greatly the rate of division except at the time when the line was nearing its cycle; and further that the effect is not permanent after feeding with thyroid is stopped." Shumway believed it obvious that the effect of the thyroid was directly upon the metabolic activities of the individual cells. He also found that the ciliates treated with thyroid were more active and slightly smaller than were the control individuals. This led him to believe that the process of metabolism was greatly accelerated by the thyroid.

Budington and Harvey (1915) obtained about the same results in their work on pedigreed cultures of Paramecium. They found it probable that "intra-cellular modifications of the Paramecium protoplasm does accompany its feeding upon and living in a medium which, among other things, brings it hurriedly to its most crucial experience, self-division." They experimented with extracts from the thyroid glands of animals representing the five classes of vertebrates and concluded that the thyroid ingredients produced essentially the same results when administered to ciliate protozoans, namely, increased division rate.

In a later paper Shumway (1917) found a 65 per cent increase in the division rate of Paramecium caudatum and Paramecium aurelia which had been subjected to a thyroid diet as compared to control animals which were on hay infusion. He also experimented with other endocrine gland products and found that the thyroid was the only one of the internally secreting glands that produced this effect. When returned to the control medium, it was also noted that the paramecia reverted to their normal division rate.

Straus (1923) found that thyroid cultures of Paramecium were more favorable to existence and reproduction than the ordinary hay infusion cultures. He noted that they needed little attention and seemed to be less dependent on oxygen, as they did not congregate at the top of the medium as do ordinary cultures. He did not elaborate on these points, however, but merely speculated that the thyroid was beneficial to the organisms.

Cori (1923) tested the influence of commercial thyroid powder and also thyroxin on the division rate of Paramecium, and found that the thyroxin effect on the multiplication rate was not as strong or as consistent as that of the thyroid extract, even though the concentrations were equivalent. The ratio of the divisions in 24 hours for Paramecium in the control, on thyroxin, and on thyroid was 100, 102, and 112 respectively.

The combined results obtained from the studies of the earlier investigators on the use of thyroid in Paramecium cultures have been cited as a corroboration of the view that the active principle of the thyroid directly accelerates cell metabolism,

thus increasing the rate of cell division. However, Riddle and Torrey (1923) found that the effect of thyroxin was manifest especially in a slight decrease in the division rate of Paramecium. This depressed rate was found to be no more than one-third that of the control (Torrey, 1924). However, it was noted that none of the organisms lacked vigor, but differed from the control animals in division rate only.

Woodruff and Swingle (1923) could find no significant acceleration of the division rate of Paramecium after administering desiccated thyroid of the turtle to the animals. They attributed the previous results of other investigators to variations in the bacterial food supply and called them erroneous.

Ball (1925) found that members of the same clone of Paramecium divided at a significantly higher rate in solutions of desiccated thyroid than in the controls when the bacterial food supply was uncontrolled. He explained this result by stating that the thyroid acted as an exceptionally suitable food for the paramecia in that it provided excellent opportunities for the growth of bacteria. However, in one group of his experiments he found the division rate of the animals living only on dead bacteria to be significantly increased by desiccated thyroid compound.

MATERIAL AND METHODS

The data in this study were taken from experiments which were conducted over a period of eight weeks. The paramecia employed were taken from a stock culture of Paramecium caudatum which had been growing in the laboratory for over five years. These organisms were isolated and counted daily during the course of each experiment.

Twenty stender dishes, five centimeters in diameter and two centimeters deep, were used in these runs. A group of five paramecia were isolated in each stender dish and one milliliter of medium was added. To each of ten dishes was added a minute amount (approximately five-tenths milligrams) of desiccated thyroid preparation (Parke, Davis and Co. Thyroid U. S. P.). The other ten dishes were used as a control. The stender dishes were then covered and kept at room temperature.

Counts were made at 24 hour and 48 hour intervals after introduction to the medium in order that results could be compared and correlated with those obtained from subsequent respiration studies. A binocular dissecting microscope with a 0.7 X lens and 10 X oculars was used in counting the animals. This was accomplished by placing the stender dish on a piece of black paper which had been ruled off into squares resembling those on a bacterial counting chamber. By placing the dish under the microscope it was possible to count the paramecia in the entire stender dish with great accuracy. The translucent animals showed up

well against the dark background which facilitated the counting procedure.

The medium employed in the reproduction experiments was taken directly from the culture jars containing the animals. The paramecia were centrifuged out, but no attempt was made to sterilize the medium or control the bacterial count in any way. The primary purpose of these trials was to compare the multiplication of Paramecium with and without thyroid treatment. Every effort was made to keep influencing factors equal between those organisms which were on thyroid and those which were not.

RESULTS

Each figure in Table 1 represents an average of ten experiments. The data were closely coherent although fluctuations did exist. A variety of possible sources of error were present, but the experiments were set up so that all influencing factors would be equal for both the paramecia receiving thyroid preparation and the control individuals.

An over-all average of the reproduction counts covered 40 series of experiments with 20 stender dishes each--altogether 800 single determinations. It was found that five of the control animals (those without desiccated thyroid) averaged 5.95 in number at the end of 24 hours, or an increase of 19.2 per cent. The thyroid-fed paramecia, on the other hand, increased in number to 9.62 or 92.4 per cent for the same period of time.

Table 1. The effect of desiccated thyroid on the rate of reproduction of Paramecium caudatum in non-sterile culture media.

Date	Number of paramecia					
	Control			On thyroid		
	Start	After 24 hr.	After 48 hr.	Start	After 24 hr.	After 48 hr.
6-15-51	5	6	17	5	8	46
6-17-51	5	7	16	5	9	49
6-19-51	5	6	20	5	10	43
6-21-51	5	5	14	5	10	39
6-25-51	5	3	15	5	8	40
6-27-51	5	4	13	5	11	47
6-29-51	5	7	16	5	10	44
7-1-51	5	7	16	5	9	50
7-4-51	5	4	11	5	7	37
7-6-51	5	5	13	5	9	36
7-8-51	5	3	9	5	7	40
7-10-51	5	6	15	5	9	44
7-17-51	5	2	8	5	4	34
7-19-51	5	5	11	5	8	44
7-21-51	5	7	21	5	12	55
7-23-51	5	8	21	5	13	47
7-25-51	5	7	19	5	9	40
7-27-51	5	9	22	5	14	48
7-31-51	5	6	20	5	11	47
Average	5	5.95	16.0	5	9.62	44.7

At the end of the 48 hour period the control organisms had multiplied to an average of 16.0 per stender dish, an increase of 169 per cent from the preceding day, or a total increase of 220 per cent for the 48 hours. During this second 24 hour interval, however, the thyroid individuals multiplied to 44.7 paramecia per dish, an increase of 364 per cent from the preceding day, or a total increase of 794 per cent over the original five ciliates.

In four different experiments the paramecia were allowed to multiply an extra day and counts were made on the 72 hour individuals. Although lack of sufficient data make these results inconclusive, it was noted (Table 2) that the pace exhibited by the organisms in their reproduction during the second day was not maintained during the third day. Whereas the control animals had shown an increase of 169 per cent during the second 24 hour interval, they increased in number only 108 per cent during the third interval. The thyroid-fed paramecia exhibited the same effect--an increase of only 102 per cent for the third day whereas they had risen 364 per cent the previous day.

It is possible that this phenomenon was chiefly due to an exhaustion of the animals' food supply. When the sample of culture medium was taken from the original culture jar, the bacteria were deprived of their immediate source of food and had to maintain themselves only on the organic particles suspended in the medium. When this supply was exhausted, the bacterial population quickly declined, creating an unfavorable environment for optimum growth of the paramecia.

Table 2. The effect of desiccated thyroid on the rate of reproduction of Paramecium caudatum in non-sterile culture media.

Date in 1951	Number of paramecia							
	Control				On thyroid			
	Start	After 24 hours	After 48 hours	After 72 hours	Start	After 24 hours	After 48 hours	After 72 hours
6-21	5	5	14	29	5	10	39	83
7-1	5	7	16	34	5	9	50	94
7-10	5	6	15	32	5	9	44	91
7-31	5	6	20	39	5	11	47	95
Mean	5	6	16.2	34	5	9.75	45	90.7

Because of the possible influence the bacteria in the medium might have had on the results, as was stressed by Woodruff and Swingle (1924), bacterial counts were made on the media to which desiccated thyroid had been added 24 hours and 48 hours previously. Counts were also made on the control medium and the results compared to find what effect, if any, the thyroid preparation had on the bacterial growth. The results of ten separate counts, as illustrated by Table 3, were striking. The control medium averaged 9,760 colonies per millliliter while the thyroid medium averaged 2,049,200 colonies per milliliter at the end of 24 hours after innoculation. Counts on the 48 hour media showed that the control medium contained 104,510 bacteria to 11,658,000 bacteria

Table 3. The effect of desiccated thyroid on the bacterial growth of the culture media.

Number of samples used for each count	Number of bacteria per milliliter of medium			
	Control		On thyroid	
	After 24 hours	After 48 hours	After 24 hours	After 48 hours
3	3,900	198,000	1,480,000	7,000,000
3	2,500	150,000	1,910,000	12,000,000
3	4,800	87,500	1,167,000	6,520,000
4	20,400	95,350	1,280,000	25,000,000
2	17,200	41,700	4,400,000	7,770,000
Average	9,760	104,510	2,049,200	11,658,000

per milliliter of the thyroid medium. Without doubt the thyroid increases the numbers of bacteria, as is shown by the 217-fold difference during the first 24 hours. This difference, however, is not so marked during the second day as the thyroid-fed bacteria increased to only 112-fold over the control microorganisms. These results must be taken into consideration, nevertheless, before any conclusions can be drawn concerning contaminated cultures.

In making experimental runs on the reproduction of ciliates, it would be expected that the animals would follow a logarithmic curve--multiplying to an infinite number. However, such was not the case in this study, although considerably more work needs to

be done on this matter before definite conclusions can be made.

A repression of the division rate, or lag, was shown by the paramecia during the first 24 hours in numerous trials. These occurred after the individuals had been transferred from old cultures to fresh culture medium. This same situation was observed by Greenleaf (1926) who attributed it to the fact that the infusorians tended to multiply faster in larger than in smaller volumes of culture media.

Although no attempt was made to measure this phenomenon critically, it was believed in comparing the paramecia with and without thyroid that the thyroid-fed individuals exhibited a higher degree of activity than did the control animals. They also appeared somewhat more transparent than did paramecia without thyroid. It was found with the use of a calibrated microscope that the ciliates with thyroid were slightly smaller, on the average, than were the control animals. The average length of the control organisms was 236 microns as compared to 229 microns for the thyroid-fed individuals.

DISCUSSION

The fact that desiccated thyroid increases the rate of reproduction of Paramecium caudatum appears indisputable; however, some different opinions exist concerning the way in which it influences this reproductive rate. The fact that rapid fission of the thyroid-fed animals is accompanied by their increased activ-

ity and transparency as well as by their slightly smaller size, seems to indicate that important internal modifications occur.

Phillips (1922) found that the bacterial content of the medium was the controlling factor in the metabolic rate of Paramecium. She discovered that when a pure culture of bacteria was present this rate was lower than when any mixture of microorganisms was employed, and stated that mixtures of bacteria appeared to be the most satisfactory food for the ciliates. Hargitt and Fray (1917) had earlier reached this conclusion, but their data were insufficient to justify their results.

The bacterial content of the medium doubtless modifies the action of the paramecia, and this factor merits consideration. This is made evident by the large increase in the bacterial count after the administration of desiccated thyroid to the medium. However, since the control and thyroid samples both came from the same culture jar, it was assumed that their bacterial content was equal when the experiments were set up. Therefore, the thyroid was responsible for the increase in the number of paramecia. This was partly due to the more favorable condition of a greater food supply for as the animals moved about in the nutrient medium they were constantly taking in food with the water absorbed, and this food, according to Calkins (1904), consists primarily of bacteria. The effect produced would thus have been an indirect one.

A review of the data reveals that in a few experiments that were carried beyond 48 hours in the same medium, the per cent of

increase of the paramecia dropped off. In no case was there an indication that the culture was dying off or that the animals were lacking in vitality. Nevertheless, this decrease in the division rate indicated that the ideal environment for optimum multiplication was not being achieved in the experiment.

SUMMARY

Research was conducted for the purpose of studying the effects of desiccated thyroid compound on the rate of reproduction of the protozoan, Paramecium caudatum. This study was performed in an effort to understand the action of thyroid on the division rate of Paramecium and to find what possible influence this growth might have on subsequent investigations on the respiration of the animal. The results of this study were as follows:

1. With an uncontrolled bacterial food supply, members of a culture of Paramecium caudatum, to which desiccated thyroid had been added in the medium, divided at a significantly higher rate than did identical animals used as a control.

2. A group of five paramecia increased in number by 19.2 per cent during the first 24 hours and by 220 per cent at the end of 48 hours.

3. Five paramecia which had been given thyroid showed increases of 92.4 and 794 per cent during the 24 and 48 hour periods, respectively.

4. Observation on Paramecium growth in medium isolated from the original culture indicated the bacterial food supply after 72 hours was being exhausted as was evidenced by a lowering rate of reproduction.

5. Bacterial counts on the culture medium showed that desiccated thyroid increased the number of bacteria 210-fold

during the first 24 hours and caused a 112-fold difference after 48 hours.

6. Rapid fission of the thyroid-fed individuals was accompanied by increased activity, greater transparency, and slightly smaller size, indicating that important internal modifications occur in the physiology of the cell.

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PART II. THE EFFECT OF DESICCATED THYROID ON THE RESPIRATION OF PARAMECIUM CAUDATUM

INTRODUCTION

Respiration is one of the essential characteristics of all living matter. It is the source of energy that every cell must have in order to carry on its complex functions, and according to Novy (1932) "may deal with the physiochemical conditions within and without the cell which underlie all functional manifestations."

There are two meanings of the word, "respiration", currently in use. The older meaning confines the term to the actual uptake of gaseous oxygen. It was later realized that oxidation may occur without employing gaseous oxygen, such as by the removal of hydrogen. The meaning of the term respiration was then broadened to include any reaction by which the cell obtained energy, whether or not it involved oxygen in its gaseous state as such. This has resulted in some confusion since the definition of the term differs with various investigators. For the purpose of this paper, however, respiration has been considered to be the uptake of gaseous oxygen.

In the case of aerobic organisms such as Paramecium, cell metabolism is evidenced by the consumption of oxygen and the elimination of carbon dioxide. The rate and intensity of this gaseous exchange varies with the composition of the medium and the kind of organism.

Although cells of Protozoa represent an excellent subject for physiological research, comparatively little work has been done on their respiration. What studies have been made on protozoan metabolism have been of a qualitative nature so that only in a few cases are there data from which the oxygen consumption of a single cell can be calculated.

Respiratory studies of Paramecium do not resemble a unified body of knowledge, but rather an accumulation of a number of more or less isolated data. This situation can be attributed to the fact that methods for measuring minute rates of gas metabolism have not, in the past, been as accurate as such investigations warrant. However, with gradual technical advances it appears likely that in the near future the development of an organized account of protozoan respiration will become a reality.

As indicated in the preceding part of this paper, extensive work has been done with thyroid on the variable effects concerning growth and division rate of Paramecium. It is also true that several publications have appeared, especially in the past decade, on respiration of the ciliates. However, after a careful search of the literature, no publications have been found dealing with the influence of thyroid substance on the respiration of Paramecium. For this reason it seemed desirable to determine if desiccated thyroid has an influence on the respiration and metabolism of Paramecium.

REVIEW OF LITERATURE

The first interest given to the respiratory exchange of the Protozoa was by Vernon (1895) when he worked on the radiolarian, Collozoum inerme. Since that time studies have been made on the gas metabolism of almost all the major groups of unicellular animals. The development of protozoological technique, however, has lagged far behind the very rapid improvement in biochemical and physiological procedure during the last half-century with the result that much of the early data which has accumulated are difficult and often impossible to evaluate.

A review of Jahn's summary (1941) of the respiratory data for different species of Protozoa reveals that no definite conclusions can be drawn from the widely varying results obtained for Paramecium caudatum.

A comparison of the findings of earlier workers is difficult because of the variety of conditions under which previous measurements have been carried out. Table 1 is a summary of the results obtained by other investigators on the respiration of Paramecium caudatum where comparable procedures were employed and where the data could be expressed in a standard way, such as the volume of oxygen in cubic millimeters consumed by one cell in one hour.

Earlier experiments on this problem were conducted on material from mixed cultures of Paramecium in media of uncertain composition. Numerous difficulties are encountered in attempts to measure the respiration of organisms in mixed suspensions since

Table 1. A summary of the results of previous investigators on the respiration of Paramecium caudatum.

Investigator	Temp.	Q_{O_2}	Method	Remarks
Lund, 1918b	21.0	.00014	Winkler	Fed
"	"	.00004	"	Starving
Zweibaum, 1921	21.0	.00074	Winterstein	Before conjugation
"	"	.00348	"	During conjugation
"	"	.00068	"	Just after conjugation
"	"	.00214	"	One week after conjugation
Necheles, 1924	19.0	.00385	Warburg	Single cells
Kalmus, 1927	21.0	.00520	Capillary	Single cells
Kalmus, 1928	17.0	.00460	Capillary	Single cells
"	13.0	.00330	"	"
Howland, 1931	20.0	.00049	Capillary	Single cells
Wood, 1938	23.0	.03500	Winterstein	
Cunningham, 1942	--	.00370	Capillary	
Pace, 1944	25.0	.00386	Barcroft-Warburg	
Pace, 1945	25.0	.00347	Barcroft-Warburg	15-19 day old paramecia
"	"	.00443	"	5 day old paramecia
Clark, 1945	25.0	.00066	Cartesian Diver	25-80 animals used. pH 6.8

the metabolism of bacteria, or other foreign life present, will alter the results to an indeterminate extent.

Bacteria-free cultures of Protozoa were first employed in measurements of respiration by Soule (1925) in his work on the parasite, Trypanosoma lewisi. Since that time more and more attention has been given to the role contamination plays in studies on respiration.

In an effort to illustrate the fact that some bacteria have a respiratory rate per gram many times that of other types of cells, Burk (1937) stated that the rate for Azotobacter was equivalent to that of a 200 pound man consuming one ton of glucose per hour.

Boell (1942) in his work on the effect of potassium cyanide and sodium azide on the oxygen consumption of Paramecium calkinsi, concluded that the respiratory mechanism of Paramecium resembles that of most plant and animal cells.

Other investigators in the field of microrespiration have shown that various factors seem to influence the physiological state of the organism and that these effects manifest themselves in fluctuations in the amount of oxygen consumption. Problems which have been investigated for Paramecium include concentration of the organisms, starvation, temperature, conjugation, age of the culture, concentration of oxygen, area of cell surface, and hydrogen ion concentration.

A few attempts have been made in an effort to relate the amount of respiration of the protozoan cell to the concentrations

of the cells employed in the experiments. Working on the protozoan, Tetrahymena geleii, Pace and Lyman (1947) found a direct relation between the concentration of the organisms involved in each study and oxygen consumption.

Lund (1918b) starved paramecia by taking them out of their laboratory medium and putting them in tap water. He placed similar organisms in a medium of tap water and yeast and noted an appreciable decrease in the respiration of the starving animals along with the disappearance of deutoplasmic food reserves from the protoplasm. The yeast-fed paramecia consumed three fold as much oxygen as the starved specimens, an increase that was independent of cell division. He also noted a difference in size between the two groups with the well-fed organisms much larger than the starved ones.

Leichsenring (1925) experimented with the effect of varying temperatures on the respiration of Paramecium. When transferred from a temperature of 20 degrees centigrade to one of 35 degrees, the ciliates showed an increase in respiration of 35 per cent. By dropping the temperature to 15 degrees, 10 degrees, 5 degrees, and 0 degrees centigrade, respiration was decreased 30 per cent, 34 per cent, 50 per cent, and 58 per cent, respectively.

Pace and Belda (1944) tried to find a relation between the cell surface of the amoeba, Pelomyxa carolinensis, and oxygen consumption. Such experiments are very delicate and subject to criticism due to the large number of variables that must be overcome. They found, however, that the rate of respiration was

closely correlated with the amount of food material present in the cytoplasm.

Pace and Lyman (1947) found that ciliates from old cultures used much less oxygen than did those from comparatively young cultures. When young organisms were employed, the oxygen consumption per unit volume of cell substance proved to be inversely proportional to population density.

MATERIAL AND METHODS

The experiments which form the subject of this paper cover a period of approximately seven months. Data on respiration was obtained directly by use of a "Warburg" microrespirometer (Plate I). The essential principle involved in this apparatus is that at constant temperature and constant gas volume any changes in the amount of a gas can be measured by changes in its pressure (Umbreit, et al. 1949). In this case the method applied to measurements of oxygen uptake which is directly indicative of the rate of respiration and metabolism of the paramecia.

Several procedures have been employed for the study of the gas exchange of respiration of microorganisms. This particular type of respirometer is essentially a microchemical one and is of comparatively recent origin. It has been used to determine the respiration of living tissues as well as that of unicellular organisms such as yeast, bacteria, and Protozoa.

Technique Employed in the Operation of the Respirometer

The Warburg instrument is suitable for two general types of use: (1) the determination of the rate of oxygen uptake, and (2) the determination of the amount of oxygen uptake. Both are usually measurable in the same determination. The rate of oxygen consumption is usually expressed in studies on respiration in terms of a quotient (Q_{O_2}) which specifies the conditions under which the rate was measured. In this study, therefore, the Q_{O_2} (or oxygen quotient per cell) equals the cubic millimeters of oxygen taken up per cell in one hour.

The Warburg apparatus (Plate II) consisted of a detachable respiratory flask, equipped with a sidearm, attached to a manometer containing a liquid of known density. The fluid used in the manometer was "Brodie's Solution" which had a density of 1.081.

The flask was immersed in a water bath at a constant temperature (25° C.) where it was shaken during the course of the experiment to promote a rapid gas exchange between the liquid being used in the flask and the gas phase above it.

The manometer consisted of two arms graduated from 1 to 300 millimeters. One was open to the atmosphere and the other closed. The closed arm was attached to the respiratory flask and also to a stopcock which was in turn closed during the course of each experiment.

The level of the liquid in the manometer was adjusted by means of a screw clamp on the fluid reservoir located at the

bottom of the arms. A "zero" point on the closed side of the manometer was chosen at 150 mm and the Brodie's Solution was always adjusted to this point with the stopcock open before recording pressure changes. The stopcock was left open for 15 minutes just before the beginning of each experiment to enable the temperature inside the respiration flask to become equal to that of the water bath and also to allow the carbon dioxide in the air already present in the gas phase to be absorbed. The experimental run began upon closure of the stopcock, after which the change in volume in the manometer was recorded every hour.

When a reading was desired the liquid in the closed arm of the manometer was adjusted again to the zero point, thus holding the volume of gas in the flask constant. The reading was then taken from the column of fluid in the open arm. A minus reading; i.e., the column of liquid had dropped below the zero mark, indicated that oxygen had been taken up by the flask. An infrequent zero or plus reading was indicative of the fact that either there had been no oxygen consumption taking place or that faulty technique had been employed in the preparation of the experimental run.

Theory of the Apparatus

If the gas volume of the flask, the volume of fluid in the flask, the temperature of operation, the gases being exchanged, and the density of the fluid in the manometer are all known, it

is possible to calculate the amount of gas consumed (or given off) providing only one gas is being exchanged. The essence of this method was to hold the gas and fluid volumes constant and to measure the decrease or increase in pressure when one gas altered in amount. Fundamentally this consisted of so calibrating the system that from the observed pressure changes it was possible to calculate the amount (in mm^3 at 0° C. and 760 mm pressure) of gas utilized or given off.

The usual practice in regard to respiratory work is to consider all quantities of gas evolved by a reaction as positive or negative (Dixon, 1943). Thus if oxygen is consumed by the paramecia it is "absorbed" and the volume of gas taken up is expressed as a negative number.

To arrive at the amount of respiration that is carried on, a formula must be worked out in order to find the quantity of oxygen consumed.

$$x = h \left[\frac{Vg \frac{273}{T} + Vf\ A}{Po} \right]$$

x = amount of gas exchanged in mm^3 at 0° C. and 760 mm pressure.

h = alteration in reading on open arm of manometer in mm.

Vg = volume of gas space in flask including connecting tube and down to zero point in closed arm of manometer in mm^3.

Vf = volume of all fluids in flask in mm^3.

T = temperature of water bath in absolute degrees.

Po = 760 mm of mercury (standard pressure) expressed in terms of manometer fluid. (Po = specific gravity of mercury divided by density of manometer fluid).

A = solubility of oxygen in the liquid in the flask, expressed in mm^3 of gas dissolved in one mm^3 of liquid when the gas is at 760 mm pressure and temperature T.

It may be noted that the expression in square brackets remains constant for a given gas with any given manometer and flask, provided the volume of liquid and the temperature remain the same. This quantity is known as the flask constant "k". Knowing the value of "k", the reading on the manometer scale is multiplied by it and the amount of gas exchanged in mm^3 of dry gas under standard conditions of pressure and temperature is obtained. Thus the formula becomes simplified to $x = h\,k$.

According to Dixon (1943), the preceding equation "is equally valid if a second gas is present in the vessel in addition to the reacting gas." For instance, if air is present in the flask instead of pure oxygen (as was the case in these experiments) the amount of nitrogen in the gas phase remains constant throughout and, therefore, cancels out from the equation. It is the pressure of the reacting gas (oxygen) which changes and it is altered by the amount "h" as before. Thus the nitrogen present does not affect the value of the flask constant.

Calibration of Flasks and Manometers

In order that the quantities of gases exchanged during an experiment be calculated, it was necessary to calibrate the volume of each flask and each manometer separately. Although the different flasks appeared identical, they were of slightly different size. Therefore, their respective volumes had to be measured accurately in order to provide reliable results of the experiments. This was accomplished by means of the mercury method described by Dixon (1943) with the use of the technique employed by Loomis (1949).

This method consisted of filling each flask and its corresponding manometer with clean mercury, so that the mercury completely filled the respiratory flask and extended down the manometer arm to the zero point on the scale. The mercury was then emptied out and weighed, and its weight divided by its density in order to find the gas volume of the flask and manometer. This volume was then used to calculate a conversion factor for each flask and its corresponding manometer. With the use of this factor all the flasks could be evaluated equally under standard conditions of temperature and pressure.

Principles Used in Respiration Determinations

In order that the uptake of oxygen be measured correctly the carbon dioxide in the gas phase must remain zero. This was accom-

plished by absorbing the carbon dioxide continuously in alkali during the course of the determination. The reaction flask was designed with a small center cup projecting up approximately two centimeters from the bottom of the flask. To this cup was added two-tenths milliliters of ten per cent potassium hydroxide. A small piece of number 50 Whatman starch-free filter paper folded in accordian-like fashion was placed in the cup and allowed to extend above the center cup a few millimeters. This increased the surface area of the alkali sufficiently to insure prompt absorption of the carbon dioxide given off during the respiratory process.

There were other factors involved with the use of the Warburg instrument that had to be controlled carefully in order that the gaseous exchange of oxygen and carbon dioxide could be accurately determined. One of these was that the temperature be held at the point desired and second and more important, that the temperature of the water bath be held uniform within 0.05° C. This latter factor necessitated vigorous stirring of the water in the bath. Due to the very small amounts of gas employed, a one degree change in temperature between two flasks would result in a large per cent of error. The Warburg machine was equipped with an electrically controlled thermostat which was capable of regulating the temperature of the bath within 0.01° C. of the desired temperature. The water was constantly circulated by a stirring motor mounted on top of the apparatus. Another factor to be considered was that of atmospheric pressure. Since it was

impossible to keep the pressure of the room constant, this variable was overcome by means of a barometer. This consisted merely of a Warburg manometer with a flask containing sterile distilled water in equal amount to that of the other flasks employed. Assuming the atmospheric pressure was the same for all the flasks being used, the changes in the gas phase of the flasks due to external pressure was corrected by the reading on the barometer. By adding or subtracting the difference in pressure recorded by the barometer, depending on whether the atmospheric pressure had risen or fallen, it was possible to measure accurately the internal pressure changes in the gas phase of the reaction flasks.

The absorption of oxygen by the respiring organisms took place almost entirely from the oxygen in solution. This was the principle reason for shaking the fluids in the microrespirometer, i.e., to obtain a fluid phase saturated with the gas phase. To fulfill these conditions the apparatus was set to give 100 shakings per minute with a stroke of five centimeters. The small amount of oxygen that was absorbed by the fluid was accounted for by a known physical constant which was employed in figuring the conversion factor of each flask.

In the present investigation the rate of oxygen consumption by Paramecium caudatum is stated in terms of the number of cubic millimeters of oxygen, reduced to 0° C. and 760 mm pressure, taken up by a single cell in one hour. Experiments were not conducted on single cells individually as the element of error would prove too great, but were made on a large number of animals and

the rate for single cells was computed. When results are expressed in this manner, the precision with which the number of organisms in each suspension is estimated largely determines the degree of accuracy obtained.

Counting of Paramecia

At the beginning of each experiment a numerical count was made on the culture. The following procedure has been found satisfactory. A uniform distribution of paramecia was assured by vigorously swirling the Erlenmeyer flask containing the organisms. By means of a calibrated one milliliter pipette, a sample was withdrawn from the flask. The technique applied here was to insert the pipette to the bottom of the flask and gradually raise it up the side while the liquid was being withdrawn. The first sample from each pipette was discarded as it served merely to wet it with the suspension. This procedure helped to correct for the tendency of the cells to stick to the sides of the pipette (Hall, 1938). By the same means a milliliter was withdrawn from each culture flask and placed into correspondingly numbered bacterial culture dishes and the fluid allowed to run around the periphery of each dish. Sonneborn (1950) stressed the need for handling the pipette very rapidly in such instances. This is due to the fact that the animals rapidly aggregate near air surfaces and swim against water currents; hence, they tend to collect quickly at the top or bottom of a measuring pipette causing uneven con-

centrations per unit volume. Approximately three-tenths of one milliliter of a weak Lugol's Solution (one gram iodine and two grams potassium iodide per liter of water) was added to the sample, killing the organisms and staining them brownish-red for counting.

This particular method was unique in that it enabled fast, accurate counting of the culture samples. The number of ciliates in each plate was counted under a binocular dissecting microscope, employing the 0.7 X objective and 10 X oculars. The results of several counts were averaged and multiplied by two in order to obtain the approximate number of cells that had previously been placed in the microrespirometer.

At the conclusion of each experiment counts were made directly on the media taken out of the respiratory flasks. By means of interpolation a reliable estimate was made on the number of paramecia in each flask for each hour the experiment had been conducted.

Preparation of Sterile Cultures

The paramecia employed in this study were all "sister" paramecia in the sense that they were originally derived from a single individual. The animal from which this clone was started was from a culture of Paramecium caudatum which had been growing in the Department of Zoology for over five years. No attempt was made to culture them aseptically in cultures of known bacterial

composition.

It was found possible to get the ciliates free of all adhering bacteria by washing them in sterile water. This procedure was carried out in sterile distilled water in 12 cm uncontaminated centrifuge tubes. After each washing the animals were centrifuged down at slow speed and the contaminated water drawn off. The paramecia were then transferred to another sterile tube and the process repeated.

Madhok and Fazal-Ud-Din (1947) proposed the theory that protozoans may be isolated in bacteria-free cultures using the principle that they move much faster through a medium than do bacteria. This was the principle employed in obtaining sterile cultures for this study. By centrifuging the animals a number of times at slow speed, so as not to injure them, the bacteria were washed off leaving the paramecia uncontaminated.

Hargitt and Fray (1917) found three washings to be sufficient, but for these studies five washings were used in the preparation of each sterile run. A bacterial examination of the wash waters showed no bacteria after the fifth washing. The paramecia so sterilized apparently were not injured in any way and seemed to suffer no loss of vigor.

Since it was desirable to keep the ciliates viable for over 48 hours, they were immediately transferred to fresh medium which had previously been autoclaved and allowed to cool to room temperature. The animals remained vigorous for only a few hours when left in the sterile state containing no food particles, but

were active for over two days in medium containing dead micro-organisms on which they could feed.

All media employed in this study were buffered to a pH of 6.8 with potassium mono basic phosphate and sodium hydroxide (KH_2PO_4·NaOH) in order that the hydrogen ion concentration would be the same in both the control and thyroid media.

EXPERIMENTAL RESULTS

The aim of the experiments on respiration was to compare the metabolism of Paramecium caudatum in the normal control medium with identical animals from the same clone which had been subjected to a medium containing thyroid 24 hours previously. Numerical counts were made on the media both before and after the respiratory runs in the Warburg apparatus. By this method it was possible to determine accurately the number of ciliates present in each experiment and thus calculate the amount of oxygen consumed by each individual.

Fifty milliliters of culture medium was added to each of two 125 ml Erlenmeyer flasks. To one of these was added 50 milligrams of desiccated thyroid gland (Parke, Davis and Co. U. S. P.) which was the equivalent of two-tenths milligrams of pure thyroxin. The untreated culture was then used as a control to compare with the culture treated with thyroid substance. Experimental runs were started at 24 and 48 hour periods following introduction to the thyroid. The primary purpose was to keep all

influencing factors equal between the two samples, except that one had been administered thyroid and the other had not.

Experiments on Paramecium in Contaminated Medium

The earlier runs were not made on sterile cultures, but the possible error induced by bacteria was accounted for by running extra manometers which contained a like amount of culture medium (2 ml) from which the ciliates had been centrifuged out. By subtracting the reading of the medium alone from that of the medium containing paramecia, it was possible to calculate the respiration of the protozoans alone.

A large number of measurements, 34 series of experiments with six to eight manometers each (altogether 256 single determinations), showed that one Paramecium required, on the average, .00314 mm^3 oxygen per hour, whereas one Paramecium which had been administered desiccated thyroid 24 hours previously averaged .00809 mm^3 oxygen per hour at 25.0° C. and pH 6.8. The data from which these results were taken are tabulated in Table 2.

These experiments were conducted 24 hours later in the same manner to find the rate of respiration of the animals after they had been fed the thyroid preparation for 48 hours. The Q_{O_2} of the control specimens was then .00361 and that of the thyroid-fed individuals was .00921. This was an increase of 8 per cent over the preceding day for the control paramecia and 12 per cent for the organisms suspended in a thyroid medium.

Table 2. The effect of desiccated thyroid on the respiration of Paramecium caudatum in non-sterile media at 25° C. and pH 6.8 when expressed in mm^3 oxygen consumed by one Paramecium in one hour (Q_{O_2}).

Date in 1951	Number of hours on thyroid	Control paramecia			Thyroid-fed paramecia		
		No. of paramecia used	mm^3 O_2 used in one hour	Q_{O_2} (mean of 3 to 4 manometers)	No. of paramecia used	mm^3 O_2 used in one hour	Q_{O_2} (mean of 3 to 4 manometers)
7-10	24	240	1.0	.00417	660	7.0	.01060
7-11	48	320	1.5	.00470	1,660	23.0	.01422
7-19	24	351	2.0	.00570	786	7.5	.00956
7-20	48	550	3.5	.00627	1,850	24.0	.01243
9-18	24	397	1.5	.00379	667	6.5	.00974
9-19	48	525	2.5	.00476	1,222	13.0	.01146
9-21	24	353	1.0	.00283	390	2.5	.00641
9-22	48	467	1.5	.00309	723	5.5	.00761
9-23	24	267	1.0	.00371	418	4.0	.00957
9-24	48	368	1.5	.00408	595	6.0	.01008
9-25	24	316	0.5	.00159	330	1.0	.00303
9-26	48	366	1.0	.00273	978	4.5	.00457
9-27	24	450	1.5	.00333	702	6.0	.00855
9-28	48	620	2.5	.00403	991	10.0	.00999
10-3	24	980	3.0	.00306	1,300	10.0	.00769
10-4	48	1,522	5.5	.00361	1,844	15.0	.00813
10-5	24	1,203	4.0	.00333	1,566	11.0	.00702
10-6	48	1,841	6.5	.00353	2,120	17.0	.00802
10-9	24	934	3.0	.00309	1,219	8.5	.00753
10-10	48	1,093	4.0	.00366	1,661	14.0	.00843
10-11	24	1,335	3.0	.00225	2,077	15.0	.00722
10-12	48	1,672	4.0	.00240	2,682	21.5	.00803
10-13	24	1,440	3.0	.00208	2,050	14.5	.00707
10-14	48	1,960	6.0	.00306	2,579	19.5	.00756
10-16	24	3,050	8.0	.00262	5,668	48.5	.00856
10-17	48	3,250	9.5	.00292	5,980	60.0	.01004
10-18	24	1,630	5.0	.00307	3,620	31.0	.00857
10-19	48	2,550	8.0	.00314	4,080	42.0	.01030
10-22	24	3,210	8.5	.00265	3,350	32.5	.00970
10-23	48	3,850	8.0	.00209	4,970	45.0	.00986
10-24	24	1,930	6.0	.00311	2,150	30.0	.00979
10-25	48	2,570	8.0	.00311	3,610	32.0	.00887
10-30	24	2,245	6.5	.00290	2,740	40.5	.00868
10-31	48	2,350	7.0	.00300	4,289	37.5	.00873
Average	24			.00314			.00809
Average	48			.00361			.00921

In spite of all known precautions, it proved impossible to exclude fluctuations in the experimental results. Small variations occurred even when identical material was used. These could be attributed to faulty counts of the animals (which correlated within three per cent) or mechanical error in reading the manometers (which could be read accurately only to 0.5 mm^3). The much greater differences that appeared in the data from experiments on different cultures may have been caused by the varying physiological state of the organisms. This was the conclusion made by Reich (1948) on similar results in his work on amoebae.

Experiments on *Paramecium* in Sterile Medium

Following the experiments on non-sterile cultures, runs were made on paramecia in uncontaminated media with the use of sterile technique. The result of 32 different trials, using six to eight manometers each (altogether 248 single determinations), showed that one *Paramecium*, 24 hours after introduction to the sterile medium, consumed .00265 mm^3 of oxygen per hour at 25.0° C. and a pH of 6.8. The average results of identical animals which had been given desiccated thyroid substance in their medium 24 hours previously, showed the Q_{O_2} of one animal was .00673 under the same conditions (Table 3).

Experiments were made on the organisms one day later in an effort to find what effect the thyroid compound would have on the rate of respiration after a period of 48 hours. The same para-

Table 3. The effect of desiccated thyroid on the respiration of *Paramecium caudatum* in sterile media at 25.0° C. and pH 6.8 when expressed in mm^3 oxygen consumed by one *Paramecium* in one hour (Q_{O_2}).

Date in 1951	Number of hours on thyroid	Control paramecia			Thyroid-fed paramecia		
		No. of paramecia used	mm^3 O_2 used in one hour	Q_{O_2} (mean of 3 to 4 manometers)	No. of paramecia used	mm^3 O_2 used in one hour	Q_{O_2} (mean of 3 to 4 manometers)
11-6	24	1,505	4.0	.00266	1,803	8.5	.00638
11-7	48	1,244	3.0	.00241	1,312	7.5	.00572
11-8	24	1,822	4.5	.00247	2,166	14.5	.00669
11-9	48	1,462	3.0	.00202	1,781	11.0	.00618
11-12	24	1,560	4.5	.00288	1,650	11.5	.00630
11-13	48	1,131	2.5	.00221	1,822	11.0	.00615
11-16	24	930	2.0	.00275	1,406	9.5	.00676
11-17	48	740	1.5	.00203	1,100	7.0	.00627
11-18	24	860	3.0	.00349	1,020	7.5	.00735
11-19	48	615	1.5	.00244	847	5.5	.00648
11-20	24	833	1.5	.00180	986	5.0	.00507
11-21	48	597	1.0	.00167	754	3.5	.00464
11-22	24	666	1.5	.00225	925	6.0	.00681
11-23	48	418	1.0	.00240	660	3.5	.00530
11-26	24	376	1.0	.00266	783	5.5	.00702
11-27	48	261	0.5	.00192	761	5.0	.00650
11-28	24	353	1.0	.00283	710	5.0	.00775
11-29	48	240	0.5	.00208	591	3.5	.00610
12-1	24	380	1.0	.00264	780	5.0	.00641
12-2	48	211	0.5	.00237	645	4.0	.00622
12-4	24	836	2.0	.00240	832	5.5	.00661
12-5	48	731	1.5	.00202	741	4.5	.00607
12-6	24	803	2.5	.00311	1,066	7.5	.00702
12-7	48	667	1.5	.00225	972	5.5	.00565
12-8	24	462	2.0	.00262	930	6.0	.00645
12-9	48	278	0.5	.00181	758	5.0	.00660
12-10	24	660	2.0	.00303	1,085	7.5	.00699
12-11	48	407	1.0	.00244	850	5.5	.00650
12-13	24	616	1.5	.00243	1,710	12.5	.00731
12-14	48	487	1.0	.00202	1,320	8.0	.00603
12-18	24	613	1.5	.00245	1,462	9.0	.00606
12-19	48	522	0.5	.00192	1,004	6.0	.00598
Average	24			.00265			.00673
Average	48			.00222			.00610

mecia employed during the 24 hour studies were not used this time, of course, as they had been killed in staining them to make counts on the culture. Ciliates from the original culture which had been in the sterile medium for 48 hours were used, however. Identical conditions were employed in these experiments in order that the results could be correlated with those of the preceding day. The average of 120 single determinations showed the Q_{O_2} of a control specimen was .00222 while the Q_{O_2} of one thyroid-fed Paramecium was .00610. This was a decrease from the 24 hour results of 12 per cent for the control animals and a drop of 11 per cent for the thyroid individuals.

It had been expected that the data for the 48 hour trials would show an increase in the Q_{O_2} over the preceding day, but this proved not to be the case. Not only was the oxygen quotient per cell less than the preceding day, but the number of paramecia per milliliter of medium had decreased. This was inversely proportional to the findings obtained on the non-sterile cultures. However, the thyroid individuals continued to show a marked increase in the rate of respiration over the control organisms, being 275 per cent higher than the corresponding organisms which had not had the thyroid.

Hutchens (1941) found that the flagellate, Chilomonas paramecium, grew rapidly in sterile cultures, reaching its maximum population in 48 hours. These animals then declined to the extent that none were viable after the third day and all were dead by the fifth day.

This was undoubtedly the reason for the reduced respiration during the second day on the sterile medium. Since the medium in which the paramecia were suspended was void of all bacterial life, the protozoans quickly consumed the amount of dead organic material present, thus exhausting their previous source of food supply. Under these conditions they decreased in population and viability until the culture died out.

Cell division appeared to taper off or stop altogether after the animals had been deprived of all bacteria in the transfer to sterile media. Lund (1918a) noticed this same effect and attributed it to the fact that the animals were under starvation conditions and were maintaining themselves on the "food reserve of the protoplasm."

Other Findings

Presence of Ammonia. Tests made with Nessler's reagent (a solution containing sodium hydroxide, mercuric iodide, and potassium iodide) indicated that there was a minute trace of ammonia present in cultures feeding on thyroid and a more appreciable amount in the untreated control cultures. These tests did not prove whether or not the paramecia produced the ammonia; however, they did show that the greater the concentration of organisms present, the greater was the amount of ammonia produced.

Specht (1934) found that the excretion of ammonia was augmented by the lack of oxygen and minimized by an abundance of it.

He concluded that the ammonia present in cultures manifests itself in a culture of microorganisms as a positive pressure and decreases the negative pressure due to oxygen consumption.

In testing culture media for the presence of ammonia it was noted that lesser amounts showed up in the medium to which thyroid compound had been added. A possible explanation of this fact is that the amino acids present in the thyroid molecule helped to counteract the presence of the free ammonia in those solutions.

In order to avoid any possible error caused by the presence of ammonia which might be produced by the respiration of Paramecium, 0.2 ml of a 0.3 Molar sulfuric acid solution was added to the side bulb of the respiratory flask. In the presence of H_2SO_4 this substance was apparently absorbed, as no precipitate was formed with Nessler's reagent on those cultures treated with the acid. Theoretically the values obtained from oxygen consumption in such instances should be increased. An examination of Table 4 reveals, however, that such changes were minute and were within the range of experimental error. This effect might have been more noticeable if a heavy concentration of cells (e.g., 100,000 paramecia per milliliter) had been employed.

That such results (Table 4) were due to the direct effects of the H_2SO_4 on the animals or on the media is highly improbable, since the acid was separated from the fluid medium in each case and was in contact only with the gaseous medium above it. The distillation of H_2SO_4 from 0.3 Molar solutions is negligible at

Table 4. The inhibiting effect of free ammonia on the rate of respiration of Paramecium caudatum in sterile control and thyroid cultures at 25° C. and pH 6.8 using sulfuric acid to absorb the free ammonia given off.

Date in 1951	Number of hours on thyroid	Cubic millimeters of oxygen consumed by one Paramecium in one hour			
		Control		On thyroid	
		No H_2SO_4	H_2SO_4	No H_2SO_4	H_2SO_4
11-6	24	.00266	.00270	.00638	.00702
11-7	48	.00241	.00233	.00572	.00640
11-8	24	.00247	.00246	.00669	.00652
11-9	48	.00202	.00196	.00618	.00606
11-12	24	.00288	.00302	.00630	.00663
11-13	48	.00221	.00237	.00615	.00623
11-16	24	.00275	.00282	.00676	.00687
11-17	48	.00203	.00226	.00627	.00631
Average	24	.00264	.00275	.00653	.00676
Average	48	.00217	.00223	.00608	.00625

25.0° C. and the form and behavior of the organisms when kept for long periods under these conditions appeared normal. However, more research needs to be done on this phase of the problem.

Influence of pH on the Rate of Respiration. In conducting experiments on Paramecium respiration it was noted that the control medium which had not been buffered was always slightly alkaline, both before and after the experimental run. The thyroid

medium, on the other hand, was almost invariably neutral or slightly acidic in its action. For this reason a few experiments were conducted on media that had not been buffered in an effort to find what effect small differences in pH had on the oxygen consumption of Paramecium caudatum. Six series were made in this study, employing eight manometers each. Four of these manometers contained ordinary culture media which exhibited a pH of approximately 7.2 when tested with bromothymol-blue indicator. The other four manometers contained a like amount of media except that it had been buffered to a pH of 6.8.

The results of the 48 single determinations (Table 5) bore out the conclusions of Issekutz and Issekutz (1944) that Paramecium is not highly sensitive to changes in hydrogen ion concentration, as the two samples failed to show any appreciable difference in oxygen uptake. The slight difference noted in the two oxygen quotients was of no real value as it was well within the limits of mechanical error in conducting the experiments. The small traces of ammonia given off by the paramecia were no doubt largely responsible for the slight alkalinity of the media. The neutral or slightly acidic action of the thyroid medium might have been due to the chemical effect of the amino acids in the thyroid molecule.

Effect of Cell Concentrations on Rate of Respiration. The experiments on Paramecium presented in this paper failed to substantiate the findings of Pace and Lyman (1947) in Tetrahymena geleii that there is a direct relation between the concentration

Table 5. The effect of the hydrogen ion concentration on the respiration of Paramecium caudatum in sterile control and thyroid cultures at 25.0° C.

Date in 1951	Number of hours on thyroid	Cubic millimeters of oxygen consumed by one Paramecium in one hour			
		Control		On thyroid	
		pH 6.8	pH 7.2	pH 6.8	pH 7.2
12-10	24	.00303	.00288	.00699	.00675
12-11	48	.00244	.00234	.00650	.00648
12-13	24	.00243	.00221	.00731	.00687
12-14	48	.00202	.00196	.00603	.00609
12-18	24	.00245	.00232	.00606	.00585
12-19	48	.00192	.00200	.00598	.00614
Average	24	.00264	.00247	.00679	.00650
Average	48	.00215	.00210	.00617	.00624

of cells employed and oxygen consumption. In the present study there was no appreciable difference noted in the rate of oxygen uptake when a large number of paramecia were used than when there was only a relatively small number employed.

Although it is quite probable that an average result derived from the total amount of a large number of cells would be more accurate than the respiratory measurement of a single cell, it appears unlikely that such a correlation exists. This seems especially evident when the results obtained by other investigators with various types of cells are taken into consideration. It is

also likely that the most accurate data would come from suspensions containing a higher concentration of ciliates, as the possible error involved would be spread out over a larger number of individuals. This would cause a negligible effect on the Q_{O_2} of a single Paramecium.

It has been suggested by Reich (1948), that an equilibrium between the gas phase and the liquid phase was not achieved by Pace and Lyman. Inadequate shaking of the manometers during the operation of their experiments might have been responsible.

Effect of Thyroid on Staining of Paramecium. It was noted during the counting of the paramecia, after they had been stained with Lugol's (iodine) solution, that the animals given thyroid substance stained a very dark reddish-brown color. The ciliates which had not been given desiccated thyroid stained only a light brown color. The reason for the difference in intensity of the stain is not known.

Experiments on Paramecium Using Different Controls. The data presented in this paper seem to justify the fact that desiccated thyroid gland produces internal modifications of the paramecia resulting in an increase in metabolic rate. However, a complete understanding of this phenomenon is not present at this time. Woodruff and Swingle (1924) proposed the theory that such cases were due merely to the factor of food supply and stated that "thyroid and other glandular material supply more food, and accordingly, for significant results, this factor must be taken

into account in devising the controls."

In an effort to find the primary cause of the increased rate of respiration exhibited by the thyroid-fed paramecia, experiments were run with controls in which paramecia were suspended in a sterile medium containing dead bacterial yeast cells. It was believed that if the element of food supply was the important factor in increased respiration, the control organisms fed on yeast would respire at approximately the same rate as the ciliates fed on thyroid. Inspection of Table 6, however, shows the yeast-fed paramecia had only a slightly higher Q_{O_2} than did the control animals which were maintaining themselves on the dead bacteria in the previously autoclaved medium.

Respiratory runs were then made on paramecia suspended in sterile media containing the free amino acids, tyrosine and glycine. Tyrosine is one of the amino acids of the thyroid molecule and it was thought that it might possibly have some effect on the metabolic activity of the Paramecium cell, and if so, might help illustrate the reason for the large increase in oxygen consumption of the paramecia suspended in a thyroid medium. Glycine was employed in order to establish whether the possible increase in oxygen uptake of the tyrosine-fed organisms was due to the direct effect of the tyrosine on the animals or merely to the nitrogen-supplying effect it would have on the medium. But it was found, as illustrated in Table 7, that both tyrosine and glycine had, if anything, an inhibitory effect on the respiration of Paramecium. Kidder and Dewey (1945) found that glycine was essential for nor-

Table 6. A comparison of the effect of dead bacterial yeast on the rate of respiration of Paramecium caudatum in sterile cultures with that of desiccated thyroid and the control animals at 25.0° C. and pH 6.8.

Date	Number of hours in media	Types of culture media used	Number of paramecia used	mm^3 O_2 used in one hour	Q_{O_2} (mm^3 O_2 per hour per cell)
12-4-51	24	control	836	2.0	.00239
12-5-51	48	"	731	1.5	.00205
12-4-51	24	yeast	861	2.5	.00290
12-5-51	48	"	841	2.0	.00251
12-4-51	24	thyroid	832	5.5	.00660
12-5-51	48	"	741	4.5	.00607
12-6-51	24	control	803	2.5	.00311
12-7-51	48	"	667	1.5	.00225
12-6-51	24	yeast	847	3.0	.00354
12-7-51	48	"	761	2.5	.00328
12-6-51	24	thyroid	1,068	7.5	.00702
12-7-51	48	"	972	5.5	.00565
12-8-51	24	control	462	2.0	.00282
12-9-51	48	"	268	0.5	.00187
12-8-51	24	yeast	635	2.0	.00315
12-9-51	48	"	460	1.0	.00217
12-8-51	24	thyroid	930	6.0	.00645
12-9-51	48	"	758	5.0	.00660
Average	24	control			.00271
"	48	"			.00206
"	24	yeast			.00320
"	48	"			.00259
"	24	thyroid			.00669
"	48	"			.00611

Table 7. A comparison of the effects of tyrosine and glycine on the rate of respiration of Paramecium caudatum in sterile cultures with that of desiccated thyroid and the control animals at 25.0° C. and pH 6.8.

Date	Number of hours in media	Types of culture media used	Number of paramecia used	mm^3 O_2 used in one hour	Q_{O_2} (mm^3 O_2 per hour per cell)
11-22-51	24	control	666	1.5	.00225
"	"	tyrosine	520	1.0	.00184
"	"	glycine	542	1.0	.00109
"	"	thyroid	925	6.0	.00621
11-23-51	48	control	418	1.0	.00240
"	"	tyrosine	357	0.5	.00140
"	"	glycine	366	0.5	.00109
"	"	thyroid	660	3.5	.00530
11-26-51	24	control	376	1.0	.00266
"	"	tyrosine	314	0.5	.00159
"	"	glycine	298	0.5	.00168
"	"	thyroid	783	5.5	.00702
11-27-51	48	control	261	0.5	.00192
"	"	tyrosine	255	0.5	.00196
"	"	glycine	214		
"	"	thyroid	761	5.0	.00650
11-28-51	24	control	353	1.0	.00283
"	"	tyrosine	280	0.5	.00180
"	"	glycine	233		
"	"	thyroid	710	5.0	.00775
11-29-51	48	control	240	0.5	.00208
"	"	tyrosine	144		
"	"	glycine	119		
"	"	thyroid	591	3.5	.00610
12-1-51	24	control	380	1.0	.00264
"	"	tyrosine	222		
"	"	glycine	201		
"	"	thyroid	780	5.0	.00641
12-2-51	48	control	211	0.5	.00237
"	"	tyrosine	96		
"	"	glycine	65		
"	"	thyroid	643	4.0	.00622
Average	24	control			.00259
"	"	tyrosine			.00151
"	"	glycine			.00064
"	"	thyroid			.00685
"	48	control			.00219
"	"	tyrosine			.00084
"	"	glycine			.00027
"	"	thyroid			.00603

mal growth in Tetrahymena, and that tyrosine was a dispensable amino acid which, in general, inhibited the normal growth of that protozoan.

DISCUSSION

In view of the numerous contradictory conclusions of different investigators, a comprehensive survey of the various papers on ciliate respiration is not possible at the present time. These irregularities are doubtless due to the variable and often improper techniques employed by different workers.

The fact remains that it is impossible to find a common basis for comparison of the results obtained for even the same species, not to mention those of closely related species. This fact was illustrated by Kidder and Dewey (1945) when they found considerable differences in the gas metabolism of various strains of Tetrahymena geleii which were frown in pure culture.

In measuring the respiration of any biological material, it is fundamentally essential that any other material that is capable of respiratory activity be either absent or well controlled. This pertains especially, in this case, to bacteria and other microorganisms which will influence all experimental results if their presence is not taken into consideration. The lack of emphasis on this fact is no doubt largely responsible for the great accumulation of data on ciliate respiration that must be either accepted at its face value or ignored completely.

Many investigators on the subject of microrespiration have based their findings on comparisons of unit weight, unit surface, or unit volume. Such conclusions seem misleading in that they do not compare the essential respiring substance of the cell. Specht (1934) stated "the highly vacuolar nature of the protoplasm in 'Paramecium' leads one to doubt the values of comparisons based on overall dimensions and weight."

Methods have also been employed in the study of protozoan respiration wherein the volume of centrifugal sediment is determined; however, the sources of error involved in such procedures make for incomparable results.

The only reliable means for measuring cell metabolism seemed to be to determine either the number of cells or their unit weight. Both methods may lead to false conclusions, but the former has the advantage that reliable counts can be made on the suspended cells. This method, therefore, seemed preferable wherever there was a question of comparing cells of a homogeneous population. The chief disadvantage of the alternative method of determining the weight of dried cells and of their nitrogen content was the difficulty of procedure. Its reliability is also only apparent, as it becomes impossible to know in each case how much of the weight is constituted by the living protoplasm and how much by food and reserve substances in the cells.

Attempts at comparing the respiration rates recorded by previous investigators on different species of Paramecium met with various obstacles which proved difficult to overcome. Tang (1941)

pointed out that the structural and physiological differences between the various types of cells are so great that it is impossible to find a common basis for the comparison of their rates of respiration. Variations of the surface area or of the volume of different cells are undoubtedly sufficient to account for the differences in the rates of oxygen consumption of various organisms.

In most of the studies performed by previous investigators, no attempt was made to control the reaction of the medium in which the paramecia were suspended. The relation between the hydrogen ion concentration and the rate of respiration of the ciliates is not known, although it was shown in this study that the medium is subject to change during the course of an experiment.

There is comparatively little data in the literature on the Protozoa relating their oxygen consumption with differences in temperature. Measurements have been made on Paramecium at temperatures ranging from 0° to 35° C. (Leichsenring, 1925) and there should be a correlation with respiration, but there is no reliable information on this problem. The temperature for the experiments included in this paper was maintained at 25.0° C., which other studies have shown to be within the optimum zone for this species of Paramecium.

Unicellular organisms make excellent material for the study of various cellular phenomena. Paramecia, as is the case of other protozoans, do not require complicated respiratory mechanisms of higher animals for the intake of oxygen. Jahn (1941)

stated that diffusion, high rate of water exchange, and protoplasmic movements are apparently sufficient to maintain a suitable level of oxygen tension in the protoplasm and to prevent the accumulation of toxic amounts of carbon dioxide.

The current paper is intended to be a contribution to the growing literature in a relatively new field of investigation. The application of such methods mentioned in this paper in dealing quantitatively with the biochemical and physiological properties of paramecia have furnished invaluable data to the field of bacteriology and other allied sciences.

A study of the measurement of gaseous exchange is not an end in itself, but is comparable to a piece of a puzzle which, when fitted together with other pieces, will furnish a picture enabling science to answer questions that have heretofore been mystifying. Such studies on the respiratory metabolism of an animal may well serve as indices of the rate at which it obtains, derives, and utilizes energy from the various substances. There also exists the possibility that such research will produce a direct relationship between certain metabolic processes and the pathogenicity of parasitic forms.

SUMMARY

1. The oxygen consumption in Paramecium caudatum is accelerated by the action of desiccated thyroid substance.

2. The extent of this acceleration is only partially dependent upon the food content available to the animals in addition to physical factors of temperature and hydrogen ion concentration.

3. Individuals from a non-sterile culture of P. caudatum respired at the rate of .00314 mm^3 per hour at the end of a 24 hour period following introduction to the medium.

4. Desiccated thyroid, when added to the non-sterile medium, increased the oxygen quotient of one Paramecium to .00829 mm^3 per hour for the same period.

5. Forty-eight hour results on the same material showed a consistent increase in both culture samples.

6. The Q_{O_2} for 24 hour sterile cultures averaged .00673 for the thyroid-fed paramecia as compared to .00265 for the controls.

7. The two groups exhibited similar differences after 48 hours on sterile media, but there was a marked decline in the respiration of both the thyroid-fed and control animals due to a decrease in available food supply.

8. Ammonia was indicated to have been given off by the paramecia during their respiration.

9. Slight changes in pH of the culture medium produced no appreciable differences in the oxygen uptake of *Paramecium*.

10. No correlation was found between the concentration of cells employed and oxygen consumption.

11. Paramecia in a thyroid medium took a deeper stain with Lugol's (iodine) solution than did the controls.

12. Sterile cultures of paramecia fed on dead bacterial yeast exhibited slightly higher numbers and a small increase in oxygen consumption over the controls, but the levels were distinctly lower than those observed in the thyroid-fed animals.

13. Tyrosine and glycine exhibited an inhibitory effect on the reproduction and respiration of *Paramecium caudatum*.

ACKNOWLEDGMENTS

The experiments described in this paper form the second of a series of studies on Paramecium. They have been carried out under the direction of Dr. E. H. Herrick, whom the writer wishes to thank for his invaluable suggestions and criticisms. Thanks are also due Dr. J. O. Harris of the Department of Bacteriology for his helpful suggestions and also for extending the facilities of the bacteriology graduate laboratory. The writer also wishes to express his gratitude to the following persons: Miss Joan Conover, medical technician senior at Kansas State College, for her help in making bacterial counts; Dr. A. T. Perkins of the Department of Chemistry for obtaining buffer solutions; and Mr. Floyd J. Hanna, college photographer, for the plates presented in this paper.

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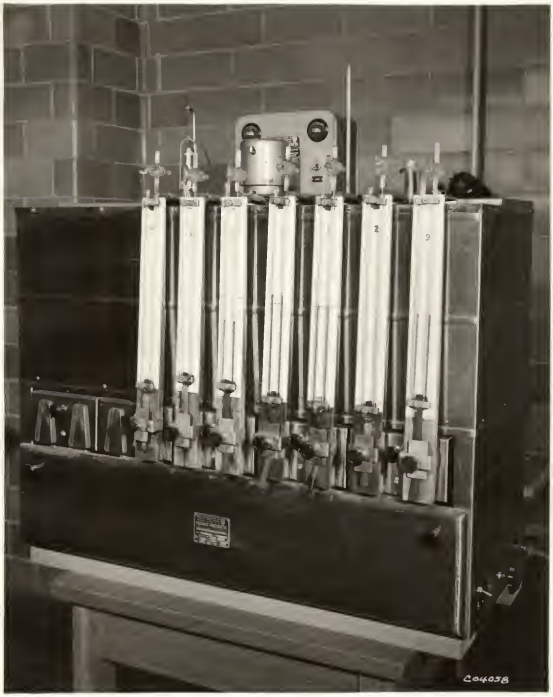
APPENDIX

EXPLANATION OF PLATE I

Photograph of the Warburg microrespirometer showing seven manometers in place. Mounted atop the machine are the electric stirrer and thermoregulator for control of the temperature of the water bath.

The respiratory flasks are currently immersed in the water bath. The thermometer is at the right of the thermoregulator, and the thermostat is hanging from a coil spring immediately behind the second manometer from the left.

PLATE I

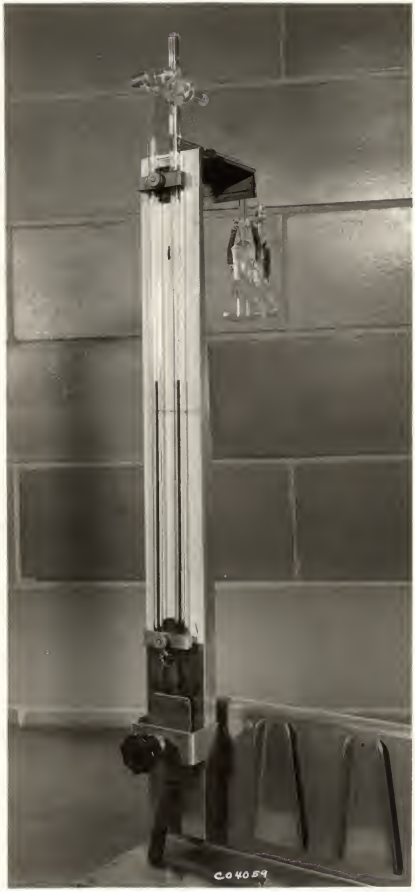


EXPLANATION OF PLATE II

Photograph of a Warburg manometer and its corresponding respiratory flask attached with the aid of small springs. The sidearm and center cup are plainly visible as parts of the flask.

The stopcock is at the top of the closed manometer arm, the Brodie's Solution is in the two manometer arms, and the fine calibration in cubic millimeter marks on the manometer arms can be seen.

PLATE II



PART I. THE EFFECT OF DESICCATED THYROID ON THE REPRODUCTION OF *PARAMECIUM CAUDATUM*

PART II. THE EFFECT OF DESICCATED THYROID ON THE RESPIRATION OF *PARAMECIUM CAUDATUM*

by

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Protozoa have long furnished biologists excellent material for physiological studies on the one cell level. Only in the past few years, however, has there been experimentation conducted on the effects of endocrine gland products on these unicellular animals.

Previous investigators have made studies on the effects of thyroid derivatives on Paramecium in regard to their specific effects on cell division. Discrepancies in their findings have appeared, however, with the result that the conclusions drawn from these earlier experiments offered little in regard to correlating them with other aspects of protozoan life, such as respiration and metabolism.

Due to the previous lack of technical equipment, the field of microrespiration is comparatively new. Studies have been made in recent years on the rate of respiration of Paramecium, but no publications have been found dealing with the influence of thyroid substance on the oxygen consumption of these animals.

For these reasons it seemed desirable to investigate the effects of desiccated thyroid on the reproduction and respiration of Paramecium in an effort to better understand its action on the animal cell as an individual unit.

Paramecia used for these experiments were taken from a stock culture of Paramecium caudatum which had not been treated aseptically in any way. The primary purpose was to compare the multiplication and respiration of Paramecium with and without thyroid treatment. Every effort was made to keep influencing factors

equal between those organisms which were on thyroid and those which were not. Observations were made at 24 and 48 hour intervals following introduction of the thyroid to the medium. The desiccated thyroid substance used in this study was Parke, Davis and Co. Thyroid U. S. P.

Data on the reproduction of the ciliates revealed that with an uncontrolled bacterial food supply, paramecia, to which thyroid had been added in the medium, divided at a significantly higher rate than did animals used as controls (Table 1).

A group of five paramecia representing the control increased in number by 19.2 per cent during the first 24 hours and by 220 per cent at the end of 48 hours. The same number of animals which had been given thyroid, on the other hand, showed increases of 92.4 and 794 per cent during the 24 and 48 hour periods, respectively.

Observation on Paramecium growth in medium isolated from the original culture indicated the bacterial food supply after 72 hours was being exhausted as was evidenced by a lowering rate of reproduction.

Bacterial counts on the culture medium showed that thyroid increased the number of bacteria 210-fold during the first 24 hours and caused a 112-fold difference after 48 hours (Table 2).

Rapid fission of the thyroid-fed individuals was accompanied by increased activity, greater transparency, and slightly smaller size, indicating that important internal modifications occur in the physiology of the cell.

Table 1. The effect of desiccated thyroid on the rate of reproduction of Paramecium caudatum in non-sterile culture media.

Number of paramecia							
Control				On thyroid			
Start	After 24 hours	After 48 hours	After 72 hours	Start	After 24 hours	After 48 hours	After 72 hours
5	5	14	29	5	10	39	83
5	7	16	34	5	9	50	94
5	6	15	32	5	9	44	91
5	6	20	39	5	11	47	95
Average	6	16.2	34	Average	9.75	45	90.7

Table 2. The effect of desiccated thyroid on the bacterial growth of the culture media.

Number of samples used for each count	Number of bacteria per milliliter of medium			
	Control		On thyroid	
	After 24 hours	After 48 hours	After 24 hours	After 48 hours
3	3,900	198,000	1,480,000	7,000,000
3	2,500	150,000	1,910,000	12,000,000
3	4,800	87,500	1,167,000	6,520,000
4	20,400	95,350	1,280,000	25,000,000
2	17,200	41,700	4,400,000	7,770,000
Average	9,760	104,510	2,049,200	11,658,000

Respiration studies on Paramecium were conducted with the aid of a Warburg microrespirometer, which proved accurate in measuring changes in gas volume to 0.5 mm^3.

Employing this apparatus, the oxygen consumption of Paramecium caudatum was found to be accelerated by the action of desiccated thyroid substance. The extent of this acceleration was seen to be only partially dependent upon the food content available to the animals in addition to the physical factors of temperature and hydrogen ion concentration.

Individuals from a non-sterile culture of P. caudatum respired at the rate of .00314 mm^3 per hour at the end of a 24 hour period following introduction to the medium, whereas identical animals fed on thyroid each consumed an average of .00829 mm^3 oxygen per hour. Forty-eight hour results on the same material showed a consistent increase in both culture samples (Table 3). In order to account for the respiration of the bacteria in the media, runs were concurrently made on media from which the paramecia had been centrifuged out and these readings subtracted from the ones taken on the samples containing the ciliates.

In order to eliminate the bacterial influence altogether, however, experiments were conducted on paramecia in media which had previously been autoclaved. Table 4 represents a summary of 248 single determinations made on sterile cultures. It can be noted that the thyroid exhibited the same influence on the animals' respiratory rate as it did in the contaminated cultures, being 254 per cent higher than the controls after 24 hours.

Table 3. A summary of 256 single determinations on the effect of thyroid on the respiration of Paramecium caudatum in non-sterile media at 25° C. and pH 6.8 expressed in mm^3 oxygen consumed by one Paramecium per hour (Q_{O_2}).

Date in 1951	Number of hours on thyroid	Control paramecia			Thyroid-fed paramecia		
		No. of paramecia used	mm^3 O_2 used in one hour	Q_{O_2} (mean of 3 to 4 manometers)	No. of paramecia used	mm^3 O_2 used in one hour	Q_{O_2} (mean of 3 to 4 manometers)
9-27	24	450	1.5	.00333	702	6.0	.00855
9-28	48	620	2.5	.00403	991	10.0	.00999
10-3	24	980	3.0	.00306	1,300	10.0	.00769
10-4	48	1,522	5.5	.00361	1,844	15.0	.00813
10-9	24	934	3.0	.00309	1,219	8.5	.00753
10-10	48	1,093	4.0	.00366	1,661	14.0	.00843
10-18	24	1,630	5.0	.00307	3,620	31.0	.00857
10-19	48	2,550	8.0	.00314	4,080	42.0	.01030
Average	24			.00314			.00809
Average	48			.00361			.00921

Table 4. A summary of 248 single determinations on the effect of thyroid on the respiration of Paramecium caudatum in sterile media at 25° C. and pH 6.8 expressed in mm^3 oxygen consumed by one Paramecium per hour (Q_{O_2}).

Date in 1951	Number of hours on thyroid	Control paramecia			Thyroid-fed paramecia		
		No. of paramecia used	mm^3 O_2 used in one hour	Q_{O_2} (mean of 3 to 4 manometers)	No. of paramecia used	mm^3 O_2 used in one hour	Q_{O_2} (mean of 3 to 4 manometers)
11-6	24	1,505	4.0	.00266	1,803	8.5	.00638
11-7	48	1,244	3.0	.00241	1,312	7.5	.00572
11-12	24	1,560	4.5	.00288	1,650	11.5	.00630
11-13	48	1,131	2.5	.00221	1,822	11.0	.00615
11-26	24	376	1.0	.00266	783	5.5	.00702
11-27	48	261	0.5	.00192	761	5.0	.00650
12-13	24	616	1.5	.00243	1,710	12.5	.00731
12-14	48	487	1.0	.00202	1,320	8.0	.00603
Average	24			.00265			.00673
Average	48			.00222			.00610

The data taken from experiments on sterile cultures of Paramecium showed a marked decline in the respiration of both the thyroid-fed and control animals after 48 hours. This was undoubtedly due to a decrease in available food supply (dead bacteria), but the ciliates on thyroid continued to consume much more oxygen than did the controls.

Ammonia was indicated to have been given off by the paramecia during the course of the experiments and a procedure was used to destroy this reaction, although the effect of this minute amount of NH_3 on the results proved to be slight.

Small changes in pH of the culture media was found to produce no appreciable differences in the oxygen uptake of the protozoans. Nevertheless, all media was buffered to a pH of 6.8 before the experiments were started.

The paramecia were counted before and after each experiment in order to determine the oxygen quotient of an individual cell. The number used in different trials varied from 200 to 6,000 and no correlation was found to exist between the concentration of cells employed and oxygen consumption per animal.

Paramecia in a thyroid medium were found to take a deeper stain with Lugol's (iodine) solution than did the controls; however, an explanation of this phenomenon is difficult at this time.

Sterile cultures of paramecia fed on dead bacterial yeast exhibited slightly higher numbers and a small increase in oxygen consumption over the controls, but the levels were distinctly lower than those observed in the thyroid-fed animals.

Experiments conducted with tyrosine and glycine showed that these free amino acids produced an inhibitory effect on both the reproduction and respiration of *Paramecium caudatum*.

SUMMARY

1. Desiccated thyroid greatly increases the rate of reproduction in *Paramecium caudatum* in non-sterile cultures.

2. Thyroid also increases the number of bacteria in the medium, thus creating a better environment for the protozoans.

3. Thyroid causes a significant rise in the oxygen consumption of paramecia in both sterile and contaminated cultures.

4. The fact that sterile cultures continue to show a large increase in respiration proves that the available food supply is not the answer, but rather is the effect of the thyroid on the cell itself.

5. Thyroid-fed individuals also showed an increase in activity, greater transparency, and slightly smaller size, indicating that important modifications occur in the physiology of the cell.

6. Well-fed paramecia (those with yeast added to their medium) respired at a significantly lower rate than did the animals on thyroid, which seems to substantiate the conclusion that desiccated thyroid is responsible for increased metabolic activities of *Paramecium*.