THE EFFECT OF STRONTIUM CHLORIDE AND ARSENIC CHLORIDE ON THE OXYGEN AND CARBON DIOXIDE EXCHANGE OF PARAMECIUM CAUDATUM

by

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A. B., John Fletcher College, 1934

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1941

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INTRODUCTION

Much work has been done recently on the oxygen exchange of various microorganisms. In these studies a wide variety of micro-respirometers have been used. Various factors have tended to make work of this type difficult, viz., such as are hard to control or those which, as yet, have not been recognized as needing controlling. These facts have led to a lack of consistency in the results thus far obtained by investigators.

Preliminary work on the oxygen exchange of paramecia was done by Wood (1938). Peterson (1941) opened up a new field by studying the effects of certain cations on the division rate and viscosity of the protoplasm of paramecia. As a continuation of the former study, it was thought valuable to observe the effects of some of these same cations on the oxygen and carbon dioxide exchange of paramecia. The purpose, therefore, of this study was to determine the normal oxygen and carbon dioxide exchange of Paramecium caudatum and then to observe any deviation from the normal exchange by the addition of $SrCl_2$ and NH_4Cl to the culture medium, since these salts were found to have a striking effect on the organisms (Peterson, 1941).

REVIEW OF LITERATURE

Culture Methods

Culture Media. Hargitt and Fray (1917) grew paramecium on hay infusions of both mixed and single strains of bacteria. Their results showed that there was very little or no difference in the growth of the animals in pure or mixed cultures. Peters (1920) reported the growth of paramecium on a bacteria-free medium. He used salts and certain extracts to support growth.

Phillips (1922) found that the difference in growth of paramecium in pure and mixed cultures of bacteria was not significant. His medium consisted of a standard timothy hay infusion. Ten grams of timothy hay were boiled for 10 minutes. This solution was cooled, brought to one liter in volume, and then filtered. This stock solution was sterilized and diluted to 0.1 percent solution for culture purposes.

Cleveland (1928) stated that it was the lack of standardization of culture media as food that accounts for the lack of agreement in the work done on paramecia. He advocated the use of pure strains of bacteria.

Other workers with bacteria free media were Glaser and

Coria (1933). They reported the successful growth of Paramecium caudatum on a medium containing no other living microorganism. Use was made of rabbit kidney, liver extract, and dead yeast cells.

Bacillus subtilis was found by Johnson (1936) to produce the greatest growth of any of the other species of bacteria tried. He also used yeast as a food. Wood (1938) also found Bacillus subtilis to be a very satisfactory food for paramecium. A bacteria-free medium which supported the growth of some ciliates of the family Frantoniidae was prepared by Brown (1940).

The findings of various workers are reported by Leslie (1940) in respect to the usability of several species of bacteria for Paramecium caudatum. Bacillus subtilis, Bacillus coli, Bacillus proteus, and Achromobacter pinnatum were found suitable foods. More investigators found Bacillus subtilis to be a suitable food than any other of the species of bacteria.

Sterilization. The first workers to develop an effective method of sterilization of paramecium were Hargitt and Pray (1917). They used a method of five washes in sterile water. In sterilizing their culture media they found that autoclaving caused the cultures to be short lived. Subsequent workers have had no trouble in supporting good cultures on autoclaved media.

Parpart (1929) improved on the method of Hargitt and Fray by increasing the number of washings to ten. The animals were left in the fifth wash for five hours to eliminate any ingested spores.

Wood (1933) followed very closely the washing method developed by the above stated investigators.

One of the newest methods was devised by Claff (1940). He used a closed system of six flasks. This method has the advantage of great distance in migration and a high degree of dilution.

Peterson (1941) freed the paramecia from bacteria by the successive washing method. This was carried out with sterile pipettes in sterile watch glasses contained in petri dishes. He found that cultures could be maintained for long periods of time on autoclaved media.

Apparatus for Measuring Oxygen Consumption

The respirometers used in micro-respiration work have been of two types, the differential volumeter and the single or non-differential volumeter. The single volumeter measures the change in volume in the experimental vessel against the barometric pressure. The control vessel is connected to a manometer. The differential type registers the volume change in the experimental vessel against a control vessel which is

sealed off from the atmosphere. This type is independent of changes in barometric pressure, but it is very sensitive to temperature changes. If the control vessel of the differential type is equal in size to the experimental vessel the movement of the index drop is only one-half of the actual change in volume of the experimental vessel. If the ratio of the control vessel to the experimental vessel is large the index drop records the total change in volume of the experimental vessel. For most purposes the differential type is the best.

The apparatus used by Thunberg in 1905 (Cunningham and Kirk, 1940) has served as a background for many types of differential volumeters. A compensating flask was used in the apparatus.

Winterstein in 1912 (Wood, 1938) developed an apparatus with two chambers. One contained the culture and was connected to a mercury manometer and also to a compensation vessel by way of a capillary tube containing a kerosene drop. A tube and clamp fastened to the manometer aided in adjusting the indicator.

Two compensating vessels were used by Krogh in 1914 (Wood, 1938). In this case, as above, the vessels were attached to a manometer, and a kerosene drop in a capillary tube was used as an indicator.

Kalmus in 1927 (Howland and Bernstein, 1931) used a straight glass tube to measure oxygen consumption of para-

mecium. A glass tube open at both ends was filled with short columns of paraffin oil, 10 percent potassium hydroxide, air, paramecium culture, paraffin oil, and air in the order stated. The last end was then sealed in a flame. The tube was cooled in a water bath. The results obtained were not consistent due to the long period required to obtain constant temperature after the glass had been heated in a flame.

Fenn (1927, 1928) used a differential volumeter which consisted of two equal bulbs connected by a capillary tube. Shunt tubes and stopcocks were placed between each vessel and the capillary tube for pressure regulation. Fenn (1927) fitted each bulb with stimulating electrodes for the study of nerves. Fenn (1928) used a chamber containing BaOH to absorb the CO_2. The amount of CO_2 was determined by its conductivity through electrodes inserted into this vessel. H_2SO_4 was used for vapor pressure regulation in the control vessel.

Howland and Bernstein (1931) used a modification of that used by Kalmus. A straight glass tube 6 cm in length and .3 mm in diameter was used. The tube was filled with successive sections of mineral oil, paramecium culture, air, KOH, and mineral oil in the order stated. In this case the tube was not sealed. It was placed in a water bath, and readings were taken of the decrease in size of the air chamber.

Kucera (1934) used a differential volumeter similar to the one used in this study. After immersing it in a water bath, he permitted it to stand one-half hour before connecting the capillary tube with the bulbs. Readings were begun after they had been connected for 15 minutes.

A sensitive gasometric apparatus was used by Linderstrom-Lang (1937). A Cartesian diver was used as an animal chamber. The reduction of the oxygen in the diver reduced the displacement volume and the diver sank to a lower level. This apparatus was very sensitive, but cannot be used for many types of work.

Wood (1938) used a respirometer which was a modified form of those used by Winterstein and Krogh. It was made of two bulbs equal in volume. The bulbs were made to fit on to ground glass joints. These joints were connected by way of a shunt tube and a capillary tube. The stopcock in the shunt tube permitted equalization of pressure in the two bulbs. The oxygen exchange was calculated from the movement of the kerosene drop in the capillary tube. Constant temperature was obtained by means of a circulating water bath.

An apparatus called "Needham's diver" was used by Hartridge (1939). It consisted of a small diver bulb with a neck and tail. The neck was coated with wax and sealed with a column of oil after the material was placed in the bulb. The flotation medium was LiCl. The flotation bulb, in which the diver was placed, was attached to a manometer tube with

coarse and fine adjustment for pressure regulation.

Heatley, Berenblum, and Chain (1939) used a closed chamber with a flexible mica wall. A beam of light was directed against the mica wall. Changes in the gas pressure on the inside changed the curvature of the mica wall and thus directed the reflected beam of light at a different angle. The beam of light was picked up on a graduated scale. The apparatus was easy to read. The accuracy was dependent upon the flexibility of the mica.

According to Cunningham and Kirk (1940), the Warburg apparatus has been the most widely used type of respirometer. This is a manometer type. The experimental vessel is attached to a "U" tube filled with some liquid. The Warburg apparatus is not reported to be very sensitive.

Thimann and Commoner (1940) constructed an apparatus which was composed of four sections. The first was an experimental vessel with a tube and stopcock leading from it. To the base of the vessel was attached a section containing a capillary tube and a shunt tube. A stopcock was inserted in the shunt tube. To this section was attached a KOH vessel, and then an experimental vessel. The control vessel was made many times larger than the experimental vessel. This had the advantage of giving the total oxygen exchange in the movement of the drop. The types which had vessels equal in size only recorded one-half the total oxygen exchange.

The last described respirometer was the type used in this study.

Oxygen Consumption of Paramecium

Barratt in 1905 quoted by Howland and Bernstein (1931) found the respiratory rate of Paramecium aurelia to be .00015 mm^3 per hour per animal.

Lund (1918) tested the effects of starvation on intracellular oxygen exchange. After starving the Paramecium caudatum for 48 hours the oxygen consumption was .00004 mm^3 per hour per individual. When fed dead yeast cells, the rate was .00014 mm^3 per hour.

Necheles in 1924 and Kalmus in 1927 and 1928, both quoted by Howland and Bernstein (1931) worked with Paramecium caudatum. Necheles found the respiratory rate to be .00385 mm^3 per hour per animal. Howland and Bernstein (1931) stated that the rate for a Paramecium caudatum was .00049 mm^3 per hour.

Wood (1938) gave the normal rate for Paramecium caudatum to be .035 mm^3 per hour. He also found an increase of 61 percent to 460 percent by the addition of desiccated thyroid to the culture.

MATERIALS AND METHODS

Culture Methods. The paramecia used in this study were Paramecium caudatum (Ehren.). They were sub-cultures of the pure strain started from one individual and sterilized by Peterson (1941).

The paramecia were cultured on a timothy hay infusion made up from Brandwein's solution (1935), wheat grains and timothy hay. The Brandwein's general culture solution is made up with the following salts:

NaCl	1.20 gm
K Cl	0.03 gm
$CaCl_2$	0.04 gm
$NaHCO_3$	0.02 gm
NaH_2PO_4	10 cc of M/15
$NaHPO_4$	40 cc of M/15

Dilute to 1000 cc with distilled water. This solution had a pH of about 7.4.

In culturing the paramecia, the Brandwein's solution was diluted 1-10 and about 250 cc to 275 cc placed in a 500 cc Florence flask. This quantity gave the maximum surface exposed to the air in the type of flask used. To this solution were added about 2 gm of timothy hay and 10 wheat grains. The flask was then plugged with cotton and autoclaved at 15-20 pounds of pressure for 15 to 20 minutes. After cooling sufficiently the medium was inoculated with a few cc of medium from another culture containing paramecia

and a single strain of bacteria.

Bacillus subtilis was the organism used as food for the paramecia. Early in the study new cultures were inoculated with Bacillus subtilis and left to stand for about 24 hours and then inoculated with paramecia, but later it was found that in transferring the paramecia enough bacteria were also carried over to produce a quick and abundant growth.

A second culture solution was prepared for use during respirometer tests. This solution was an aqueous solution of "Cerophyl"*. One tablet of "Cerophyl" was placed in a small amount of water and left to dissolve. The volume was then brought to 200 cc and filtered (600 - 1000 cc were made up at one time). This solution was sterilized at 15 to 20 pounds pressure for 15 to 20 minutes. After cooling sufficiently it was inoculated with Bacillus subtilis and incubated on the top of an electric oven for 24 hours at a temperature of about 40 degrees C. This culture was then stored in a refrigerator at 0 - 2 degrees C. Small portions were removed as needed. Peterson (1941) found that the low temperature caused the bacteria to form spores and thus a controlled supply was at hand for quantitative studies.

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* "Cerophyl" is a dried form of cereal grasses in tablet form as a source of vitamins. The product is manufactured by the American Butter Company, Kansas City, Missouri, and it was supplied by Dr. J. S. Hughes of the Department of Chemistry.

Phillips (1922) used a stock solution of timothy hay infusion. This solution was tried, but the results obtained were not satisfactory so the procedure was discontinued.

The glassware used in the culturing of these paramecia was sterilized either in an autoclave at 15 to 20 pounds pressure for 15 to 20 minutes or in a hot oven for an hour or more.

The Respirometer. The respirometer used in this study was a differential volumeter such as was used by Thimann and Commoner (1940). It was manufactured by Messrs. McAlister and Bicknell, Cambridge, Massachusetts. The apparatus used in this study, however, differed slightly from the one used by Thimann and Commoner. They used a capillary index tube with a diameter of .2 to .3 mm. The apparatus used in this study had a capillary index tube with a diameter of .87 mm. This reduced the sensitivity approximately 27 times. The entrance to their capillary tube was not capillary in size, but in the apparatus used here it was capillary in size. This feature hindered the manipulation of the kerosene drop.

The mount and constant temperature water bath were modified considerably. The glassware was mounted on a board 17 inches long and 2 inches wide. This board was perforated with holes to allow for free circulation of the water about the apparatus. Two steel strips were fastened to the board at right angles to each other, and bent up along the sides of the control vessel as supports for it. Hooks

were bent on the ends of these strips to permit a rubber band to be put around the supports and hold the control vessel firmly in place. Another steel strip was bent in the form of a "U" and attached to the board for a support to the capillary tube near the end where the KOH vessel was attached. To each end of this board was attached a board in upright position, and at a suitable distance on each upright board was attached another board at right angles to the upright. These boards rested on the rim of the inner bath and permitted the apparatus to be submerged into the water to the required distance.

The water bath in the first part of the experiment consisted of a wash boiler. Fair results were obtained when the apparatus was placed in the constant temperature chamber of the Department of Entomology. The boiler was found to be too small to keep constant enough temperature. A large water bath was then built for this purpose. The outer bath was about 3 feet square and 2 feet deep. The walls were composed of galvanized sheet iron on the outside and copper on the inside. Between the sheet iron and copper was a layer of insulation material. A smaller inner bath was placed in the larger bath and supported about 7 inches from the bottom. This tank was 24 inches long, 10 inches wide and 12 inches deep and constructed of copper. The respirometer was immersed in the water of the inner bath to a depth of about 6 inches. This was well above the control vessel stopcock.

The tanks were placed in a basement room to secure as constant a temperature as possible.

Technique in Using the Respirometer. To insure good results the first requisite was to have the capillary tube thoroughly clean. It was first washed with acetone to remove any grease. Then it was cleaned with a warm solution of "Calgonite", a commercial washing powder. The tube was finally rinsed with hot distilled water. It was dried by drawing air through it by means of a vacuum jet. This cleaning process needed repeating only if the kerosene drop showed signs of sticking or if water got into the capillary tube. The cleaning solutions were injected into the tube with a capillary pipette.

The indicator used in the capillary tube was kerosene colored red with Sudan III. The kerosene was previously dehydrated with calcium chloride. The drop was difficult to insert into the capillary tube until a technique was developed. The drop was inserted in the following manner. A small volume of kerosene was taken into a capillary pipette with a smooth end. The pipette was introduced as far as possible into the end of the capillary tube which joined the control vessel. Enough kerosene was injected to pass through the first capillary tube and the enlargement of the junction of the shunt tube, and fill the main capillary tube to a length of 1 to 2 cm. By tilting, the drop in the main tube was allowed to run well toward the middle. The shunt cock

was open during this procedure. Most of the excess kerosene which lodges in the first section of capillary was removed by placing a pipette with a smooth end as far as possible into the opening and withdrawing the kerosene with a rather sudden release of the pipette. Too sudden a release would also disturb the drop in the main tube. Any kerosene that could not be removed with the pipette was removed by successive insertions of a wire about the size of the capillary.

Usually when the kerosene passed from the enlargement at the junction of the shunt tube to the main capillary tube it was taken up in sections, that is, air bubbles formed between successive sections of kerosene. This was not desirable for accurate readings so two methods were devised to get the droplets into a single drop. The first method was to run the droplets to the end into which they were injected. Then a very fine wire was inserted into the droplets. The droplets collected about the wire and by lowering the end into which the wire was inserted successive droplets ran down and collected on the wire. The air bubbles passed out of the tube. When all were collected in this way the wire was gradually removed, and as it passed through the enlargement the kerosene stayed behind in a single drop. The second method was to run the droplets to either end by tilting. Then the stopcock was closed and a finger was gently placed over the opposite end. The tube was then tilted with the free end up and pressure applied with the finger. This pushed the droplets

to the end of the capillary and liberated the air bubbles. As it was held with the free end up the kerosene was not taken up in the outer tube and when the finger was released the kerosene returned to the main tube in a single drop.

Both methods worked and can be used for different situations, but the last method proved to be the best in most cases.

The KOH vessel was coated on the inside with paraffin to keep the KOH from creeping to the ground glass joints. This was done by placing a small bit of low melting paraffin in the vessel and bringing the vessel to a flame several times until the paraffin melted and then the vessel was rotated so as to cover the desired area.

In assembling the apparatus the control vessel was placed in position and held by means of a rubber band about the top of the steel clamps. With the stopcocks open the outer parts were then connected in order. The joints were sealed with petroleum jelly and held together with rubber bands. The steel mounts were found to rust and discolor the glass so they were coated with paraffin.

Before any animals were placed in the apparatus, it was checked for variation. It was found the drop did not move when the temperature did not vary more than one degree C.

The number of paramecia placed in the experimental vessel was determined in the following manner. A number of samples of cultures were selected as nearly identical to

those used as could be determined under a wide field binocular microscope. These were placed in a Syracuse dish and killed with picro-sulphuric acid fixative according to Guyer (1936). They were then counted under a wide field binocular microscope. The bottom of the dish was ruled to aid in counting.

The salts used in this study were $SrCl_2$ and NH_4Cl. The stock solutions consisted of 0.5 M $SrCl_2$ and 1.0 M NH_4Cl. For the quantitative studies these solutions were diluted to the desired concentration with the "Cerophyl" culture solution. Peterson (1941) found M/99 $SrCl_2$ to be favorable to the growth of paramecia, and M/70 NH_4Cl was found to be quite toxic. For this reason, these concentrations were selected for this study.

Introducing the paramecia into the salt solution was carried out in the following manner. A few cc of the "Cerophyl" culture solution were taken from the refrigerator and warmed to the approximate temperature of the room. This was then used in making the proper dilution of the stock salt solution. Paramecia were removed in as concentrated a form as possible from a general culture flask. These were introduced into a Syracuse dish and washed several times in successive dishes with the desired "Cerophyl" salt solution. The proper number were then taken up and placed in the experimental vessel.

Different methods were tried to concentrate the paramecia, but as yet no sure method has been devised.

After the apparatus was assembled containing paramecia and KOH in their respective bulbs, it was immersed in the water bath while both stopcocks were open. It was left in the water for an hour or more to permit it to come to the temperature of the water. The stopcocks were then closed and readings begun. The capillary tube was graduated in mm to a length of 15 cm. A white strip was mounted under the capillary tube to facilitate reading the instrument.

Occasionally it was necessary to move the drop of kerosene back without dismounting the apparatus. This was accomplished by opening both stopcocks, and then as one hand was placed on the KOH vessel, the other was used to close the stopcock in the shunt tube. The hand warmed and expanded the air in the KOH vessel and pushed the drop back. When the desired point was reached the stopcock was again opened. The apparatus was allowed to come to constant temperature and the cocks closed and readings begun again. A piece of wood was shaped to turn the stopcocks so the warmth of the hand did not effect the temperature of the apparatus.

Before removing the apparatus from the water, both stopcocks were opened. The shunt cock was opened first to establish an equilibrium within the apparatus so that the kerosene drop was not blown to pieces upon opening the control vessel cock. After removing it from the water the parts to be disconnected were thoroughly dried before removing any of the vessels. If this precaution was not taken water often

got into the capillary tube.

For absorption of the CO_2, 0.3 cc of 10 percent KOH was placed in the bottom of the KOH vessel. This was changed after each 100-200 mm movement of the kerosene drop to be certain that it did not become saturated. After removing the apparatus from the water and removing the vessels, the KOH was quickly poured into a small flask and stoppered. The KOH was then titrated with N/50 H_2SO_4 during which time the flask was immersed in an ice water bath to prevent the escape of any CO_2. The first titration was carried to neutrality with phenolphthalein as the indicator. This was called titration A. At this point methyl orange was added as an indicator and titration was continued to the end point of the methyl orange. This was called titration B. The number of cc of acid used in titration B was used to calculate the CO_2 present. The formula used was $CO_2 = 2BMN$ where B is the cc of acid used in titration B, M is the mg equivalent weight of CO_2, and N is the normality of the acid. The value obtained is in terms of gm of CO_2.

RESULTS AND DISCUSSION

The results of this experiment are listed in Table 1 which shows the number of grams and cubic millimeters of O_2 and CO_2 exchange per hour for each paramecium in the various media indicated. The respiratory quotient (R. Q.) was determined by the following formula:

$$R.\ Q. = \frac{mm^3/hr.\ of\ CO_2}{mm^3/hr.\ of\ O_2}$$

The hay infusion and "Cerophyl" groups in Table 1 were both "salt free" control groups. A higher respiratory rate was obtained in the "Cerophyl" and it was used as the medium in the remaining studies on the effects of salts. These figures represent an average of from two to five cultures. When a series was run with a small number of cultures more readings were taken per culture to make the number of determinations about the same.

Each paramecium consumed .02 mm^3/hr. of O_2 when in the hay infusion culture. This figure is .015 mm^3/hr. less than that found by Wood (1938). Using a modified form of the Winterstein and Krogh respirometer the value he determined was .035 mm^3/hr. His culture medium was similar to the one used in this study. His value was much greater, however, than any others recorded. The nearest approach to Wood's value is that of Necheles in 1924 quoted by Howland and

Bernstein (1931). Necheles found the O_2 consumption to be .00385 mm^3/hr. for each paramecium. This value is about one-tenth of that found by Wood (1938). Necheles' value is almost 8 times that of Kalmus in 1927 and 1928 quoted by Howland and Bernstein. Kalmus found the O_2 consumption to be .00049 mm^3/hr. per paramecium. The great variation in results by the different investigators gives us no standard for comparison. In this study paramecia were found to give off .018 mm^3 of CO_2 per hour when in hay infusion culture. Given in terms of weight each paramecium used .29 x 10^{-7} gm/hr. of O_2 and gave off .36 x 10^{-7} gm/hr. of CO_2.

The "Cerophyl" control group used O_2 at the rate of .039 mm^3/hr. for each paramecium. This was an increase of .019 mm^3/hr. over that of the hay infusion, and an increase of .004 mm^3/hr. over the value found by Wood (1938). The increased respiratory rate in "Cerophyl" was probably due to a better bacterial growth in this culture. The rate of CO_2 exchange in this culture was almost twice that in the hay infusion. The value found was .035 mm^3/hr.

For both the "salt free" controls the respiratory quotient was .90. This value lies between that of protein and that of carbohydrate metabolism. The fact that both controls had the same respiratory quotient leads one to believe that the increased rate of gaseous exchange in the "Cerophyl" was not due to any direct effect of the medium on the paramecium but to an increased bacterial growth which provided a greater

food supply for the animals.

To test the effect of $SrCl_2$ on the respiratory rate of paramecia, readings were taken on a single culture of 200 animals for a period of 85.6 hours. The average rate for the first 47 hours is recorded under $SrCl_2$ (A) in Table 1. The O_2 exchange for group (A) showed an increase of .009 mm^3/hr. for each paramecium over the "Cerophyl" control group giving a value of .048 mm^3/hr. for each paramecium. This is a substantial increase, representing an increase of about 23 percent, and since the same culture medium was used except for the $SrCl_2$, this salt, and especially the Sr^{++}, must be responsible for the increase. The CO_2 exchange showed a corresponding increase with a value of .043 mm^3/hr. These values give a respiratory quotient of .89 which is .01 below that of the controls.

At the end of 47 hours, the paramecia were fed two loops of bacteria from an agar slant. Readings were then taken for an additional 36.6 hours. The average rate per paramecium after being fed is given under $SrCl_2$ (B) in Table 1. The O_2 consumption increased to .076 mm^3/hr. for each animal which is an increase of .028 mm^3/hr. or 58 percent over the same group before being fed, or an increase of .037 mm^3/hr. or 95 percent over the "Cerophyl" control group. This increase was due to a food situation, but it shows the activating effect of $SrCl_2$ in that available food is rapidly used in metabolic processes. The CO_2 given off after being fed was .063 mm^3/hr.

for each animal. This was an increase of .02 mm^3/hr. or 47 percent over that before being fed and an increase of .028 mm^3/hr. or 80 percent over the "Cerophyl" control group. The respiratory quotient fell to .83 in this group.

Figure 1 shows the varying rate of O_2 and CO_2 exchange per hour under the influence of M/99 $SrCl_2$. There was a gradual increase in the rate for the first 17 hours. The increase was from 5.23 mm/hr. to 5.76 mm/hr. These figures are in terms of the movement of the index drop. After 17 hours a gradual decrease in rate set in. This decrease was quite uniform giving almost a straight line on the graph. At the end of 47 hours the rate had fallen to 2.33 mm/hr. At this point the paramecia were fed as indicated above. The rate then showed a very rapid increase for the next 11.5 hours. The rate at the end of 11.5 hours after being fed was 13.77 mm/hr. A rapid decrease was then evident for 4 hours at the end of which the rate was only 6.42 mm/hr. This was a decrease of about half the rate in 4 hours. At the end of 15.5 hours after being fed the rate showed a gradual decline. This rate of decline was very similar to that which set in after 17 hours in the series before being fed. At the end of 85.6 hours from the beginning or 35.6 hours after being fed the rate had fallen to 4.64 mm/hr. The culture began to show signs of degeneration at this time so no further records were used.

The effect of M/70 NH_4Cl on the O_2 and CO_2 exchange of paramecium was exactly the reverse of that shown with $SrCl_2$. "Cerophyl" was again used as the culture medium. The figures are the average of five readings from two cultures. A long series on a single culture could not be made with this salt because the cultures died in about three days. The average O_2 exchange was found to be .026 mm^3/hr. This value is .013 mm^3/hr. below the "Cerophyl" control, and it is just a little more than one-half that of the $SrCl_2$ (A). The CO_2 exchange in the presence of NH_4Cl was .019 mm^3/hr. This was a decrease of .016 mm^3/hr. from the "Cerophyl" control. The respiratory quotient here also took a decided fall with a value of .73. This value is in the range of fat metabolism. The toxic effect of NH_4Cl or more particularly the NH_4^+ was evidenced in two ways. There was a decided decrease in the respiratory rate, and the cultures died in about three days. The cultures were inspected at times of changing the KOH to insure discontinuing the readings when signs of degeneration were evidenced.

Figure 2 is a comparison of the values obtained for the O_2 and CO_2 exchange in the various media used in this study. The values for both the O_2 and the CO_2 of the hay infusion were lowest and also their difference is smaller than that for any of the other media. The value for the O_2 is .02 mm^3/hr., and the CO_2 is .018 mm^3/hr. The difference is .002. The respiratory quotient calculated from these figures is .90.

The O_2 for the "Cerophyl" was almost twice that obtained with the hay infusion, and the CO_2 showed a corresponding increase. The values of .039 mm^3/hr. of O_2 and .035 mm^3/hr. of CO_2 give a difference of .004. The respiratory quotient remains the same with a value of .90 even though the difference was twice that for the hay infusion the absolute values were also about twice the former values leaving the ratio constant.

A further increase of both values was found in the $SrCl_2$ (A). The difference between the O_2 and CO_2 here increases by .001 making it .005. The values of .048 mm^3/hr. and .043 mm^3/hr. for the O_2 and CO_2 respectively were not enough to keep up the respiratory quotient making its value .89. This, of course, is a very small drop. After the $SrCl_2$ group was fed ($SrCl_2$ (B)) the marked increase of O_2 and CO_2 was not proportional to the former cultures. The difference of .013 between .076 mm^3/hr. of O_2 and .063 mm^3/hr. of CO_2 made a drop of .06 in the respiratory quotient. It could be possible that the very rapid rate of respiration increased the CO_2 content in the KOH vessel faster than it could be absorbed, and thus a low reading was obtained in $SrCl_2$ (B).

The drop in both the CO_2 and O_2 in the NH_4Cl medium indicates a toxic condition. We find here a greater difference between the two values than any other group except the $SrCl_2$ (B). The absolute values being small, however, reduced the respiratory quotient to .73. This is .17 lower than the

"salt free" control. This respiratory quotient falls in the range of fat metabolism. The NH_4^+ likely has an effect to increase the use of fats in the metabolic processes.

In making these determinations the effect of the bacteria on the readings has been considered. Determinations were made of the O_2 and CO_2 exchange of the bacteria in "Cerophyl". From the records thus far obtained, it has been calculated that 31.8 percent of the O_2 and 30.4 percent of the CO_2 was actually due to the paramecia present. These percentages were used in compiling the data in Table 1.

A comparison of the results of this study and that of other workers has been made. The fact that there is so much variation indicates that a number of factors had not been controlled. One reason for the increased respiratory rate per paramecium in this study is no doubt due to the size of the animals used. The length for Paramecium caudatum given by Kudo (1939) is 200-260 micra. The strain used in this study averaged around 230 micra in length. A heavy growth of bacteria in the "Cerophyl" no doubt played a part in the increase.

So far as known, there have been no values for the CO_2 exchange published for paramecium. The results obtained in this study compare well with the values obtained for the O_2.

Peterson (1941) found that the paramecia, when placed in M/99 $SrCl_2$, diminished in size to about one-half in a few days, and that they moved with a peculiar jerky, angular

movement. The same observation was made in this study. The Sr^{++} probably has some effect on the pellicle of the paramecium.

The high respiratory rate of the paramecia in M/99 $SrCl_2$ may have a partial explanation in the fact that Peterson (1941) found a low viscosity of the protoplasm in this salt. A low viscosity would aid in rapid metabolism and gaseous exchange. Another factor which probably aids in this rapid respiratory rate is the ratio between the surface and the volume of the paramecium. In the normal paramecium of the size used in this study, 50 by 280 microns, the ratio of the surface to the volume is approximately 1/6. After they were in the $SrCl_2$ for a few days their size was approximately 25 by 140 microns. This made the ratio of the surface to the volume 1/3. This means that in the normals each unit of surface served 6 units of volume, and in the $SrCl_2$ group each unit of surface served 3 units of volume, or just one-half as much. In the "Cerophyl" group the O_2 exchange was 18 x 10^{-7} mm^3/sq. micron of surface per hour. The corresponding value for the $SrCl_2$ (A) was 87 x 10^{-7} mm^3/sq. micron, and for the $SrCl_2$ (B) it was 138 x 10^{-7} mm^3/sq. micron. There was increased exchange per unit of surface, but each unit of surface had fewer units of volume to serve. By dividing the above figures by the number of units of volume served per each unit of surface one gets the amount of O_2 exchange for each unit of volume served by each unit of surface. The values thus ob-

tained are 3 x 10^{-7} mm^3 for the "Cerophyl", 29 x 10^{-7} mm^3 for the $SrCl_2$ (A) and 48 x 10^{-7} mm^3 for the $SrCl_2$ (B). The ratio of these numbers is 1/9.6/16. It can be seen that the increase in O_2 exchange was greater than the decrease in the number of units of volume served by each unit of area. It is believed that the ratio of the surface to the volume had a part in the increase, but it is also believed that other factors are involved.

Peterson (1941) observed that NH_4Cl had a very toxic effect on the paramecia when the culture was exposed to the air, but when the culture was stoppered tightly the CO_2 content of the air rose and the toxic effects of the salt disappeared. He also found that paramecia grown in NH_4Cl when stained with Sudan III (a specific stain for lipid) showed more free lipid than the controls. This was attributed to the ionization of NH_4Cl to NH_4OH. The dialysis of this through the cell membrane would cause an excess alkalinity in the cell with a resulting fatty degeneration. In the present study a respiratory quotient of .73, which is within the range of fat metabolism, was found when the paramecia were grown in NH_4Cl. At least part of the toxic effect of NH_4Cl may have been due to fatty degeneration.

Table 1. The respiratory exchange of paramecia in various media.

	O_2			CO_2		R. Q. = $\frac{\text{vol. } CO_2}{\text{vol. } O_2}$
	gm/hr.	mm^3/hr.	mm^3/hr./sq. micron of surface	gm/hr.	mm^3/hr.	
Hay infusion	0.29×10^{-7}	.020	9×10^{-7}	0.36×10^{-7}	.013	.90
"Cerophyl"	0.56×10^{-7}	.039	18×10^{-7}	0.69×10^{-7}	.035	.90
$SrCl_2$ (A)	0.69×10^{-7}	.048	87×10^{-7}	0.86×10^{-7}	.043	.89
$SrCl_2$ (B)	1.09×10^{-7}	.076	138×10^{-7}	1.35×10^{-7}	.063	.83
NH_4Cl	0.37×10^{-7}	.026	12×10^{-7}	0.38×10^{-7}	.019	.73

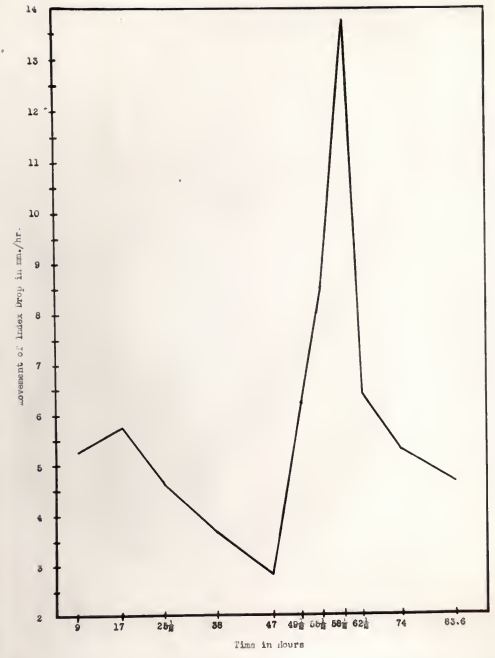

Fig. 1. The O_2 consumption of paramecia in $SrCl_2$, and the effect of food supply.

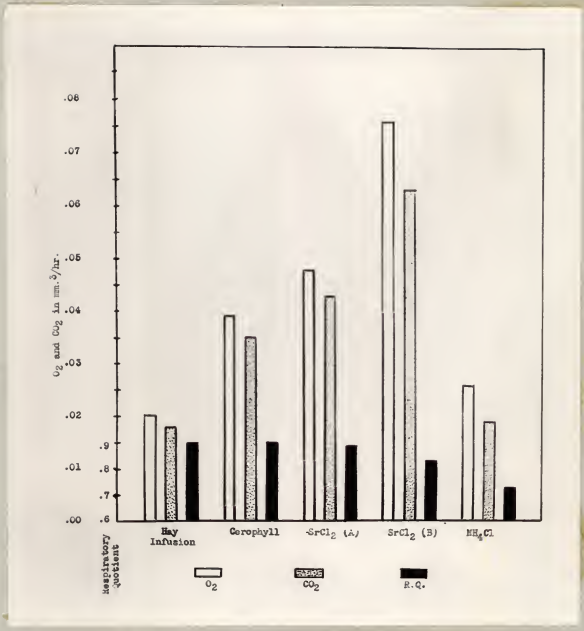

Fig. 2. The comparison of the respiratory exchange of paramecia in various media.

SUMMARY

1. The purpose of this study was to determine the effect of $SrCl_2$ and NH_4Cl on the O_2 and CO_2 exchange in Paramecium caudatum. A differential respirometer with the control vessel many times larger than the experimental vessel was used to measure the gas exchange.

2. The paramecia used in this study were cultured under sterile conditions and fed a single species of bacteria, Bacillus subtilis.

3. Controls were run in two types of media. The O_2 consumption of a paramecium in timothy hay infusion was .02 mm^3/hr., and the CO_2 production was .018 mm^3/hr. In "Cerophyl" each paramecium consumed .039 mm^3/hr. of O_2 and produced .035 mm^3/hr. of CO_2. The O_2 exchange was 13×10^{-7} mm^3/hr. per square micron of surface. In both media the R. Q. was .90. The "Cerophyl" medium was used in the experiments on the effects of salts.

4. M/99 $SrCl_2$ increased the O_2 and CO_2 exchange to .048 mm^3/hr. and .043 mm^3/hr., respectively. This was an approximate increase of 23 percent for both. The R. Q. was .89. An additional increase of 58 percent for the O_2, giving a value of .076 mm^3/hr., and an increase of 47 percent for the CO_2, giving a value of .063 mm^3/hr., was noted when the paramecia were fed bacteria from an agar slant. In this

case the R. Q. was .83. Before feeding the O_2 exchange was 87 x 10^{-7} mm^3/hr. per square micron of surface, and after feeding the value was 138 x 10^{-7} mm^3/hr. per square micron of surface.

5. M/70 NH_4Cl decreased the O_2 consumption 33 percent and the CO_2 production 46 percent. Each paramecium consumed .026 mm^3/hr. of O_2 and produced .019 mm^3/hr. of CO_2. The O_2 exchange was 12 x 10^{-7} mm^3/hr. per square micron of surface. The R. Q. in this salt was .73.

ACKNOWLEDGMENT

This study was conducted under the direction of Dr. E. J. Wimmer, Professor of Zoology. The sterile paramecia were supplied by Mr. Ralph Peterson, while the Department of Bacteriology generously supplied the Bacillus subtilis. Dr. H. W. Brubaker of the Department of Chemistry suggested the method for determination of the CO_2.

LITERATURE CITED

Brandwein, P. F.
The culturing of fresh-water protozoa and other small invertebrates. Amer. Nat. 69: 628-632. 1935.

Brown, W. G.
Growth of protozoan cultures. II. Leucophrys patula and Glaucoma pyriformis in a bacteria-free medium. Physiol. Zool. 13: 277-288. 1940.

Claff, C. L.
A migration-dilution apparatus for the sterilization of protozoa. Physiol. Zool. 13: 334-341. 1940.

Cleveland, L. R.
The separation of Tritrichomonas of man from bacteria; measurement of its growth and division rate in pure cultures of various bacteria. Amer. Jour. Hyg. 8: 256-278. 1928.

Cunningham, B. and Kirk, P. L.
A new form of differential microrespirometer. Jour. Gen. Physiol. 24: 135-149. 1940.

Fenn, W. O.
The gas exchange of nerve during stimulation. Amer. Jour. Physiol. 80: 327-346. 1927.

Fenn, W. O.
A new method for the simultaneous determination of minute amounts of carbon dioxide and oxygen. Amer. Jour. Physiol. 84: 110-118. 1928.

Glaser, R. W. and Coria, N. A.
The culture of Paramecium caudatum free from living microorganisms. Jour. Parasitol. 20: 33-37. 1933.

Guyer, M. F.
Animal Micrology. Chicago. The University of Chicago Press. 331 p. 1936.

Hargitt, G. T. and Fray, W. W.
The growth of paramecium in pure cultures of bacteria. Jour. Exp. Zool. 22: 421-454. 1917.

Hartridge, H.
Survey of methods and apparatus for micro-gas analysis. Jour. Sci. Instruments. 16: 317-324. 1939.

Heatley, N. G., Berenblum, I. and Chain, E.
A new type of microrespirometer. Biochem. Jour. 33: 53-67. 1939.

Howland, R. B. and Bernstein, A.
A method for determining the oxygen consumption of a single cell. Jour. Gen. Physiol. 14: 339-343. 1931.

Johnson, H. H.
Studies on the nutrition and reproduction of paramecium. Physiol. Zool. 9: 1-14. 1936.

Kucera, W. G.
Oxygen consumption in the male and female fly, Drosophila melanogaster. Physiol. Zool. 7: 449-458. 1934.

Kudo, R. R.
Protozoology. Ed. 2. Baltimore. Charles C. Thomas, Publishers. 689 p. 1939.

Leslie, L. D.
Nutritional studies of Paramecium multimicronucleata. II. Bacterial foods. Physiol. Zool. 13: 430-433. 1940.

Linderstrom-Lang, K. U.
Principle of the Cartesian diver applied to manometric technique. Nature, 140: 108. 1937.

Lund, E. J.
Quantitative studies on intracellular respiration. Amer. Jour. Physiol. 47: 167-177. 1918.

Parpart, A. K.
The bacteriological sterilization of paramecium. Biol. Bul. 55: 113-120. 1928.

Peters, R. A.
Nutrition of the protozoa. The growth of paramecium in sterile culture medium. Jour. Physiol. 53: CVIII-CIX. 1920.

Peterson, R. E.
The effects of some cations upon the growth rate and colloidal nature of the protoplasm of Paramecium caudatum. Unpublished thesis. Kans. State Col. of Agr. and Appl. Sci. 122 p. 1941.

Phillips, R. L.
The growth of Paramecium in infusions of known bacterial content. Jour. Exp. Zool. 36: 135-183. 1922.

Thimann, K. V. and Commoner, B.
A differential volumeter for micro-respiration measurements. Jour. Gen. Physiol. 23: 333-341. 1940.

Wood, C. S.
Part I. Methods of maintaining protozoan cultures.
Part II. A micro-respirometer for determining the metabolism of Paramecium caudatum (Ehren.) and Pelomyxa carolinensis (Wilson). Unpublished thesis. Kans. State Col. of Agr. and Appl. Sci. 46 p. 1938.