STUDIES ON A RACE OF PARAMOECIUM POSSESSING EXTRA CONTRACTILE VACUOLES

I. AN ACCOUNT OF THE MORPHOLOGY, PHYSIOLOGY, GENETICS AND CYTOLOGY OF THIS NEW RACE

ROBERT T. HANCE

Zoölogical Laboratory, University of Pennsylvania

THREE PLATES AND TWELVE CHARTS

CONTENTS

I. Introduction.....	288
II. Technique.....	288
III. Morphology.....	291
a. General description.....	291
b. The vacuoles.....	292
IV. Observations on the behavior of the vacuoles.....	294
a. Percentages—range of vacuole number.....	294
b. Change of number during the life time of the individual.....	297
c. Number of vacuoles in offspring.....	298
d. Effect of various conditions on vacuole number.....	299
V. Physiology.....	303
a. General.....	303
b. Resistance.....	307
c. Rate of vacuole contraction.....	307
d. Conjugation.....	312
VI. Genetics.....	314
a. Inheritance of vacuoles for four generations.....	314
b. Effect of selection.....	316
c. The immediate and temporary effect of location of the vacuoles in the parent form on inheritance by offspring.....	320
d. The effect of conjugation.....	320
e. Attempts to cross the two races.....	323
VII. Cytology.....	323
VIII. Discussion.....	324
IX. Summary.....	325
X. Bibliography.....	327

I. INTRODUCTION

While examining a number of paramoecia taken from a laboratory culture in the early part of January, 1915, one individual was seen to have three instead of the usual two contractile vacuoles. Subsequent examination of the stock from the original culture showed that a large percentage of the animals possessed the extra organs. Several animals with three vacuoles were therefore isolated and pure lines started from them. It soon became evident that the potentiality for one supernumerary vacuole was not only inherited but that the offspring might possess even more than one extra contractile organ. A preliminary account of the behavior of these organs in heredity was published some months ago (2).

The discovery of such a marked variety of a form which has served as a basis for so much valuable study opens an interesting field for investigation. Parts of this report must necessarily be of the most preliminary sort and various portions will be completed from time to time and published separately. The finding of the new race of paramoecium affords opportunity for comparison with the common race and for a study of the physiological activities of the two varieties. The following points will be considered more or less completely in the following paper:

1. The extra vacuole gives a definite character in the Protozoa whose inheritance can be traced. The existence of two races differing as far as is known only in this one character gives an opportunity of crossing the two races by conjugation and of determining whether inheritance in protozoa is Mendelian as it is in the metazoans.

2. A comparison of the two races may be helpful in the analysis of the function of the contractile vacuole.

3. Some light may be thrown on the process of conjugation through a study of the behavior of both races.

II. TECHNIQUE

A separate pipette was used for each culture to guard against cross contamination and was hung in a special holder attached

to the jar when not in use (3). In following the offspring of a single individual for several generations each of the daughter cells was isolated in a watch glass and as fast as the individuals divided a record was made of the number of vacuoles in each and they were again separated. The syracuse watch glasses that were used were sterilized in boiling water to which a little clean paraffin had been added. This deposited an invisible coating on the glass which was, however, sufficient to prevent the drop of hay infusion from spreading over the surface as it would on perfectly clean glass. On the paraffined surface the fluid rounds up and presents the minimum surface for evaporation (3). It was found that the most satisfactory pipette for this work had a short tip of almost hairlike fineness. The method of making these has been described (3).

It is rather difficult to find a method of slowing down the animals for the purpose of making accurate observations without killing them. The first method used was to place an individual in a small drop of water and over this a cover glass was lowered with the aid of a fine pair of forceps. The cover had a drop of hardened balsam at two corners which had been filed down to the proper thickness and which prevented it from crushing the animal. The excess fluid was drawn off with a piece of filter paper. To recover the animal the slide was tilted over a watch glass and by means of a stream of hay infusion the cover and whatever was under it were flushed into the crystal. This method was very laborious and slow, and the chances of losing the paramoecium great. The following method has proved to be the better: An individual is placed on a slide in a fairly large drop of the medium. The liquid is drawn off with a pipette under the dissecting microscope, the animal being kept as nearly as possible in the center of the drop. Finally, enough of the fluid is removed so that the adhesion of the surface film to the slide exerts sufficient pull to hold the animal quiet. In the center of the drop the pressure is least and it seldom causes a paramoecium to burst. Toward the edges, however, the chances are very great that the animal will succumb almost instantly to the greater pressure. In such a preparation the

pressure at the center tends automatically to lessen as the surface tension slowly draws the liquid from its close adhesion to the slide and rounds it up. This takes a sufficient length of time, however, to allow accurate observations to be made under the compound microscope. Occasionally the pressure is relieved too quickly and the paramoecium is given enough fluid to move about in, which makes observation with the high power impossible and the process has to be repeated. As soon as the examination has been made, a drop of fresh hay infusion is added. To drop this liquid immediately on top of the animal after it has been under pressure may frequently crush it and the safer way is to allow the fresh fluid to flow from the side into the drop containing the animal. The paramoecium may then be picked up in a pipette and placed in its respective watch glass. When examining paramoecia in pure lines a separate pipette is used for each line.

Under the 16 mm. lens when the animal is compressed, the vacuoles stand out with diagrammatic clearness appearing as so many holes punched through the cell and as a rule are to be found close to one side and almost invariably lying in a straight line. This latter characteristic was one of the criteria for differentiating between the contractile vacuoles and food vacuoles when these were so numerous as partly to obscure the former. The comparative lack of refraction of the pulsating organs was another basis of distinction. When, however, the refraction of the food vacuoles was almost identical with that of the contractile vacuoles under the low power, the 4 mm. lens was swung into place and the contraction of the supposed contractile vacuoles was watched for. It has been observed that the vacuoles in animals raised in watch glasses are more difficult to see than they are in animals living in larger amounts of medium. In the watch glasses the food vacuoles have a lack of refraction which gives them an appearance very much like the contractile vacuoles whereas the food vacuoles in animals raised in the large culture jars frequently appear very nearly black.

Cytological technique. For measuring and drawing the whole paramoecium Worcester's fluid was used (10 per cent formalin

saturated with mercuric chloride). Animals fixed in this way can be immediately cleared in glycerine which brings out many of the internal parts very well, and the vacuoles, if they happen to be expanded at the time of fixation, are visible. For whole mounts and sectioning, paramoecia were fixed in Schaudinn's, Gilson's, Flemming's and Worcester's solutions. As comparatively little time has been devoted to cytological studies I am not prepared at present to offer any criticism of the fixatives.

To Dr. M. H. Jacobs I am indebted for his constant interest, advice and criticism during the progress of the work recorded here and I also feel under great obligation to the other members of the Zoological Department of the University of Pennsylvania whose many kind suggestions have been of great assistance to me.

III. MORPHOLOGY

a. General description

In general form, the animals of this race are apparently identical with the common slipper-shaped *Paramoecium caudatum*. The newly discovered individuals average rather larger than any of the representatives of the two-vacuolated race I have found about Philadelphia. Chart 1 shows a curve plotted to illustrate the range in size. The peak of the curve is at $233\ \mu$ and is very nearly midway between the two extremes. On this same chart are plotted the ends of two curves formed by animals taken about Philadelphia. The range of one race is from $153\ \mu$ to $207\ \mu$ and in the other from $106\ \mu$ to $173\ \mu$. In the collection of slides belonging to the Department I have found paramoecia somewhat larger than the new race ranging from $197\ \mu$ to $325\ \mu$. The extremes of all the races studied by Jennings were $50\ \mu$ and $332\ \mu$.

In certain cultures where the individuals were unusually favorable for study a band slightly darker than the surrounding protoplasm could be seen across the center of the animal in the region where the constriction appears at the time of division (figs. 4 and 6). It is interesting to note that in these cultures

Chart 1 On the axis of abscissas are marked the various sizes in μ while on the axis of ordinates are placed the percentages. The heavy solid line represents the multivacuolated race of paramoecium while the light broken line indicates the larger sizes of a small two-vacuolated race and the light unbroken line is based on figures obtained from fixed preparations of two-vacuolated animals in the Laboratory collection.

the percentage of animals with three and four vacuoles was very high. The consideration of this phenomenon will be left for future work.

b. The vacuoles

1. *Location.* So exactly are the contractile vacuoles located that a single straight line parallel to the outline of the animal might be drawn passing through the center of each. In a very few cases I have found one or two vacuoles slightly displaced from the usual straight row condition (fig. 9). In the majority of individuals the extra vacuoles are located in the posterior part of the animals as can be seen from the drawings. In a few

instances, however, one extra vacuole was found in the anterior end (fig. 3), but I have never found more than two vacuoles located in the foremost half of the body. It is a curious fact that, rare as this condition is in the normal medium of hay infusion, in cultures to which a little sea salt has been added paramoecia with two vacuoles in the anterior end have been found to be much more numerous. As yet no plausible explanation for the increase of this rare arrangement has been found—if, indeed, it is not merely a coincidence.

There can be no doubt that the contractile vacuoles are very definitely located organs and fixed in position for a given individual. When an animal is swimming the vacuoles can be seen to turn with the cell and always maintain the same position in relation to each other. As far as I have been able to determine, both the radiating canals and what appears to be the excurrent pore or tube aid in holding the vacuoles in position and particularly the latter. That there is a connection with the outer wall of the animal can be seen when a paramoecium bursts directly opposite to a fully expanded vacuole, when the vacuole, drawn by the outflowing protoplasm, can be seen straining at some retaining fastening.

2. *The vacuoles.* Under normal conditions the vacuoles in this race measure on the average about $10\ \mu$ in diameter with a capacity of 500 cubic micra. There may be some variation in this size due to the age of the vacuole, i.e., the length of time since it had appeared, but I think that it is safe to say that the diameter of fully formed vacuoles is very nearly the same. There can be no question that these vacuoles originate separately as they generally lie at some distance from each other and no cases have been seen when a vacuole appeared as though it had been separated by division from another vacuole. The average size of the vacuoles in the common race which I have observed is $11\ \mu$ with a cubic content of 664 cubic micra. In my fixed preparation the vacuoles are surrounded by what appears to be a definite morphological membrane (fig. 16). When animals have been kept at a temperature a few degrees above freezing for a few hours the vacuoles expand and do not contract and it is

interesting to note that the anterior vacuole becomes from three to four times the diameter of those in the posterior end. This, in the cases studied, was not true for the common two-vacuolated race, neither vacuole, exceeding the other in size.

IV. OBSERVATIONS ON THE BEHAVIOR OF THE VACUOLES

To sum up briefly our knowledge of contractile vacuoles:

The contractile vacuole is a pulsating organ surrounded by a membrane of physiological importance, belonging to the ectoplasm. These vacuoles form an osmotic system and the evidence at hand shows them to function in part as excretory and respiratory organs.

a. Percentage of variation in contractile vacuole number

After the pure lines that had been started had multiplied considerably examination was made of a number of individuals in each line. Apparently these high numbers of vacuoles were not in every case passed on to the offspring for in all of the cultures, whether started with three or four vacuolated paramoecia, there were considerable numbers of two vacuolated forms. But on the contrary in the pure lines started with three vacuolated forms there were found individuals possessing four contractile organs. Observations made on one culture in the early days of the work showed a range of variation in vacuole number in the following percentages.

	<i>per cent</i>
Two vacuoles.....	8.6
Three vacuoles.....	65.7
Four vacuoles.....	25.7

Later and more accurate observations were made for a period of thirty days on one culture (covering 663 individuals) and the results for that time averaged showed the proportion to be:

	<i>per cent</i>
Two vacuoles.....	16.13
Three vacuoles.....	66.48
Four vacuoles.....	16.43
Five vacuoles.....	0.904

In both of these tables, and in all of the following work, the three vacuolated animals show themselves to be in the excess in nearly all cases. The statement made in my first note on this race that there was an apparent tendency for the vacuole number to settle down with three as the standard must be retracted. When the note was written I was not familiar with the response of the vacuole number to various conditions (rate of division, age of medium, etc.).

The last table shows that a five-vacuolated paramoecium appeared. Animals with five vacuoles, while not rare, seldom have been found in a percentage greater than the one indicated. The cause of the limited production of these extreme forms will be discussed under Section VI. Recently four paramoecia have been obtained with six contractile vacuoles and one with seven (figs. 10 and 12). It is a striking sight to see a paramoecium with six or seven vacuoles in a row all swelling and contracting regularly. The six vacuolated condition is very rare and in over a year I have not seen more than a half dozen animals thus equipped, and the majority of these I have found recently. The only cases seen in 1915 were very doubtful as the contractile vacuoles were hidden by food vacuoles of similar refractiveness.

The above results were obtained on cultures maintained under relatively similar conditions and it is interesting to compare them with the average of the results obtained from a number of cultures under observation for a much longer time and under much more varied conditions. The data from which charts 2, 3, 4 and 5 were made served as a basis for these calculations. These data were obtained on paramoecia under exceedingly varied conditions—in cultures ranging from freshly made up infusions to cultures that were very old, in cultures where plenty of medium was present and in cultures that were undergoing rapid evaporation, in culture during conjugation and after the process had stopped. The averages given below are based on an examination of 9818 animals.

	<i>per cent</i>
Two vacuoles.....	23.5
Three vacuoles.....	61.3
Four vacuoles.....	13.4
Five vacuoles.....	0.22
Six vacuoles.....	0.042
Seven vacuoles.....	0.0001

Chart 2 This chart gives a graphic illustration of the comparative numbers of two, three, four, five and six-vacuolated animals. This chart was prepared from data on ten thousand animals living under all conditions of medium.

Chart 2 illustrates these percentages graphically. In comparison with the twos, threes and fours, the fives and sixes, and especially the latter, scarcely appear above the base line.

b. Variability in vacuole number in the life cycle of the individual

While making the above studies it became evident that the time of day apparently had some influence on the various percentages. This suggested that the period in the life cycle of the individual at which the observation was made might play a rôle in determining the general percentage of the various vacuole numbers. (In this paper reference to the life cycle of the paramoecium always refers to the period between transverse divisions.) A single individual showing two vacuoles was therefore isolated and allowed to divide several times. Examination was then made of all the offspring and each was isolated in a separate watch glass. At intervals of from three to five hours the animals were again examined with the following results.

As can be seen, paramoecia possessing two contractile vacuoles increased this number in several cases before division. In some of the individuals the number was not increased. However, continued work with the two vacuolated forms has demonstrated that they have not lost their power of producing multi-vacuolated daughters. In one case I have followed individuals through three generations (representing fifteen animals in all) without a three vacuolated form appearing and then the extra vacuolated animals were produced in just a large percentages as in other lines. This point will be more concretely illustrated under 'selection,' page 316.

The potentiality for the higher number of contractile vacuoles is maintained although several generations may be passed before it exerts itself or, better, before the conditions are such that it must exert itself.

c. The number of contractile vacuoles in the offspring

When normal individuals get ready to divide, and when the beginning of the constriction has appeared, two new vacuoles are added so that immediately after division the daughter cells are fully equipped with excretory organs. In this new race, as a rule, two vacuoles are added at this time, although cases appear when only one seems to be formed. In a certain percentage, apparently more than two have been added before division but whether the vacuoles in excess of two were added before or after division cannot be ascertained from my data, as the observations were not, as a rule, made until some time after separation had taken place, in which case the extra vacuoles might have been formed after division. In a few cases division occurred without the formation of any new vacuoles.

For 225 animals in:

15.11 per cent—one vacuole was added
 54.66 per cent—two vacuoles were added
 30.22 per cent—more than two vacuoles were added

From this it is evident that in at least 50 per cent of the cases the usual normal addition of two new vacuoles prevails as in the common race.

With these facts in hand, the number of vacuoles possessed by the offspring of the individuals of this new race may be understood. It is practically impossible, however, to predict with certainty what number of contractile vacuoles the offspring of any particular paramoecium will have immediately following division. Some of the possibilities observed up to date are illustrated below.

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)	(n)
2	2	2	3	3	3	3	4	4	4	5	5	5	6
∧	∧	∧	∧	∧	∧	∧	∧	∧	∧	∧	∧	∧	∧
2 2	2 3	3 3	2 2	2 3	3 3	4 2	2 3	4 2	3 3	4 2	3 3	5 2	4 2

As stated above, as a rule two vacuoles are added at the time of division (one to the anterior end and one to the posterior end). a, e, i, j, and m represent cases where presumably such an addition has taken place. In b, c, f and g more than two vacuoles have been added although as pointed out above this may be due to post divisional additions rather than to additions at the time of constriction. d, h, and l represent cases where only one vacuole, has been added, while n indicates that there has been no increase whatever.

d. Effect of various conditions on the vacuole number

Division rate. Early in the course of these observations it appeared that in newly started cultures of fresh hay infusion where the animals were increasing rapidly the percentage of two-vacuolated forms was frequently very high. On the other hand, in older cultures where the division rate was presumably slower the percentage of multi-vacuolated animals outnumbered the twos. To test this point more accurately, a number of cultures were set up to see whether this condition was the rule. From the time the cultures were well established records were made of the percentage of individuals possessing two, three, four or five vacuoles in each jar every day, or every few days, extending over a period of from two to three months. These records supplied the data for drawing up the accompanying graphs. The results of these experiments have been plotted in various ways to show conclusively that the mode of behavior is essentially always the same.

In all the charts the three-vacuolated animals are represented by a heavy broken line, the twos by a heavy unbroken line and the fours by a light unbroken line and when the fives are drawn in a light broken line is used. The figures placed across the various curves in all the charts except No. 5 indicate the number of vacuoles in the form which the curve represents. Chart 3 represents a single culture. It can be seen at a glance that although the twos are at first more numerous their line drops rapidly until it meets the axis of abscissas on March 10 while

the threes and fours rise to a certain point which is maintained with more or less fluctuation to the end. Chart 4 is drawn from figures based on the average of three cultures and shows the various curves to follow practically the same paths that they did in the preceding graph, and although the twos in this case

Chart 3 In this chart and in all succeeding ones the axis of abscissa represents time in days and the axis of ordinates represents percentages. The description of this chart is in the text, page 299.

Chart 4 This graph is described on page 300.

did not begin in the majority, they nevertheless drop toward the base line noticeably. Chart 5 is a striking confirmation of the two previous charts. Six cultures were followed and the results plotted on the one chart. The fours play a comparatively unimportant part and will not be discussed. The X formed by the crossing of the unbroken line representing the two vacuolated animals falling from high on the axis of ordinates to the axis of abscissas by the dotted curve of the three-vacuolated paramoecia rising from low percentages to higher ones is very marked.

Chart 5 The description of this chart is on page 301.

The numbers on the various curves indicate the number of the culture and not the number of the vacuoles as on the other charts. The same method of designating the various forms is used as on the other graphs.

Still more interesting are the results obtained from March 15 on and represented on this graph. Cultures 1, 3 and 5 were undisturbed while fresh hay infusion was added to cultures 2, 4 and 6. The results for each three cultures were averaged and plotted. 1, 3 and 5 continued as before while 2, 4 and 6 due to the acceleration of the division rate caused by the addition of fresh infusion show a rapid increase of two-vacuolated paramoecia with a corresponding drop in the threes. By April 6, however, the rate of increase had slowed down and the curves of the one set of cultures were very close to the corresponding curves of the other set. Chart 12 while drawn primarily to illustrate the

effect of conjugation shows conditions similar to the above. Chart 6 illustrates graphically the relation between the division rate and the percentages of animals possessing various numbers of vacuoles. The very heavy line represents the division rate and it can be readily seen how the percentage of two-vacuolated

Chart 6 This graph compares the fluctuations of the number of two, three and four-vacuolated paramoecia in a single culture for a period of nearly two months with the daily division rate. The division rate was obtained by isolating a number of single animals in separate watch glasses in fluid filtered from the main culture. At stated intervals an average of the number of divisions that had occurred in all the watch glasses was struck. The figures in circles at the extreme left of the chart indicate the number of divisions and the broad black band traces the division rate across the graph. The other figures are the same as have been used on previous charts. It is evident from this chart that in general while the threes and fours increase as the division rate decreases there is apparently another factor at work that cannot be accounted for on this basis.

animals rises and falls with the increase or decrease of the fission rate.

Age of the culture. To determine whether paramoecia divide more rapidly in fresh hay infusion than in an old, worn out medium, liquid was filtered from a culture nine months old. A few drops of this fluid was placed in several watch glasses and a paramoecium taken from the same culture as the liquid was isolated in each. From this same old culture single paramoecia were placed in similar amount of fresh hay infusion. In six

days all of the animals isolated in the fresh medium had divided twice producing four animals, while one of the animals in the old culture had divided once, one had not divided at all, and two had died. Even in the new medium the division rate was apparently very slow, but the animals were so starved that it took them several days to return to the normal condition. After starved paramoecia have been abundantly fed I have always found their division rate to increase to normal.

This experiment was repeated using animals that were not starved. The division rate for a period of five days was 2.7 in the old infusion against 5.6 divisions of sister paramoecia in freshly made hay medium. A more complete account of the effect of various media will be given under Physiology.

V. PHYSIOLOGY

a. General

It has been over two years since the original animals of the new race were discovered and they not only show no indication of dying off but have given much evidence of being a line superior in vitality to any of the two-vacuolated paramoecia with which they have been compared. Cultures of this new race have been kept in various laboratories of the Zoological Department of the University of Pennsylvania and in the course of time, since no particular care was taken to prevent the mixture of various cultures, the multi-vacuolated animals found their way into the general cultures and it was not long before they had displaced the common form. For some time this year it was impossible to find a normal two-vacuolated paramoecium in any of the cultures maintained in this laboratory. This extra vacuolated race is made up of rather large animals and under many adverse conditions these paramoecia surpass the common races in viability and vigor.

One of the characteristics of the race in question that was first observed was the extreme resistance it seemed to possess to culture media which were so old that they would probably not have supported the common race of caudatum. Several cultures made up at the beginning of the summer recess came

through the hot weather in a very healthy condition although the cultures had remained in a room and in which the temperature was at times quite high. Cultures are being carried along at present that are nine months old and although most of the animals have died there are still a few very active paramoecia remaining.

The division rate for this race has a somewhat wider range than I have observed in the case of other races. Under normal conditions (fresh or nearly fresh hay infusion) these animals divide from one to three times in twenty-four hours. When the culture medium is older the rate may drop below this. There have been times, however, when the animals under observations divided as rapidly as five times in twenty-four hours and this rate was maintained for six or seven days. This rate of division is much more rapid than is usual for the two-vacuolated races.

In an attempt to determine whether or not the rapidity of division is the sole agent in the production or inhibition of the formation of extra vacuoles, several single individuals were isolated in watch glasses containing hay infusions that differed widely in age and condition. Three sets of experiments were set up in each kind of infusion and in three days the number of divisions that had occurred and the percentage of two, three and four-vacuolated animals that were present was recorded for each of the three sets of experiments and for each type of medium. The results obtained in each infusion were averaged and are given below. All the experiments were repeated to determine whether the results of the first set would be constant. The cultures from which the animals for this experiment were drawn was started from a single individual. The number of divisions given is for a period of three days.

1. Medium filtered from culture nine months old

NUMBER OF DIVISIONS	EXPERIMENT A	NUMBER OF DIVISIONS	EXPERIMENT B
3 {	Two vacuoles, 44 per cent Three vacuoles, 48 per cent Four vacuoles, 8 per cent	2.7 {	Two vacuoles, 31 per cent Three vacuoles, 61 per cent Four vacuoles, 8 per cent

2. Medium filtered from culture from which paramoecia were obtained to conduct these experiments. Culture was about one week old

NUMBER OF DIVISIONS	EXPERIMENT A	NUMBER OF DIVISIONS	EXPERIMENT B
4.3 {	Two vacuoles, 81 per cent Three vacuoles, 18 per cent Four vacuoles, 1 per cent	3.7 {	Two vacuoles, 30 per cent Three vacuoles, 70 per cent

3. Hay infusion freshly made up

NUMBER OF DIVISIONS	EXPERIMENT B
5.6 {	Two vacuoles, 82 per cent Three vacuoles, 18 per cent

4. Medium filtered from culture containing many animals which had been allowed to evaporate (concentrate) and had been refilled with fresh hay infusion four times. The concentration of the metabolic products present was doubtless quite high and the culture had a very strong odor. At the time this experiment was started fresh infusion had been added to the culture about twenty-four hours

NUMBER OF DIVISIONS	EXPERIMENT B
4.5 {	Two vacuoles, 37.5 per cent Three vacuoles, 50 per cent Four vacuoles, 12.5 per cent

The foregoing data point strongly, I think, to the fact that rapidity of division plays a very important rôle in influencing the production of the extra vacuoles. Apparently one of the chief requirements for the production of the extra vacuoles for which this race possesses the potentiality is sufficient length of time between divisions. When the rate of division is rapid the two-vacuolated animals tend to predominate as illustrated by Experiment A, 2; and Experiment B, 3. With a slower rate of multiplication there appear more three and four-vacuolated paramoecia as the results of Experiments A, 1 and B, 1 and 2 show. Moreover, in old cultures where the division rate is low two-vacuolated animals are very scarce when not absolutely lacking. While there is some slight variation in the results of the corresponding sets of experiments I am not at present inclined to

believe that this fact interfered with any of the above statements, as the variability of individuals may readily account for this. In general all of the results indicate that extra vacuoles appear as a rule when the rate of fission is relatively slow. Chart 6 compares graphically the division rate with the percentages of two, three and four-vacuolated, animals.

There are, however, enough exceptions to the general rule to suggest the probability of some other factors playing a part and very likely, not an unimportant part in determining the number of vacuoles. As yet, I am not certain what this factor or these factors may be, but Experiment B, 4 gives at least the basis of a working hypothesis. In this particular experiment although the division rate was high the number of two-vacuolated paramoecia developed was 43.5 per cent under the number produced in Experiment A, 2 where the rapidity of division was approximately the same. The difference between the experiments lay in that the fluid used in Experiment B, 4 contained many times the amount of metabolic waste products present in Experiment A, 2. From this result the tentative suggestion may be made that although the rate of division seems to be the primary agent in influencing the vacuole number, the presence of waste products in the environment has an activating effect on the production of extra vacuoles. Indeed if the amount of the waste products of metabolism is sufficient it may in part offset the effect of a rapid division rate.

Merely slowing down the rate of division is not sufficient, as this was tried by placing six paramoecia in a constant temperature room at 12°C. In four days two had divided only once and the rest not at all while the controls at room temperature had each divided three times. When the animals in the cold room were examined it was found that the vacuole number had not increased at all. Under these conditions although division did not occur for four days, the temperature lowered the metabolic activities of the paramoecia and made the production of extra vacuoles either impossible or unnecessary.

b. Resistance

This race has proved itself to be more resistant to all the abnormal conditions it has been subjected to than have the common animals.

Heat. Dr. Jacobs has found that under conditions where the ordinary paramoecia are killed at temperatures of 40° to 42° C. the race in question may survive an exposure to 44°. Animals that have been living in a hay infusion to which a small percentage of sea salt has been added resist high temperatures for very much longer periods of time than are paramoecia taken from pure hay infusion. My experiments along this line are, as yet, not sufficiently extensive to base conclusions.

Cold. A few animals of the multi-vacuolated race withstood an air temperature of

1° to 2° for 45 hours
-3° to -4° for 2½ hours

The common race was killed in the first case between five and thirty hours and in a trifle over an hour in the second experiment. In this time, although ice crystals were formed, the fluid did not freeze solid at this low temperature.

Distilled water. Mr. Mitchell Carrol, of the Zoological Laboratory of the University of Pennsylvania, has maintained this race of paramoecium in distilled water for fifteen generations, covering a period of forty-four days. In similar experiments of my own these animals showed the same ability to live in distilled water.

c. Rate of vacuole contraction

A few observations on the rate of vacuole contraction under varying conditions have been made on this new race of Paramoecium to determine whether there is any essential difference between this and the common stock. Two methods have been employed. In the first method each vacuole as it contracted was called off to an assistant who noted the time. Several observations were made on each vacuole and then the records obtained for each vacuole were averaged. In all forty-four animals were studied at 23°C. with an average of three observations per vacuole per animal.

	FIRST VACUOLE	SECOND VACUOLE	THIRD VACUOLE	FOUR VACUOL
Two vacuolated animals.....	9	9		
Three vacuolated animals.....	10	13	13	
Four vacuolated animals.....	11	12	14	9

The second method employed, though not so accurate as the first since only one observation per vacuole per animal was obtained, gave results which are approximately the same as the first. The time of contraction was taken with a stop watch from the instant the vacuole disappeared until it disappeared again. With animals possessing three or more vacuoles two watches were frequently used as the paramoecium would often dry up if each contraction was timed separately. The results obtained at each temperature are given below. The figures are based on observations of thirty-five paramoecia.

The well known fact that the rapidity of vacuole contraction increases with the temperature is again brought out in these tables. It is evident in the multi-vacuolated race that there is a

Multi-vacuolated race. Time in seconds; temperature in Centigrade

	FIRST VACUOLE	SECOND VACUOLE	THIRD VACUOLE	FOURTH VACUOLE	TEMPERA- TURE
					<i>deg. C.</i>
Two vacuolated animals..... {	8	13			22.0
	9	10			23.0
	10	9			24.0
Average.....	9	11			
Three vacuolated animals..... {	13	12	12		20.0
	11	12	13		20.5
	8	8	10		24.0
	10	12	12		22.5
Average.....	10.5	11	11.7		
Four vacuolated animals..... {	12	14	13	12	20.5
	10	9	13	12	23.0
Average.....	11	11.5	13	12	

Common two-vacuolated race

FIRST VACUOLE	SECOND VACUOLE	TEMPERATURE
10.7	10.9	20.5
10.2	9.8	22.0
10.0	10.6	25.5
Average....10.3	10.4	

progressive lessening of rate of pulsation from anterior to posterior end. This is not so evident in the records of the two-vacuolated race as both vacuoles appear to beat in almost identical rate. While the observations were not taken at the same temperature, the relative frequency of the various vacuoles in each case would remain approximately the same.

An interesting condition appears in the first table by averaging the average time of contraction of the vacuoles in each group. The result is:

	<i>seconds</i>
Two-vacuolated group.....	10
Three-vacuolated group.....	11
Four-vacuolated group.....	12

There is a steady slowing down of the rate of pulsation as the number of vacuoles increases. Whether this will be borne out by more extensive studies remains to be seen.

1. *The effect of salt solutions on the rate of contraction.* The work of other investigators has shown that marine protozoa seldom possess contractile vacuoles and that when fresh water forms are subjected to saline solutions the rate of contraction decreases greatly and in some cases the vacuole disappears entirely (6). This race was subjected to salt solutions of various strengths to determine whether it was possible to cause some of the extra vacuoles to disappear permanently. Enough sea salt was added to hay infusion to bring the percentage of salt to 0.06 per cent or to 0.1 per cent. The animals could not withstand an immersion in a solution as strong as 0.5 per cent immediately but they survived very well if the percentage was allowed to in-

crease slowly through evaporation.¹ The salt slowed the contraction rate but did not in any way influence the number of vacuoles. The most marked effect in this direction was while the animals were still unaccustomed to their environment. After they had become somewhat acclimated, to the new conditions after which the rate of pulsation became more normal although it never regained the usual rapidity. This may account in part for the varying results noted below.

	FIRST VACUOLE	SECOND VACUOLE	THIRD VACUOLE	TEMPERA- TURE	CONCEN- TRATION OF SALT
				<i>deg. C.</i>	<i>per cent</i>
Two vacuolated animals..... {	25	25		20.0	0.06
	27	23		21.5	0.1
	34	23	45	20.0	0.06
Three vacuolated animals..... {	14	19	18	21.5	0.5
	26	19	18	21.5	1.0

2. *The effect of cold on the rate of contraction.* Cold slows the rate of contraction markedly and extreme cold, i.e., 1° to 2°C. apparently brings contraction almost to a complete stop.

3. *The volume of the vacuoles of the two races.* In making this comparison the volume of the animals was calculated in the following manner. The percentage of difference in weight of a paramoecium modeled in clay and a cylinder of the same length and diameter made of the same material was determined. The volume of a cylinder the length and diameter of a living paramoecium was calculated and the volume of the paramoecium was obtained by reducing the volume of the cylinder by the determined percentage of difference between the clay models.

¹ It might be noted here that cultures containing a small percentage of salt thrived wonderfully and the infusion seldom became as murky or as bad smelling as the untreated cultures. It seems likely that the salt prevents the great growth of bacteria producing putrefaction, but it seems to interfere in no way with the supply of food for the paramoecia. Animals treated in this way are more tough and when they burst under pressure the protoplasm oozes out in a thick mass instead of the usual thin stream. Frequently the animal is able to clear itself of the extruded mass and the mutilated area is covered over. Under normal conditions the ejection of the protoplasm is so rapid that there is no possibility of this recovery. This treatment might be of assistance with individuals to be operated on.

The diameter of the vacuoles was obtained from a number of fixed preparations some of which were sectioned.

Multi-vacuolated race

Average length.....	233 μ
Average width.....	51 μ
Volume.....	284, 493 cubic μ
Capacity of each vacuole.....	500 cubic μ (diameter, 10 μ)

To deliver themselves of a quantity of liquid equal to the volume of the animal, at a temperature of 22°C., the

2 vacuolated animals contract 284 times in 47 minutes at a 10 second rate
3 vacuolated animals contract 190 times in 35 minutes at a 11 second rate
4 vacuolated animals contract 142 times in 28 minutes at a 12 second rate
5 vacuolated animals contract 114 times in 25 minutes at a 13 second rate
6 vacuolated animals contract 95 times in 22 minutes at a 14 second rate

The rate for the five and six vacuolated animals has been based on statistics given above (page 309).

Common race. Large individuals

Average length.....	265 μ
Average width.....	53 μ
Volume.....	397, 690 cubic μ
Capacity of each vacuole.....	664 cubic μ (diameter, 11 μ)

At the same temperature these animals require forty-nine minutes to pump out a quantity of water equal to the volume of the animal.

Small individuals

Average length.....	180 μ
Average width.....	53 μ
Volume.....	194, 260 cubic μ

This lot would require twenty-three minutes for the same operation.

It is evident from these figures that the new paramoecia are superior to the larger forms in the quantity of liquid they can excrete in a given time but in general are not quite equal to the smaller races in this respect. I have been unable as yet to obtain a wild race as large as these new animals to compare their respective resistances and this will form an interesting part of future work. I have not attempted as yet to determine whether there is any correlation between resistance to various substances and the number of vacuoles but the addition of vacuoles in the individuals as the culture ages and the conditions are presumably becoming more unfavorable is a response that is very suggestive.

d. Conjugation

A cycle of conjugation was awaited for over a year and then a method of bringing it about at will was hit upon accidentally. Two cultures which had been started with 200 cc. of hay infusion were allowed to stand uncovered. In seven days the fluid had evaporated to one half and a strong epidemic of conjugation was observed. The experiment was repeated successfully eight times, conjugants always appearing when the fluid had been reduced to approximately one half its original volume. With the containers used this usually required a week.

From these results it seemed probable that conjugation had been produced by the rapid concentration of metabolic products. To test this theory further a number of cultures were set up. Some jars were covered and others were allowed to evaporate. The jars used were much smaller than the original containers and the liquid surface exposed was not more than one third as great. Consequently the evaporation was much slower and three weeks were required to reduce the liquid to the point that one week produced in the larger jars. In three jars that contained (1) pure hay infusion, (2) hay infusion plus 0.05 per cent sea salt, and (3) hay infusion plus 0.1 per cent sea salt, although evaporation did not take place conjugation began in (3) in thirty-six days, in (2) in thirty-eight days and in (1) in thirty-nine days. These cultures had been started in drinking glasses. Three cultures similarly made at the same time were allowed to evaporate. No conjugants appeared. When the medium became very low in the container it was replenished with fresh infusion and again allowed to evaporate. This was repeated five times and no sign of conjugation was observed.

During the year previous to these experiments cultures had been made up using 500 or 600 cc. of liquid and in this time as has been stated no conjugation took place. Conjugation has been obtained in eighteen cultures when the exposed surface of the fluid was sufficient to permit rapid evaporation at room temperature or when the quantity of fluid was small and the multiplication of the animals rapid.

It is of considerable interest to note that when conjugation did take place in cultures to which a minute quantity of sea salt had been added the epidemic did not last nearly as long as in the untreated infusions. In one set of experiments conjugation lasted for twelve days in the unsalted culture while in the culture containing 0.1 per cent sea salt it died out in three days. Another time an untreated medium maintained conjugation for thirty-three days against thirteen and nineteen days in two salt cultures. The length of one of the periods of conjugation is figured on chart 7.

Chart 7 This chart was drawn from records obtained from a single culture and illustrates the percentage of conjugants to unfused forms found during a cycle of conjugation. The figures in the circles at the extreme left of the chart indicate the percentage of conjugants and the heavy black band traces the fluctuations of the percentage of conjugants across the graph. The other curves represent either individuals or conjugants and are labeled on the chart. It will be noted that when the number of conjugants is high the percentage of three and four-vacuolated forms decrease and increase as conjugation dies out.

In brief, conjugation has been obtained in cultures that have been started with single individuals under the following conditions.

1. Conjugation appeared in practically all cases when the culture was evaporated rapidly causing the metabolic products to concentrate considerably.

2. Conjugation appeared in cultures having a comparatively small amount of fluid supporting a large number of paramoecia.

3. No conjugations were seen in some cultures undergoing the same treatment as 1 and 2 particularly when the concentration was not rapid.

4. No conjugation took place in cultures made up with a large amount of fluid.

5. When a slight amount of sea salt was present conjugation sometimes occurred a little before the control of plain hay infusion showed signs of this condition and in all cases the epidemic of conjugation in the saline solutions died out before it did in the pure hay media.

As a discussion of the various theories of conjugation in relation to the results given above is somewhat foreign to the main subject of this paper a full consideration of the phenomena will be published separately.

VI. GENETICS

The account that precedes this section has dealt with the general behavior and inheritance of the contractile vacuoles. In this section will be considered the inheritance of the extra organs for several consecutive generations and the effect of selection and of conjugation on the race.

While these experiments might have been carried on for more generations the technical difficulties were so great as to make further pursuance of this line of investigation unpracticable. In this part of the work the animals had to be examined with the 4 mm. objective in many cases to be certain of the number of vacuoles they possessed, and to do this the animals could be allowed so little fluid to move in that many times I have lost them through drying thereby breaking the continuous line of succession. Indeed, dozens of experiments were started before the results illustrated below were obtained.

a. The inheritance of the contractile vacuoles for four generations

Lines were started with one two, one three and one four-vacuolated paramoecium. As rapidly as they divided the num-

ber of vacuoles in the daughter cells was recorded and the animals were separated and again allowed to divide when the operation was repeated. The results of these experiments are shown in the charts 8, 9 and 10. The last generations on chart 8 and 9 show practically 50 per cent of twos and threes. The same generation on chart 10 shows a much higher percentage of twos. A study of these charts will, I think, convince the reader that

Chart 8 This figure illustrates the mode of inheritance for four generations from a two-vacuolated progenitor. The heavy figures indicate the number of vacuoles for any descendant.

the number of vacuoles any one individual may possess is no indication of its potentialities—that the fewer-vacuolated paramoecia may give rise to animals with more vacuoles and vice versa. The last statement was demonstrated by starting cultures with several of the animals possessing different numbers of vacuoles from the fourth generation of these lines. After the lines had multiplied considerably examination showed the percentages to be in no way different from those given throughout this paper. In the case of experiments such as these the two-

vacuolated forms tend to predominate, as fresh hay infusion was used for each generation. As has been pointed out before, fresh medium causes the animals to divide more rapidly and that rapidity of division may be said in a general way to keep the number of vacuoles low.

Chart 9 In this case the line was started with a three-vacuolated paramoecium.

b. Effect of selection

Selection has been carried on in three ways.

First method. Individuals possessing two, three, four and five vacuoles were isolated. When they had divided, the number of vacuoles in each was recorded and, in the case of the two-vacuolated line, the paramoecium with the lowest number of vacuoles was allowed to continue while in the three, four and five-vacuolated lines the highest vacuolated animals were always selected. The individuals not selected for continuing the lines were discarded. This plan was carried on for a period of from nine to fourteen generations and the results are given on chart 11. Here the vacuole number of the progenitor is placed at the

top of the line in a diamond-shaped frame. Directly beneath it is the number of vacuoles in the selected descendents. To the left of this line is placed the number of vacuoles in the sister cell. At first sight it would seem that selection has some effect but figuring the percentage of animals with twos, threes, fours and fives in each line it is found that they vary little more than 10 per cent.

Chart 10 A four-vacuolated paramoecium gave rise to paramoecia possessing the number of vacuoles indicated on this chart.

Second method. The same difficulty was experienced with selecting by the first method as was found in following all the animals for several generations, i.e., the danger of losing an important individual through drying. By the second method each animal was allowed to multiply for a considerable number of generations. A large number would then be examined and the percentage of twos, threes, fours and fives noted. One would then be isolated to start a new line. In an experiment where selection was carried on for nearly three months according to

this method at the time of writing no tendency to produce more high-vacuolated animals is evident.

Chart 11 This figure illustrates the effect of selection and is described on page 316.

Analysis of Chart 11

	TWO VACUOLES	THREE VACUOLES	FOUR VACUOLES	FIVE VACUOLES
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Line 2.....	53	37	10	
Line 3.....	50	27	23	
Line 4.....	40	37	15	7
Line 5.....	41	41	7	10

Experiment A was started with a two-vacuolated paramoecium and selection was always made of animals with the least number

of vacuoles and the line carried on with them. The last examination of this culture showed the percentages to be as follows:

	<i>per cent</i>
Two-vacuolated paramoecia.....	13
Three-vacuolated paramoecia.....	78
Four-vacuolated paramoecia.....	9

Experiments B and C were carried on with selection for animals with the most contractile vacuoles.

Experiment B started with a three-vacuolated paramoecium gave rise to:

	<i>per cent</i>
Two-vacuolated paramoecia.....	31
Three-vacuolated paramoecia.....	69

Experiment C started with a four-vacuolated paramoecium produced:

	<i>per cent</i>
Two-vacuolated paramoecia.....	43
Three-vacuolated paramoecia.....	57

In Experiment A where the selection was for the animals having the least number of vacuoles the percentage of multi-vacuolated paramoecia produced was greater than in B and C. It might seem that the two-vacuolated animals of Experiment A were possessed of a potentiality to produce more multi-vacuolated offspring than were the paramoecia of B and C. As far as is known the culture conditions in all three experiments were the same. As has been stated several times before, the extensive data that have been gathered on this race under all conditions leads me to feel confident that in experiments such as A the greater number of multi-vacuolated animals were produced because of some characteristic of the culture medium which either slowed down the division rate (allowing more time for vacuoles to develop) or had some other influence on the animals which produced extra vacuoles and which as yet I have been unable to discover. In B and C this factor was presumably absent or was not so potent.

c. Immediate and temporary effect of location of the vacuoles in the parent form on inheritance by offspring

When a paramoecium possessing the most common arrangement of vacuoles (the supernumerary vacuoles located posteriorly) divides, the anterior half receives fewer vacuoles than the posterior half. For instance, an animal with three vacuoles—one anterior and two posterior—divides. Just before division a new vacuole is added to each end which results in a daughter paramoecium from the anterior half with two vacuoles while the daughter arising posterior to the constriction has three vacuoles. The paramoecium springing from the posterior half starts its life cycle with more vacuoles than does its anterior mate but the anterior animal, however, as had been emphasized before, has not lost the power of producing paramoecia with the higher number of vacuoles.

d. Effect of conjugation

When conjugation was first observed it seemed that there was what might be termed a selective mating or more exactly that there were more two-vacuolated animals conjugating at the beginning of the epidemic than multi-vacuolated individuals. Further work and the final plotting of the results showed that this was not the case. Neither was there a tendency for threes to pair only with threes or fours with fours but there was a heterogeneous mating in approximately the same percentage as the frequency of the various number of vacuoles in the individual units. Chart 12 illustrates these points. Conjugants were found paired in nearly all possible combinations: 2×2 , 2×3 , 2×4 , 2×5 , 3×3 , 3×4 , 3×5 , 4×4 and 4×5 . During an epidemic of conjugation the proportions of these various combinations on any one day followed in a fairly close manner the percentage of single animals having two, three and four vacuoles in the same culture. This relation becomes clearer when graphically represented as on chart 12. Here the lines representing the various combinations in conjugation may be seen to follow rather closely the rise or fall of the lines rep-

representing the vacuole number in the single paramoecia. More of the multi-vacuolated paramoecia conjugate at the end of the epidemic than at the beginning, apparently, not because of different physiological characteristics but because more of these forms appear as the culture ages.

The period of conjugation was awaited with great interest as furnishing an opportunity for determining the nature of this

Chart 12 This graph is reproduced to show the manner in which the various combinations of paramoecia in conjugation follow closely the percentages of two, three and four-vacuolated paramoecia on any day. Note that as conjugation ceases on February 2 the percentage of three and four-vacuolated paramoecia increase. Further discussion is to be found on page 320 under "Effect of Conjugation."

new character. If the paramoecia were homozygous then we should expect no change in the inheritance of the potentiality for extra organs. But should they be heterozygous, individuals might result which were pure for the lower number of vacuoles. I have used the words 'might result' in the last sentence, since, as far as we know, conjugation between heterozygous paramoecia would present equal possibilities for the offspring of the exconjugants to be heterozygous as it would for a homozygous line

to appear. However, this line is evidently pure for this character since exconjugants when separated produced offspring which showed no variation in the percentage of vacuoles from the number possessed before conjugation began as is indicated by the following observations. Single conjugants of the various combination of vacuole number were isolated and allowed to separate and divide a number of times and then examined. The results were not different from those obtained on unconjugated forms. To take two typical cases in which the conjugants were isolated, and allowed to separate and divide for a week or more.

Conjugant 2×3 :	<i>per cent</i>
Two-vacuolated paramoecia.....	31
Three-vacuolated paramoecia.....	69
Conjugant 2×4 :	
Two-vacuolated paramoecia.....	32
Three-vacuolated paramoecia.....	69

The number of two-vacuolated forms here is slightly higher than the average due probably to the rapid division stimulated by fresh hay infusion.

In one instance the exconjugants were allowed to divide twice, producing eight animals in all, and then each animal was isolated in a separate watch crystal and allowed to multiply for some time. This was done to determine whether any difference in potentiality would crop out due to the redistribution of the micronucleus. No change was observed.

No effect can be ascribed to conjugation other than a temporary increase in the percentage of two-vacuolated forms and a corresponding decrease of the threes and fours. This may indicate that conjugation speeds up the division rate somewhat as it has been shown before that increased rate of division results in individuals with fewer contractile vacuoles than is the case when the division rate is slow.

Chart 5 beginning with March 19 when conjugation began in cultures 1, 3 and 5 shows a slight rise in the 'two' line (marked 1-3-5) and a fall in the 'three' line for the same cultures both, however, returning to very nearly the same point on April 6

as they were on March 15. Conjugation had by this time dropped to less than 0.5 per cent of the population.

e. Attempts to cross the two races

One of the most interesting points to be developed in connection with this new race of paramoecium is the effect of crossing with the common form. This is the first time to my knowledge that two forms of protozoa with definite individual characters have been available for crossing. I have made one hundred and fifty attempts to cross the two forms with no success. The difficulties that beset the mating of these two paramoecia are many. Cultures of both races have to be entering into a period of conjugation at the same time. Single individuals from both races must be placed in as small amount of fluid as possible so that they will have every opportunity of coming together. The chances that either or both of the mated paramoecia are in a condition which makes conjugation necessary are small. The matings must be made up fresh at least, every twenty-four hours for when either of the individuals divide there is no way of telling to which race an animal belongs. In spite of these obstacles I am confident that the crossing will ultimately be achieved after sufficient number of matings have been made and perhaps after certain methods of technique have been determined which are not evident at present.

VII. CYTOLOGY

My studies on the cytology of these paramoecia are not sufficient as yet to permit a detailed account. There is one micronucleus present (fig. 15) which indicates that this race is a variety of *Paramoecium caudatum*. In all the preparations made I have had great difficulty in staining the micronucleus and in the majority of cases so far I have been unable to make the stain remain in it. The depression in the macronucleus where the micronucleus usually lies is frequently visible but it appears empty. I do not believe that this appearance is due entirely to faulty technique, as the same methods give excellent results when used on the common race.

VIII. DISCUSSION

In the course of some experiments by Dr. M. H. Jacobs on the effect of high temperatures on paramoecia, various lots of these animals were ejected into hay infusions at temperatures ranging from about 36° to 42°C. Those that survived this treatment were thrown into a battery jar containing hay infusion. In this jar the new animals were discovered. I am still of the opinion (stated in my preliminary note in *Science* (2)) that these new paramoecia may have been produced as the result of the high temperature, although I have been unsuccessful as yet in a number of attempts to reproduce this race artificially. It would be a most striking coincidence if this multi-vacuolated character has been in existence for any length of time that its discovery should be delayed until found in the culture made up of the descendents of heat treated animals.²

In this new paramoecium we have a form which lends itself to a study of the processes of heredity in the protozoa. The character is seemingly not at all comparable to those reported for *Diffugia* by Jennings (5). Since the vacuoles are, however, very definite structures and a marked variation from the common form, their study and comparison is exceedingly interesting and offers many possibilities. The data possessed at present are insufficient to make a discussion of these hereditary processes of value. Selection experiments, though incomplete, seem to indicate as far as they have been carried that the character cannot be affected by selection in either direction. It will be impossible to determine whether the tendency or potentiality for extra vacuoles is germinally controlled until we have succeeded in crossing the two strains. There is some evidence at present that points to the cytoplasm as the portion of the cell

² Since this paper was sent to press I have received Shumway's paper (11) in which he has reported the formation of extra contractile vacuoles in thyroid fed animals. None of his specimens had more than three vacuoles and the inheritance of the extra vacuole in the form studied has not been demonstrated. The percentage of extra-vacuolated animals among his thyroid fed paramoecia (20 to 30 per cent) is considerably less than I have found in my race (77 per cent).

that controls the production of the vacuoles since their production seems to be controlled by so many conditions. Whether these conditions act on the cytoplasm directly or on the cytoplasm through the nucleus there is no possibility of determining with the knowledge available at present.

IX. SUMMARY

1. This race which is a variety of *Paramoecium caudatum* is considered possibly to have arisen under experimental conditions when certain animals were subjected to high temperatures.

2. The animals of this race range in length from 167 μ to 307 μ and are unusually strong and resistant.

3. A slightly denser portion extends across the middle of some of the individuals as a band.

4. The extra vacuoles are generally located in the posterior end of the cell.

5. The number of vacuoles possessed by individuals of this race varies from two to seven.

6. The number of vacuoles may increase during the life time of the individual.

7. Various conditions affect the number of vacuoles present in the individual, such as the rate of division and the age of the culture medium.

8. Though extra vacuoles may not appear for several generations for the above reasons, the potentiality for this organ has not been lost.

9. The division rate ranges between one and five times in twenty-four hours.

10. Besides being influenced by the rate of division the vacuole number may apparently be increased in the individual by the presence of katabolic products in the environment.

11. The resistance of this race to heat, cold, distilled water, etc., is very high.

12. The rate of pulsation of the vacuoles is slightly slower than is that of the vacuoles in the common race but the new animals are in general superior to the common race in their powers of excretion.

13. Conjugation is believed to be caused by the concentration of katabolic products in the environment.

14. It may be said that a potentiality for *extra* contractile vacuoles rather than for a definite number of the organs is passed on from generation to generation and that this potentiality is effected by various conditions during the life time of the individual.

15. Selection has no effect in raising or lowering the vacuole number.

16. The location of the vacuoles in the parent has an immediate but temporary effect on the number possessed by the offspring.

17. Conjugation has no effect upon this new character.

X. BIBLIOGRAPHY

- (1) CALKINS, G. N. 1909 Protozoology. Lea and Febiger.
- (2) HANCE, R. T. 1915 The inheritance of extra contractile vacuoles in an unusual race of *Paramoecium caudatum*. Science N. S., 42, October 1.
- (3) 1916 Notes on handling protozoa in pure line work. Trans. Amer. Mic. Soc., 35, no. 2, April.
- (4) JENNINGS, H. S. 1910 What conditions induce conjugation in *paramoecium*. Jour. Exp. Zool., 9.
- (5) 1916 Heredity, variation, and the results of selection in the uniparental reproduction of *Diffugia corona*. Genetics 1, September.
- (6) MINCHIN, E. 1912 Protozoology. London, Arnold.
- (7) WOODRUFF, L. L. 1908 The life-cycle of *paramoecium* when subjected to a varied environment. Amer. Nat. 42, 520-526.
- (8) 1910 Two thousand generations of *paramoecium*. Arch. f Protisk., 21.
- (9) 1911 The effect of excretion products of *paramoecium* on its rate of reproduction. Jour. Exp. Zool., 10, No. 4, 557-581.
- (10) WOODRUFF, L. L. AND BAITSELL, G. A. 1911 The reproduction of *Paramoecium aurelia* in a constant culture medium of beef extract.
- (11) SHUMWAY, W. 1917 The effect of a thyroid diet upon *paramoecium*. Jour. Exp. Zool., vol. 22, no. 3.

PLATE 1

EXPLANATION OF FIGURES

The figures are reproduced at a magnification of $480\times$ with the exception of figure 16 which is $1120\times$. Figures 3, 4, 5, 6 and 8 were drawn from living animals slightly flattened under pressure. The others were outlined from fixed preparations.

In preparing these figures an attempt has been made to reproduce all the various characteristics of the contractile vacuole observed. In general when the vacuoles are expanded the radiating canals are not visible but in favorable specimens or in animals under pressure they appear as thread-like rays as shown in figures 2, 3, 4, 5 and 10. Note that in most cases the vacuoles lie in a line parallel to the outline of the body. The anterior end of the paramoecium is toward the upper part of the page in all the plates. In all the figures the contractile vacuoles are not stippled.

1 Paramoecium with three contractile vacuoles arranged in the most common manner.

2 Paramoecium with two vacuoles.

3 Paramoecium with extra vacuole in the anterior end—a rare grouping.

4 Paramoecium with four vacuoles in the usual position but with one vacuole slightly displaced to the right.

5 Paramoecium with four vacuoles in common arrangement.

6 Paramoecium with four vacuoles in the unusual grouping of two in the anterior end and two in the posterior end. Note the slightly darker area across the middle of the animal.

PLATE 2

EXPLANATION OF FIGURES

- 7 Paramoecium with five vacuoles.
- 8 Paramoecium with five vacuoles two being located in the anterior end.
- 9 Paramoecium with five vacuoles, two of which are slightly displaced to the right. This displacement is rare.
- 10 Paramoecium with six vacuoles.
- 11 Paramoecium dividing. Before constriction occurred this was a three-vacuolated animal. A new vacuole has been added to each half.
- 12 Paramoecium with seven vacuoles.

PLATE 3

EXPLANATION OF FIGURES

13 A paramoecium dividing. This animal before division had four vacuoles arranged as in figure 6. A new vacuole has been added to each end.

14 A conjugating pair of paramoecium. One animal has three and the other two vacuoles.

15 Drawn from a fixed and stained preparation showing the macronucleus and the single micronucleus.

16 Drawing made with the aid of an oil immersion lens showing the vacuoles to be surrounded by a morphological membrane.

17 A paramoecium dividing. Before division began this animal had five vacuoles arranged as in figure 7. A new vacuole has been added to either end.

18 A paramoecium with a greatly enlarged vacuole. This condition has been rare in my cultures. Occasionally the animals recover from this condition but more frequently they die with the vacuole still distended.

19 The mouth of this paramoecium has been forced out under pressure.

13

14

15

16

17

18

19