UROLEPTUS MOBILIS ENGELM.

II. RENEWAL OF VITALITY THROUGH CONJUGATION

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ONE CHART AND ONE FIGURE

In a previous paper I have described the morphology and the cytology of division and conjugation stages of the rare hypotrichous ciliate which forms the subject of the present paper.¹ Lending itself admirably to the cultural method which has been employed, *Uroleptus mobilis* is the most satisfactory organism for experimental work I have yet encountered. Paedogamous conjugation, in epidemic form, occurs readily, under the proper conditions, in the culture medium. Ex-conjugants, upon isolation, live and thrive in this culture medium in practically 100 per cent of cases—a rare phenomenon among the hypotrichs. Asexual reorganization, or parthenogenesis, called by Woodruff and Erdmann 'endomixis', occurs at fairly definite periods, under the protection of a cyst membrane. Such reorganizations, therefore, are advertised by the form assumed by the organisms and do not interfere with the study of comparative vitality throughout the life cycle.

Starting with a single individual which was isolated immediately after conjugation, on November 16, 1917, I have followed the life history of thirteen different series, all beginning as ex-conjugants of pairs of normally conjugating individuals, and all were progeny of the original ex-conjugant which was isolated November 16, 1917. Three additional series, derived from encysted individuals, have also been studied in similar isolation cultures. The different series were started at various periods

¹ Calkins, 1919. *Uroleptus mobilis*, Engelm. I. History of the nuclei during Division and Conjugation. Jour. Exp. Zool., vol. 27, no. 3, p. 293.

of the life history of parental series and at different stages of vitality, so that I have abundant data for the study of comparative vitality of parent and offspring. It is with pleasure, based upon admiration for the genius of that gifted pioneer in this field of research, Edouard Maupas, that I can say these data convincingly prove the truth of his conclusions that conjugation in ciliates restores vitality and prevents the phenomena accompanying 'old age' which we include under the terms senescence and natural death.

METHODS AND RECORDS

On October 3, 1917, a rich culture in an old hay infusion was found to contain a large number of individuals of *Uroleptus mobilis*. The normal structure of this rare ciliate is described and its systematic position given in my earlier paper. It is illustrated again in figure 1 of the present paper. Attempts were immediately made to cultivate the organism on the usual hay-infusion culture medium. A better medium was obtained by mixing boiled flour water, hay infusion, and spring water, and this was used until the middle of January, 1918. It was in this medium that the first pairs of conjugating individuals were found and isolated, giving the ex-conjugant destined to form the first, or A series. A still better medium was substituted on January 18th. This was obtained by boiling 100 mg. of chopped hay with 130 mg. of flour in 100 cc. of spring water, and diluting this, when twenty-four hours old, with an equal part of fresh spring water. This standardized medium, made fresh each day, has been used exclusively throughout the experiments.

Series and lines

As used here, the term 'series' is applied to an aggregate of individuals, all derived from a single individual and representing its protoplasm. An ideal way to study vitality of such protoplasm at different age periods would be to follow all of the progeny throughout the life cycle. As this is obviously impossible, I follow as many individual representatives of that protoplasm as time and space will permit. My practice is as follows: When

an initial individual divides, each of the two cells is isolated as the beginning of a 'line;' when these divide, two more lines are started, and one more is added at the next division. Each day a record is made of the number of divisions during the twenty-four hours in each line, and a single individual is isolated with a capillary pipette from each line and transferred to fresh culture medium. The vitality is measured by the division rate, the average number of divisions per day in all five lines for a given period representing the vitality of the series for that period. I have daily records, extending from November 16, 1917, to date, of sixteen series and eighty lines of *Uroleptus*.

Conjugation tests

In every line of a series at the time of the daily isolation there are two or more individuals in the culture dish according to the number of divisions that have occurred. After one is transferred the unused individuals are either thrown away or placed in a similar culture dish containing about 1 cc. of the fresh culture medium. Representatives of all five lines of a series are collected in this way and stored in a moist chamber as 'stock.' Here they accumulate by division until a large number are present. Once a week this stock is washed into a Syracuse dish containing several cubic centimeters of fresh culture medium and set aside as a conjugation test. The number of individuals increases rapidly until, in three or four days, there are many hundreds or even thousands of organisms. These Syracuse dishes are examined carefully every other day for a period of two weeks and without any fresh medium being added. During these two weeks the limited food is gradually exhausted, and by the end of the period conjugation would have occurred if the internal conditions of the organisms were suitable for it. The date of the appearance of conjugations is recorded and the extent of conjugations, up to epidemic frequency. At the end of the period the individuals are small, inactive, and starved, and, other tests being under way, they are discarded.

Encystment tests

Encystment, with its accompanying asexual reorganization, has never occurred in my isolation culture dishes. It does occur, however, at certain periods of the life cycle in the Syracuse dishes during the conjugation tests. Once encysted, the organism cannot be coaxed out until after a longer or shorter period in a dried state. Such encystments are particularly abundant just prior to the first epidemic of conjugation in a series, and, in some tests, practically all of the individuals encyst without conjugation. In such cases the Syracuse dishes are set aside and the culture medium is allowed to evaporate. Such Syracuse dishes with the dried cysts are then stacked away for future experiments. After several weeks or months of storage, fresh culture medium is added to the Syracuse dish containing the dried cysts. In some cases the reorganized individuals emerge by the end of a week; in others, two or three weeks may be required. Series B and M were derived from such encysted individuals. The cytology of reorganization during encystment will form the subject of a later paper.

The measure of vitality

Reproduction by division is an indication that cell structures are functioning normally, and the rate of division is an index of the condition of metabolic activities of the protoplasm, or a numerical index of the protoplasmic vitality of a series for a given period. The relative activity of a given protoplasm at different periods or of parent and offspring protoplasm for the same period may be obtained by averaging the number of divisions per day in a series for successive periods of the same length of time, e.g., five days, ten days, or sixty days. If we compute the average division rates in this way for successive ten-day periods from start to finish of a series and plot the results, we obtain a curve indicating the relative vitality of the protoplasm of a series at different periods throughout the life cycle. Furthermore, since all series and all lines are fed daily at the same time and with the same standardized culture medium, if we plot a number of curves representing different series which have originated at different

times for identical calendar periods, we can tell whether fluctuations in vitality are due to conditions of the environment or to inherent vitality of the protoplasm. If due to environmental conditions, a correction may be made which will indicate, approximately, the comparative vitality, had the environmental conditions been normal.

Again, if we plot the curves for different series and start them all from the same ordinate, we have a means of measuring the vitality of different series at similar stages of the life cycle. Such curves enable us to find the cyclical incidence of important phases of the life history, such as conjugation and encystment, and to establish the limits within which they occur.

These methods have been used in working out the results described in the following pages. In maintaining the cultures and keeping up the records, as well as in working up the statistical data, I have been fortunate in having the assistance from time to time of Miss Mabel L. Hedge and of my colleague Prof. Louise H. Gregory, whose helpful interest I gratefully acknowledge.

GENERAL HISTORY OF THE CULTURES

The fact should be emphasized at the outset that these experiments deal, in the main, with one bit of protoplasm which emerged from the processes of conjugation on November 16, 1917, reproduced abundantly by division, underwent paedogamous conjugation repeatedly, underwent encystment, and is still living with a vigor equal to that at the beginning. Some of this protoplasm has been maintained in isolation cultures whereby conjugation has been prevented and in which encystment does not occur. Such protoplasm invariably dies, the phenomenon of natural death being the last stage of a decreasing vitality—a decrease which begins to show early in the life cycle. Other parts of this protoplasm have been allowed to conjugate among themselves, the subsequent isolation cultures showing the effects of such conjugation upon such protoplasm. Still other parts of this protoplasm have been allowed to encyst and to undergo processes of asexual reorganization within such cysts, and the effects of such reorganization have been ascertained.

Also, the fact should be emphasized again, that throughout the entire history of the protoplasm, with the exception of a few weeks at the outset, the same standardized culture medium, made fresh each day, has been used. Waning vitality cannot be attributed to deleterious food conditions, for, on the same day with the same food, one portion of the protoplasm may be at the lowest ebb of vitality, while other portions are in the full swing of metabolic vigor, all portions being equally old in point of time. The difference in vigor between them cannot be due, therefore, to environmental or external conditions, but must be attributed to the internal conditions following conjugation.

This protoplasm has been studied in fourteen different series as follows: The A series, or parent race, started as an ex-conjugant from a pair of 'wild' *Uroleptus mobilis* on November 16, 1917, and died on September 18, 1918, in the 313th generation. The C series, or first filial series (F_1), started on February 4, 1918, as an ex-conjugant from a pair of individuals in approximately the 78th generation of the A series. It died out on December 30, 1918, in the 348th generation. The D series, or second filial series, started as an ex-conjugant on March 9, 1918, from the A series in the 137th generation and died out on October 13th in the 271st generation. An E series was also started at the same time from the same source, but was discarded after the 50th generation. The F series, or first F_2 series, started as an ex-conjugant on March 25, 1918, from the C series in the 86th generation (the grandparent A series was in the 155th generation). This series died December 21, 1918, in the 317th generation. The H series, or third F_1 series, started as an ex-conjugant on April 24, 1918, from the A series in the 237th generation, and died out on January 16, 1919, in the 277th generation. The I series, or first F_3 series, started as an ex-conjugant on July 7, 1918, from the F series in the 143rd generation (the F_1 grandparent C series was in the 224th generation and the great-grandparent A series was in the 278th generation). This series is still living in the 321st generation, but has stopped dividing and will die shortly. The J series, or fourth F_1 series, started as an ex-conjugant on August 20, 1918, from the A series in the 311th

generation, and is still living in the 254th generation. The L series, or first F_4 series, started as an ex-conjugant on November 3, 1918, from the I series in the 199th generation (the grandparent F series was in the 294th generation, the great-grandparent C series was in the 347th generation, and the great-great-grandparent A series was dead). This series, also, is active, and in the 196th generation. The M series started from a cyst of the F series which had encysted on April 27th while in the 45th generation, remained dry for more than six months, and was recovered from the cyst on November 18, 1918. The N series was started on December 12, 1918, as an ex-conjugant from the J series in the 188th generation. The O series was started as an ex-conjugant on January 10, 1919, from the M series in the 105th generation. The P series was started on January 12, 1919, as an ex-conjugant of the L series in its 115th generation. The Q series was started on January 19, 1919, as an ex-conjugant of the I series in its 316th generation. Finally, the R series was started as an ex-conjugant on January 19, 1919, from the J series in its 245th generation.

In addition to these fourteen series representing one protoplasm, two other series, representing a different initial protoplasm, have been followed throughout the life history. Of these the B series started from an encysted 'wild' *Uroleptus mobilis*. This individual encysted on November 9, 1917, remained dry from December 1st until January 24th, when it was recovered from the cyst. A filial series, the G series, started as an ex-conjugant from the B series in the 115th generation on March 23rd, and died out on January 5th, 1919, in the 291st generation.

In computing the division rate of a series for a given period, e.g., ten days, the number of divisions in all five lines for the ten days are added and the sum divided by five. The value of such an average depends somewhat on the extent of variation in number of divisions in each of the five lines. In table 1 the individual line records for six consecutive ten-day periods from June 27th to August 25th and for all the series under observation during this sixty-day period are given. Since the different series were started from ex-conjugants at different times, this sixty-

TABLE 1

Actual numbers of divisions in ten-day periods in all lines of series A, C, D, F, H, and I

	LINES	PERIOD						TOTALS 60 DAYS	AVERAGE DIVISION RATE PER LINE IN 10 DAYS
		6/27-7/6	7/7-7/16	7/17-7/26	7/27-8/5	8/6-8/15	8/16-8/25		
A series.....	1	6	5	10	3	2	0	26	5.86
	2	9	10	12	4	2	1	38	
	3	11	8	12	5	2	0	38	
	4	9	9	10	7	4	0	39	
	5	10	6	13	5	1	0	35	
C series.....	1	11	10	16	5	7	10	59	10.90
	2	11	12	19	6	10	10	68	
	3	12	13	17	10	9	9	70	
	4	8	12	18	11	10	9	68	
	5	8	10	18	7	9	10	62	
D series.....	1	13	13	19	14	6	5	70	12.83
	2	13	15	17	14	11	9	79	
	3	14	15	17	6	10	12	74	
	4	14	15	22	12	13	13	89	
	5	13	15	17	10	10	8	73	
F series.....	1	12	13	20	7	10	12	74	13.16
	2	12	14	21	12	9	12	80	
	3	14	15	20	11	12	11	83	
	4	13	14	19	10	7	13	76	
	5	13	15	19	15	8	12	82	
H series.....	1	16	15	23	12	11	14	91	15.26
	2	15	17	19	14	15	15	95	
	3	14	16	24	12	13	16	95	
	4	13	15	22	12	11	16	89	
	5	13	17	21	11	9	17	88	
I series.....	1	18	20	16	10	12	12	88	17.16
	2	16	23	19	11	14	16	99	
	3	18	23	20	12	15	17	105	
	4	20	24	22	13	17	17	113	
	5	20	23	20	13	17	17	110	

day period covers almost every stage of a typical life cycle. It includes a late stage of the A series and the initial stage of the I series, while the others are intermediate.

The five different lines of a series thus give consistent records in number of divisions in the same calendar period, so that an average of all five lines gives a fairly accurate idea of the vitality of the protoplasm of a series for that period. Table 2 is a list of such averages for all series in the same consecutive calendar ten-day periods.

The averages given in table 2 are based on the actual records of daily divisions in all series, the individuals being isolated daily and fed on the same fresh, standardized culture medium. In some periods, notably in periods 20 and 21, and again in 25, the averages are conspicuously out of proportion with those before and after. These low averages, occurring in all series at exactly the same time, but in different phases of the life cycles, are obviously due to external conditions. The 20th and 21st periods fell on May 28th to June 17th. On the 29th of May the cultures were transferred from New York to Woods Hole, where a different natural water was used in making the culture medium, and the temperature also was considerably lower than it had been in New York. This unusual variation in the sequence of averages makes no difference in the life cycle of a given series, but if we wish to compare similar stages in the cycles of all series, some of which include this period of adverse conditions while others do not, it is obvious that a correction of the lower averages is imperative. Such corrections are made for all series in the 20th and 21st periods by averaging the 18th and the 22nd to obtain the 20th, and the 19th and 23rd to get the 21st. In making such corrections the records of the individual lines are used and the rate for each line is corrected; these corrected line averages are then averaged to obtain the corrected average for the series. The same method is employed for other periods in which corrections are necessary. In table 2 such corrected averages are included in brackets, and the corrected averages are used in plotting curves of the life cycles and for comparison of different series in similar phases of the cycle.

TABLE 2
Average division rates, all series, in the same ten-day periods

10-DAY PERIODS	A SERIES	B SERIES FROM WILD CYST	C SERIES FROM A 78	D SERIES FROM A 137	F SERIES FROM C 86	G SERIES FROM B 115	H SERIES FROM A 237	I SERIES FROM F 143	J SERIES FROM A 311	L SERIES FROM I 199	M SERIES FROM CYST OF F 45	N SERIES FROM J 188	O SERIES FROM M 108	P SERIES FROM L 116	Q SERIES FROM I 316	R SERIES FROM J 245
1	9.8															
2	6.6															
3	4.8															
4	5.4															
5	7.8															
6	21.0															
7	18.6	22.4														
8	20.8	21.0	18.6													
9	18.0	17.0	18.8													
10	12.6	14.2	16.2													
11	13.2	14.2	16.8	16.0												
12	14.8	15.6	17.2	18.2												
13	14.6	13.4	15.6	16.0	13.4											
14	13.2	13.0	14.2	17.0	13.8											
15	15.0	14.4	16.4	17.6	16.4	17.4										
16	13.6	13.8	15.6	18.4	19.8	19.4										
17	17.0	13.8	17.4	19.0	20.2	21.0										
18	12.8	15.0	18.0	20.0	19.6	19.6	19.6									
19	7.4	12.2	13.4	15.2	15.8	15.6	16.8									
20	4.0	4.0	5.4	7.6	6.2	7.0	7.6									
	(11.0)		(14.4	16.6	16.8	15.8	17.8)									
21	5.6	5.6	6.2	7.2	9.2	8.0	10.0									
	7.0		(12.8	14.4	14.0	13.6	15.8)									
22	9.0	7.6	10.4	13.4	12.8	12.0	14.8									
23	7.6	4.8	11.4	14.6	14.2	12.0	16.0	18.4								
24	11.2	7.2	17.6	18.4	19.8	18.8	21.4	22.6								
25	4.8	2.6	7.8	11.2	11.0	7.4	10.0	12.4								
			(10.6	12.6	13.8	11.8	16.4	19.4)								
26	2.2	1.0	9.0	10.4	15.2	15.0	18.0	11.8								
27	0.2	1.8	9.8	9.4	12.0	12.6	13.6	15.0	17.2							
28	0.6	0.8	8.6	3.4	11.8	12.2	15.6	15.8	16.6							
29	0.2	0.0	7.6	1.4	10.8	12.4	15.4	14.8	18.6							
30	0.0	0.0	6.0	0.8	12.0	15.2	17.8	17.6	17.4							
31		0.0	6.8	0.4	14.6	16.8	17.8	18.0	20.4							
32		0.0	4.8	0.2	9.8	11.6	11.0	11.6	17.2							
33			2.4	0.0	9.6	13.2	15.4	16.4	15.8							
34			0.6	0.0	12.0	9.2	12.6	17.4	17.2							
35			0.0		8.0	3.6	7.4	14.0	14.6	16.0						
36			0.4		5.8	2.4	2.4	12.8	12.4	13.2	8.4					

TABLE 2—*Concluded*

10-DAY PERIOD	A SERIES	B SERIES FROM WILD CYST	C SERIES FROM A 78	D SERIES FROM A 137	F SERIES FROM C 86	G SERIES FROM B 115	H SERIES FROM A 237	I SERIES FROM F 143	J SERIES FROM A 311	L SERIES FROM I 199	M SERIES FROM CYST OF F 45	N SERIES FROM J 188	O SERIES FROM M 108	P SERIES FROM L 116	Q SERIES FROM I 316	R SERIES FROM J 245
37			0.4		3.8	0.2	1.6	13.8	14.4	16.2	17.0					
38			0.0		2.8	0.2	1.6	20.8	20.6	23.2	24.8					
39					0.0	0.8	0.4	15.8	17.0	18.6	19.8	17.2				
40							1.2	13.0	12.6	16.0	18.0	17.8				
41							0.0	14.2	13.0	16.4	24.6	23.6				
42								9.6	6.6	17.4	18.6	17.8	15.2	16.0		
43								3.0	3.6	18.6	18.8	20.4	22.6	19.8	13.2	19.6
44								0.0	0.8	11.2	13.4	13.8	16.8	14.8	5.8	13.8
45								0.2	0.2	12.6	12.4	13.6	16.6	15.0	1.2	14.0

Incidence of conjugation and encystment

Conjugation tests in all series have been carried out at weekly intervals beginning with the sixth ten-day period, during which the standardized culture medium was introduced. The records of these tests give the dates on which pairing occurred. With only one exception, every series derived from an ex-conjugant failed to give any evidence of conjugation before the fiftieth day. The one exception was the I series in which a single pair of conjugating individuals were observed in a test made during the third ten-day period. In the C and D series the first pairing occurred in the fifth ten-day period; in the H and L series the first pairing occurred in the sixth period, and in the J and F series the first conjugation occurred in the seventh and eighth periods. In all of these cases, except the I series, conjugations were of epidemic frequency, i.e., hundreds of pairs were present in the testing dishes. It is apparent, therefore, that the first sixty days, approximately, represent a period of immaturity so far as conjugation is concerned, although, during this period, vitality as indicated by the division rate is at its maximum. After conjugation begins in a series, the succeeding tests are usually positive unless some adverse condition renders the test inconclusive.

During the period of immaturity the history of the conjugation tests was the same for all series. Two or three thousand indi-

viduals would accumulate by division in the Syracuse dishes during the first seven to eight days when food was plentiful. With the transition from rich feeding to starvation, conjugation should occur, provided the protoplasm is ready for it. During this period of immaturity, however, no conjugations take place, and at the end of two weeks the individuals are inactive and greatly reduced in size through starvation.

Conjugation tests made during the period of maturity give a very different result. The individuals multiply as before during the first week. Then they begin to collect in a single group until a dense, white agglomeration is formed which sometimes measures half an inch in diameter. Such agglomerations are invariably followed by an epidemic of conjugations. Since each conjugation test is started with a few individuals of the same series that are left over after a daily isolation, the conjugations that occur must be between closely related individuals of the same age and with an identical previous history.

In diagram 1 the ten-day averages given in table 2 are plotted for the purpose of comparing the life cycles of the first eight series. The ordinates represent the average numbers of divisions of a single line in ten days. The abscissas represent the successive ten-day periods. The positions of the curves in the first period indicate the average division rate of each line during the first ten days of life after conjugation, while the positions of the curves in the tenth represent the average numbers of divisions between the 90th and the 100th days after conjugation, etc. The period during which conjugations occurred to furnish the filial series is shown on the curve of the parental series by a letter which indicates the filial series. Thus the points marked C, D, H, and J on the curve of the A series indicate the periods when filial series C, D, H, and J were started as ex-conjugants from pairs in the A series.

The successful and negative conjugation tests are shown in diagram 1 by + and - signs, while the inconclusive tests due to lack of material or to adverse conditions under which the tests were made are indicated by question marks. The dotted line connecting the first positive signs in different series indicates that

DIAGRAM 1

the protoplasm of each series represented by the curve to the left of this line was in a condition of sexual immaturity. Sexual maturity, or ability to conjugate, persists after the first sixty days practically to the end of the cycle. This is well illustrated by the A series where conjugations occurred regularly up to one month prior to the death of the race. Usually, however, the conjugation tests in the later periods of the cycle are inconclusive, owing to the low division rate and the resulting scarcity of material.

The cysts of *Uroleptus mobilis* are small and spherical and the walls are smooth. When once encysted, the organisms do not emerge from their cyst membranes until weeks afterwards, and then only after an intervening period in a dried condition. Such dried cysts, when placed in fresh culture medium, will give up their contained organisms in from five to twenty days. During such encystment, or prior to it, the protoplasm of the individual undergoes asexual reorganization ('endomixis') and, after emerging, begins its life cycle with an initial vigor similar to that of an ex-conjugant. The B and M series, for example, started from cysts.

Encystments have never occurred in the isolation cultures and the uniformity of the curves is sufficient evidence to show that no other process of parthenogenesis takes place in these cultures. In conjugation tests, however, encystment seems to be due to the same conditions under which conjugation is possible. In such tests the date of the appearance of cysts is always recorded. Such dates are indicated on diagram I by circles above the symbols for the conjugation tests.

As shown in the diagram, encystment usually takes place among some individuals of a conjugation test before the first conjugating individuals appear (series D, F, G, H, and J). In some cases (G and J) encystment occurs in such tests made during the first ten days of the cycle. In other cases (C and I) encystment and conjugation both appeared for the first time in the same test. I have no comment to offer as to the significance of these results, but I hope to get further light on the subject with continued observations.

RESULTS OF THE EXPERIMENTS TO DATE

The physiological effect of conjugation or fertilization in living things has been interpreted, in the main, along two lines of theory. One of these, and the older, may be indicated by Bütschli's term *Verjüngung* and by Maupas's term *rajeunissement*—terms indicating that the primary effect of conjugation is to restore the lagging vital activities to an optimum. The other theory, first fully elaborated by Weismann, assumes that the union of germ plasms (*amphimixis*), brought about by conjugation, is a source of variation.

These two theories are not reciprocally exclusive, and it is possible that both are correct, although neither has yet been conclusively established.

Bütschli interpreted conjugation in the protozoa as a means whereby waning vitality is restored to full metabolic activity. The problem thus suggested involves three fundamental questions: 1) Does the protoplasm of a single individual protozoon and its progeny by division undergo a progressive waning of vital activities leading to 'old age' and finally to natural death? 2) Does conjugation actually restore such weakening protoplasm to a condition of full metabolic activity? 3) If conjugation accomplishes this, what is the explanation of the result?

The first of these questions has been answered in the affirmative by the experiments of Maupas and of subsequent investigators. The second has never been answered conclusively, although strong experimental evidence has accumulated in support of the affirmative. The third question, obviously, is dependent on the second, and at best can be answered only hypothetically on the basis of our present knowledge.

1. *Does the protoplasm of a single individual Uroleptus mobilis and its progeny by division undergo progressive waning of vitality and natural death if conjugation and parthenogenesis are prevented?*

Table 2 and diagram 1 based upon it show clearly enough that this question is answered in the affirmative. Series A, C, D, F, G, and H, all started as ex-conjugants, show the same initial

vitality and a progressively waning vigor leading to death, while other series, obtained from these by conjugation, fed at the same times on the same standardized culture medium, are now living actively. Counting from the day on which the first division of the ex-conjugant occurred to the day on which the last division occurred, the protoplasm of the A series divided during 267 days; that of the C series during 294 days; the D series, 215 days; the F series, 256 days; the G series, 253 days, and the H series, 245 days. The total number of divisions and the average division rate per individual in each series are shown in table 3.

The C series had the greatest vitality in regard to endurance, while the F series had the greatest vitality in regard to division

TABLE 3

	SERIES					
	A	C	D	F	G	H
Number of division days.....	267	294	215	256	253	245
Number of divisions.....	313	348	271	317	291	268
Average division rate in any ten day period.....	11.72	11.83	12.14	12.38	11.50	10.93

energy. In the latter case the individuals in each line divided on the average 12.38 times in ten days, while in the C series the average was 11.87, and in the H series it fell to 10.93. The difference between F and C is too slight for comment, but that between F and H or between D and H may have some significance in connection with the problem as to whether the offspring vary in vitality according to the age of the parents at the time of conjugation. I have not enough data at present to throw much light on this problem, but the data from which table 3 was derived may be further analyzed to show how the differences between the different series are distributed in the life cycles.

It is shown above that the first conjugations in a series occur, as a rule, from fifty to seventy days after the first division of the ex-conjugant which gives rise to the series. Sixty days, therefore, may be chosen as approximately the period elapsing before the first conjugation in a series, and a period representing the stage

of immaturity of the protoplasm. Adopting sixty days as a unit period, it is possible to work out from the daily records the division rates in all series for the first, second, third, and fourth sixty days of each series to date. These are shown in table 4 in which the mean division rate of a series per day and its probable error have been worked out by Davenport's formulae as shortened by Crampton. For comparison with the preceding tables, these rates are divided by five and multiplied by ten (multiplied by two) to give the number of divisions which each of the five lines of protoplasm of a series is capable of undergoing in ten days. The G series is omitted from this table, as it represented protoplasm which did not come from the original A series. Three other series, I, J, and L, are introduced, although only one period of the last is involved. The mean for the first sixty days of the A series is not included, since this represents the period of experimentation with the culture media at the outset of the work; the standard culture media was first used with this series ten days before the beginning of the second sixty-day period.

The remarkable uniformity of the division rates during the first sixty days in all series regardless of the source, or calendar period, or age of parent, indicates that every ex-conjugant composed of a portion of the original protoplasm derived from A begins its life cycle with a definite optimum division energy indicated by 17.1 to 17.9 divisions per line in ten days. This is the average rate for sixty days, and the rate for the first or second ten day period may be lower or higher than the average for sixty days. Referring to table 2, we find a higher rate than the mean for sixty days in the first ten-day periods of series C, H, and I; while it is lower than the mean for the first sixty days, in the first ten-day periods of series D, F, J, and L. These averages for the first ten days may be even less than the averages for the same calendar periods of the parental series, a fact which furnishes the kind of evidence that has been used by some experimenters as an argument against rejuvenescence by conjugation. Thus in the first ten-day period of the C series, the division rate was 18.6 while that of the parental A series in the same period was 20.8. Again the F series, with

[illegible]

only 13.4 divisions in its first ten-day period, was considerably lower than that of the parent C series, which was 15.6 for the same calendar period. These fluctuations disappear by using the longer, sixty-day period, where in every case the mean division rate of the filial series is greater than that of the parental series for the same period (table 5).

The mean division rates for the second sixty-day periods show a decreased vitality in every series (table 4). Here, again, there is a remarkable uniformity of results, although variations are more marked than in the first sixty-day period. The decrease in the vitality of the H series in this second period is so slight that it would not be noticed were it not for the decrease in all series—a decrease which is especially noticeable in the F and J series.

In the third sixty-day period there is a similar decrease in vitality over the second sixty-day period in all series, and the variations in different series become still greater. Compared with the second sixty-day period, the decrease in vitality is again slight, but compared with the first sixty-day periods, the decrease in vitality becomes sufficiently large to indicate an indisputable waning of vitality in all series.

In the fourth sixty-day periods the vitality shows a marked drop in all series that have passed through this age, and here the variations in different series are extreme. The D series, for example, drops from an average of eleven and a half divisions to seven-tenths of one division in ten days. On the other hand, the F series dropped only from twelve and six-tenths to nine and seven-tenths divisions in ten days.

The precipitous fall in vitality manifested during this fourth period of sixty days is continued into the fifth period. Only a few of the series outlive this sixty-day period, indeed, only the protoplasm of the C series was alive at the end of three hundred days, that of the A series dying during the last week of this fifth period. The decline in vigor and early death were most marked in the F and H series, the former dying four weeks, the latter, three days after the end of the fourth period.

Maupas found that the last individuals of a race were deformed, reduced in size, and degenerate through loss of micronuclei and

cirri. In *Paramecium caudatum* ('04) I found that the micronucleus is degenerated through hypertrophy, while the cortex showed degeneration mainly through the entire absence of trichocysts. Similarly, the last individuals of a series of *Uroleptus mobilis* show evidences of morphological as well as physiological degeneration. They become greatly reduced in size and the micronuclei entirely disappear, probably by absorption. The macronuclei do not disappear, but show characteristic changes indicating degeneration. The eight separate nuclei usually remain independent, but show evidence of an attempt to fuse as they do prior to division in a normal individual. Their chromatin contents, however, are quite different from the normal; instead of an equal distribution of uniform granules, it masses together to form an intensely staining nuclear body similar to those which form from the degenerating macronuclei subsequent to conjugation (fig. 1, 2, 3, 4; cf. fig. 87, and 89, Calkins, '19).

Unlike Maupas's hypotrichs, *Uroleptus* does not show cytoplasmic changes further than reduction in size: the membranelles and cirri do not disappear and the contractile vacuole keeps up its activity.

In this degenerate condition the last individuals of a series are unable to divide—division, apparently, being impossible without a micronucleus. They sometimes show a remarkable tenacity of life, however, in this final phase, indicating that metabolic activities may go on despite degeneration. Thus the last individual of the A series lived for thirteen days after the final (313th) division: the last of the D series for eighteen days; the last of the G series for fourteen days; the last of the H series for fifteen days. The most remarkable cases of longevity of the single individual occurred in the C and the B series. The last individual of the C series lived in its 348th generation for thirty-six days without dividing again, and the last one of the B series, formed as a product of the 257th division on August 31st, lived, without dividing again, until October 9th, a period of forty days. These last individuals, like all others, were transferred daily to fresh standardized culture medium, and their death was not due to violence.

Fig. 1 *Uroleptus mobilis*. Normal individual and individuals degenerated through old age. Camera drawings, same magnification.

1 Normal individual with typical nuclear complex of eight macronuclei and four micronuclei.

2 Individual from the F series in the 315th generation. Four micronuclei still present, but greatly reduced. The macronuclei have undergone granular degeneration. The F series died out in the 317th generation.

3 A prematurely degenerated individual from the C series in the 316th generation. The micronuclei have entirely disappeared. The macronuclei show the characteristic granular degeneration. The C series died out in the 348th generation.

4 Individual from the A series in the 313th generation (a sister cell of the last individual of the series). All of the nuclei have degenerated, the macronuclei by granule formation, the two remaining micronuclei by hypertrophy.

Bearing in mind the fact that, during these periods of declining vigor and death, other filial series derived from their protoplasm through conjugation were living with full vigor, although handled precisely alike and fed at the same times with the same culture medium, we are led to the conviction that waning vitality and natural death are inevitable attributes which are inherent in *Uroleptus* protoplasm, and that the conclusions of Maupas and of subsequent investigators in regard to this phenomenon are fully confirmed.

2. Does conjugation between two closely related individuals of Uroleptus of the same age result in checking this waning vitality and in restoring the protoplasm to full metabolic activity?

The conjugation tests are made with individuals of the same age and of the same series, collected on a certain day from the excess individuals after the isolations are made for that day. These excess individuals, sister cells of those continued in the isolation cultures, represent protoplasm the history of which is identical with that of the protoplasm of the isolation cultures. They are placed in a Syracuse dish with an abundance of culture medium. Here they multiply, and their progeny, in the course of two or three weeks, will conjugate, provided they are sexually mature.

One such pair of conjugating individuals is isolated in fresh culture medium. The two individuals will have separated as ex-conjugants in twenty-four to thirty-six hours, and one of these is isolated to start a filial series. Thirteen such filial series have been started; five of these have completed their full cycles and have died out; eight, in various stages of vitality, are still under observation.

The question above has already been answered positively by the results shown in table 4. Every ex-conjugant from the A protoplasm, regardless of the phase of vitality in which the conjugation occurred, shows an average division rate during the first sixty days of life of 17.1 to 17.9 divisions in ten days. Table 4 also shows that this is the highest average division rate of all sixty-day periods of the life cycle. Environmental conditions of food, treatment, etc., being the same for parental and filial

series, the only possible explanation of the restoration of vitality in filial series lies in the process of conjugation by which such filial series are started.

Although table 4 shows that all ex-conjugants have practically the same optimum division rate at corresponding periods of the life cycle, it does not show the actual differences in metabolic activity between parent and filial series in identical calendar periods. If conjugation does not restore vitality, the division rate of a filial series should not differ from the division rate of the parent series from which it has been derived; on the other hand, if it does restore vitality, the extent to which it is restored will be indicated by the difference in the average division rates of parental and filial series in identical calendar periods.

These differences in average division rates of parent and offspring series are clearly shown in table 5. Here two interesting phenomena are evident: first, reading across, the table shows that the discrepancy between parent and offspring increases with the age of the parent series at the time of conjugation; second, reading down, it shows that, while both series are losing vitality, the parent series is losing it more rapidly than the filial series.

In regard to the first of these phenomena, it will be noted that the C series came from the A series when the latter was in the 78th generation and that, during the first sixty days of the filial series, the protoplasm of each line had the power to divide 1.53 times more in ten days than that of each line of the parent A series in the same period. The D series came from the A series when the latter was in the 137th generation, that is approximately fifty generations older than the protoplasm which gave rise to the C series. The discrepancy now between the parent series A and the filial series D amounted to 3.03 divisions in ten days during the first sixty days of the D series. That is the protoplasm of each line of the offspring series had the power to divide 3.03 times more in ten days than that of each line of the parent A series. The filial H series came from the same parent A series when the latter was in the 237th generation, or about 150 generations older than when the C series was formed. The discrepancy between parent A and offspring H series now

amounted to 4.8 divisions in ten days. Finally, the J series came from the same parent A series when the latter was in the 311th generation, i.e., the protoplasm was about 230 generations by division older than it was at the time when the C series came off. The discrepancy in division rates between the young J series and the old parental A series now amounted to 17.6 divisions in ten days. This means that, if the protoplasm contained in the two weakened cells of the parental A series had not conjugated in the 311th generation, it might have divided during the subsequent sixty days at the rate of twenty-five hundredths of one division in ten days, but having conjugated, it actually divided at the rate of 17.9 times in ten days.

These results indicate that the protoplasm of *Uroleptus* under these cultural conditions has a certain optimum capacity or potential of metabolic activity which is gradually exhausted, but which can be restored by conjugation. The greater the exhaustion, the more remarkable the restoration. As recharging a storage battery restores its potential of active energy, so conjugation restores the potential of vital energy. If the battery is recharged before the old charge has been drawn upon, its potential of activity would scarcely be affected. An analogous condition is shown by the *Uroleptus* protoplasm after encystment, and after conjugation occurring in an earlier period of the life cycle (e.g., the A and C series, or the C and F series). If, however, the battery is nearly exhausted before it is recharged, the difference in potential between the newly changed condition and the exhausted condition would be marked. The analogue to this is the J series and the parental A series. The restored potential of vitality in *Uroleptus* protoplasm is a 'charge' of vital energy which is capable of metabolic activities through a period of from 260 to 300 days, or vitality sufficient to produce individuals to the number of 2 to the 300th + power.

In regard to the second of the phenomena shown by table 5, it is interesting to see that, as the protoplasm of a series grows older, the potential of vitality is exhausted at an increasingly rapid rate. Thus, the discrepancy between the division rates of parental and filial series is greater during the second sixty-day

TABLE 5
Comparative vitality of parents and offspring

	AGE OF PARENT.....	C FROM A		D FROM A		H FROM A		J FROM A		F FROM C		I FROM F	
		SERIES	SERIES	SERIES	SERIES	SERIES	SERIES	SERIES	SERIES	SERIES	SERIES	SERIES	SERIES
First 60 days of offspring.	78 generations			137 generations		237 generations		311 generations		86 generations		143 generations	
	2/7 to 4/8			3/8 to 5/7		5/22 to 7/21		8/16 to 10/15		3/28 to 5/27		7/7 to 9/5	
	Mean daily division rate all five lines	8.6333	7.8667	8.5833	7.0666	8.6666	6.2666	8.9666	0.125	8.6000	8.1000	8.5833	6.5666
	Average division rate per line for 10 days	17.2666	15.7334	17.1666	14.1333	17.3332	12.5332	17.9332	0.2500	17.2000	16.2000	17.1666	13.1332
	Increase per line of filial series in each 10 days	1.5332		3.0333		4.8000		17.6832		1.0000		4.0334	
Second 60 days	Dates	4/8 to 6/7		5/7 to 7/6		7/21 to 9/19				5/27 to 7/26		9/5 to 11/4	
	Mean daily division rate all five lines	7.9167	6.6000	8.1666	5.2833	8.5333	1.5500	Parent dead.		7.7834	6.6667	7.9834	5.6666
	Average division rate per line for 10 days	15.8333	13.2000	16.3333	10.5600	17.0666	3.1000			15.5668	13.3334	15.9668	11.3332
	Increase per line of filial series in each 10 days.	2.6333		5.7733		13.9666				2.2334		4.6336	

Third 60 days	Dates	6/7 to 8/6		7/6 to 9/4		9/19 to 11/18		7/26 to 9/24		11/4 to 1/3	
	Mean division rate all lines	6.4166	4.3650	5.7834	2.234	Parent dead		6.3333	4.0166	7.5166	2.0800
	Average division rate per line for 10 days	12.8332	8.7300	11.5668	4.4680			12.6666	8.0332	15.0333	4.1600
	Increase per line of filial series in each 10 days.	4.1032		7.0988				4.6334		10.8733	
	Dates							9/24 to 11/23			
Fourth 60 days	Mean daily division rate all five lines	Parent	dead	Parent	dead			4.8833	1.2500	Parent	t dead
	Average division rate per line for 10 days							9.7666	2.5000		
	Increase per line of filial series in each 10 days.							7.2666			

period of the filial series than during the first sixty days. Indeed the discrepancy between parent and offspring in successive sixty-day periods increases in geometrical proportion. Thus the differences between the A and C series for the first, second, and third sixty-day periods are indicated by 1.53, 2.63, and 4.10 divisions in ten days. The differences in the A-D series are 3.03, 5.77, and 7.09. In the C-F series, the differences for four successive sixty-day periods are 1.0, 2.23, 4.63, and 7.26, which is a geometrical increase of the disparity in division energy of parent and offspring protoplasm.

When we consider that we are dealing here with one protoplasm derived from the protoplasm of the single individual ex-conjugant that was isolated on November 16, 1917, these results offer conclusive evidence that conjugation rejuvenates or restores vitality to an optimum when that vitality is reduced through continued metabolic activity. Many series have died, but I have under cultivation to-day protoplasm of the L, N, P, O, and R series which is directly descended from the original ex-conjugant A and which is living with the same metabolic vigor as that shown by the A series during its most vigorous period. Yet there has been no change in the standardized culture medium with which this protoplasm has been fed, and no variation in the daily treatment. The continued vitality is due solely to the successive conjugations which have taken place between representative bits of this protoplasm.

Since one condition, viz., starvation, is found in the conjugation tests and not in the isolation cultures, the objection might be raised that changes may be set up in the protoplasm due to such starvation or to some other condition of the conjugation tests which would result in a restoration of vitality, thus making conjugation an accessory phenomenon without effect on rejuvenescence. To test this point twenty individuals which had reached this starvation point as shown by reduced size, and all taken from a conjugation test, but without having conjugated, were isolated and carried on in isolation cultures as though they were ex-conjugants. An ex-conjugant from the same test, and obtained from a pair that were isolated while conjugating, was likewise carried on at the same time in five lines as series U.

The results (Table 6) show that conditions of the conjugation test have no stimulating effect on the protoplasmic activities. Indeed there is evidence of a depressing effect as indicated by the average division rates for sixty days. The rates for non-conjugants are not only lower than that for the ex-conjugant (U Series) but, in all cases, are lower than that of the control parental series. All were descendants of the same series (L series) and all were of the same age. The starved individuals are grouped in four aggregates of five each in table 6, as follows:

TABLE 6

	PARENT RACE L SERIES (5 LINES)	EX-CONJUGANT FROM L SERIES U SERIES (5 LINES)	NON-CONJUGANTS FROM SAME CONJUGATION TEST AS U SERIES. TWENTY INDIVIDUALS IN 4 GROUPS OF 5 EACH			
			Group 1	Group 2	Group 3	Group 4
Average division-rate first 10 days.....	8.4	9.2	7.0	7.6	7.4	6.8
Second 10 days.....	5.4	14.0	5.8	6.6	6.6	6.8
Third 10 days.....	10.0	13.4	4.4	5.6	6.8	5.4
Fourth 10 days.....	10.4	14.0	7.0	6.6	8.4	5.4
Fifth 10 days.....	7.6	13.6	6.6	6.8	7.2	7.2
Sixth 10 days.....	5.6	13.6	7.8	7.6	6.4	7.4
Average division-rate 60 days.....	7.9	12.96	6.4	6.8	7.1	6.5

There is some evidence, by no means complete as yet, that vitality of the more recent series lacks the endurance of the earlier series. This is apparent in diagram 1, where the curves of the filial series are progressively shorter from the C series to the J series. Both I and J are now in the last stages of metabolic vigor and no further divisions in either series will occur. The I series is now 235 days old, and the J series 200 days, the former having divided 322 times, the latter 253 times. The shortened cycle appears to be accompanied by greater intensity of division energy than in the earlier filial series, the I series having an average division rate of 13.2 divisions in ten days, the J series, 12.6 (table 3). In a few months the cycles of the L, N, O, P, and R series will be complete and will furnish more adequate data for conclusions on this interesting point.

The Q series, coming from the I series in the 316th generation, has been queer from the start, dividing only twenty-three times in thirty-three days, and it will soon die out. The exceptional history of this series is probably due to faulty reorganization after conjugation, for the nuclear complex is quite abnormal. If this result is due to the age of the parent series at the time of conjugation, the history of the Q series is an interesting contradiction to that of the J series where the parent series was relatively even older at the time of conjugation. Defective reorganization is possible after any conjugation, but the chances of such defective reorganization are probably greater with increasing age of the parent.

While the several series described above were all derived from one ancestral protoplasm of the A series, two other series, B and G, came from a different source. The B series was started from an encysted *Uroleptus mobilis* which had encysted in 'wild' stock before the A series was started. It emerged from the cyst on January 25, 1918, and lived until October 9th, dividing 258 times between January 25th and September 1st, or 11.8 divisions in ten days on the average. Many epidemics of conjugation occurred, but only one ex-conjugant was isolated. This one formed the G series, the B series being in the 115th generation at the time. The same resultant renewal of vitality and continued life of the filial series was observed as with the A protoplasm, the G series starting with an optimum division rate of 18.06 in ten days for the first sixty days, running through 291 generations and dying out January 4, 1919.

3. *Does reorganization during encystment ('endomixis' or parthenogenesis) restore waning vitality to full metabolic vigor?*

Unfortunately, I have insufficient data to draw positive conclusions on this subject. Only two series, B and M, were derived from cysts, and of these only the M series is pedigreed. This series was started on November 18th from a cyst that was formed by an individual of the F series in its 45th generation, on April 27, 1918, and remained encysted for six months. In

the first period of sixty days after emerging from the cyst, it had a higher division rate than any other representative of the A protoplasm, the protoplasm of each line dividing, on the average, 19.8 times in ten days. If the vitality of the encysted individual was the same in potential as that of the same protoplasm in the isolation cultures, we would expect the division rate of the isolation series during the sixty days subsequent to the 45th generation to be practically the same as that of the protoplasm from the cyst. This expectation, however, was not realized, for during this period the F series divided only 17.6 times in ten days—a difference in potential of 2.2 divisions in ten days. The difference between the division rate of the F series for its first sixty days and the M series for its first sixty days was 2.6 divisions in ten days (table 4).

The difference between the division rates of the parent F series and the offspring M series by parthenogenesis is not actually as large as the figures indicate. The M series was maintained under the conditions of a constant temperature of 24°C., while the F series was maintained under the conditions of laboratory temperature which varied from 19° to 22°C. That the difference in rate is not due solely to these different conditions, however, is shown by a comparison of the M series with the L series (cf. Table 2). The latter came from the I series and the I series from the F series. Both L and M, therefore, had the same ancestry. Furthermore, L and M were started at approximately the same time, L as an ex-conjugant, M from a cyst, and both series were maintained under the same temperature conditions. The L series, during the same sixty days as above for the M series, had an average division rate of 18.8 in ten days as against 19.8 for the M series.

The unpedigreed B series, which came from a cyst, may be compared with the C series which started as an ex-conjugant at about the same time as the B series. The first sixty days of the B series gave an average division rate of 17.4 divisions in ten days, while that of the C series was 17.2.

So far as the evidence thus far obtained is concerned, it appears that the initial vitality after encystment and partheno-

genesis is as great as, or even greater than, that after conjugation. It remains to be seen whether this high potential has the same capacity of endurance as that obtained from conjugation. The M series is not old enough at the present time to furnish evidence. The B series divided only 258 times, while its contemporaries, the A and C series, divided 313 and 348 times, respectively.

GENERAL

It is not my intention to formulate here any theory in explanation of the phenomenon of rejuvenescence. The facts concerning it may be grouped in two categories: one, physiological, the other, morphological.

In the first place, the results presented in this paper show that in *Uroleptus mobilis* the physiological processes of metabolism are not capable of unlimited activity. The limits vary from the time of conjugation or encystment to between 268 (H series) and 349 (C series) generations by division. Within these limits there is a progressive weakening of metabolic vigor from an optimum shown during the first three months after conjugation. This weakening, furthermore, increases by geometrical progression, i.e., it is cumulative, as shown by the geometrical increase of the difference in vitality between filial and parental series in successive sixty-day periods (table 5). Such weakening protoplasm, if not allowed to conjugate, inevitably dies, as does the somatic protoplasm of metazoa.

A second physiological fact is equally well established by these *Uroleptus* experiments. The same protoplasm is transformed from a condition of metabolic weakness to a condition of optimum metabolic vigor by the process of conjugation. The effect of conjugation is clearly indicated by the extreme case of the J series. Here the protoplasm, at the time of conjugation, had only enough metabolic vigor to divide twice in ten days. The cells that conjugated were both composed of this weak protoplasm. Ten days later if they had not conjugated, each might have been able to divide at the rate of 0.25 times in ten days, or once in forty days; but, having conjugated, one of them, the

J series, was able to divide at the rate of 17.9 times in ten days, or 71.6 times in forty days, and there is no reason to doubt that the other ex-conjugant of this pair would have had the same vigor.

The experiments also indicate that there is a limit to the extent to which this protoplasm can be rejuvenated. It might be inferred, with reason, that if two weak individuals are transformed by conjugation into individuals capable of dividing seventeen times in ten days, then conjugation between two individuals from a series in which physiological weakness is not yet perceptible, would result in an ex-conjugant capable of dividing more than seventeen times in ten days. The inference, however, is not supported by the facts. A good illustration is the relation between the F and C series (cf. Table 5). The F series came from C when the latter's vitality was indicated by 17.2 divisions in ten days. Each of the two individuals conjugating at this time, would have had the ability to divide at the rate of 16.2 times in ten days during the ensuing sixty days, if they had not conjugated. Having conjugated, they were able to divide at the rate of 17.2 times—a difference, or extent of rejuvenescence, indicated by only one division in ten days. Since all ex-conjugants, under the conditions of the experiments and regardless of the state of vitality of the parent protoplasm, return to this same optimum of vitality measured by 17+ divisions in ten days (table 4), it is evident that the protoplasm of *Uroleptus* will hold only a certain charge, so to speak, or potential of metabolic vigor, as a result of conjugation. This optimum, of course, is subject to change by changes in the environmental conditions—heat, for example, increasing it.

A third physiological fact is also indicated by these experiments, although the numerical support is not as adequate as that in support of rejuvenescence through conjugation. This is the fact of rejuvenescence following encystment and parthenogenesis, in which no nuclear interchange occurs. The B series and the M series came from cysts. The ancestry of the former is unknown, but during its first sixty days in culture, its metabolic vigor was measured by a division rate of 17.4 times in ten days.

This is the same optimum as that following conjugation. The M series is pedigreed. It came from the F series in its 45th generation, and after a period of six and a half months was recovered from the cyst. During the sixty days subsequent to the date of the 45th generation, the F series divided at the rate of 17.6 times in ten days, while the protoplasm of the M series during its first sixty days of culture divided at the rate of 19.8 times in ten days. The difference (2.2 divisions) is undoubtedly greater than it would have been had the temperature conditions remained the same. As explained on page 151, the M series was cultivated under conditions of higher temperature than the laboratory, whereas the F series was cultivated under the laboratory temperature. The L series, which came from the F series through conjugations and which was started at approximately the same time as the M series, was cultivated under the same conditions as the M series. Its division rate during this same period of sixty days was 18.8—one full division higher than the usual ex-conjugant. In this case of the M series, therefore, the rejuvenating effect of parthenogenesis was even greater than that of conjugation. Whether the endurance of the parthenogenetic protoplasm differs from that of the protoplasm following conjugation remains to be seen.

Parthenogenesis through encystment appears to be an attribute of high vitality, and the ability to encyst is apparently lost at an early date (diagram 1). In the C series it did not occur after the 160th day; in the F series, not after the 110th day, and in the D, I, and J series it did not occur after the 80th, 60th and 20th days, respectively. I do not know what this means, but it is certainly true that no internal reorganization without encystment has occurred thus far, for in every series the physiological depression is continuous and progressive, and death invariably follows. Conjugation, with rejuvenescence, however, is possible almost to the end of the cycle. Encystment, apparently, is not possible near the end of the cycle, but it does occur even in the first ten days after conjugation. Conjugation, on the other hand, does not occur until from thirty to seventy days after the previous conjugation. I am aware of published state-

ments to the contrary in connection with other ciliates, and it may well be that this condition of sexual immaturity noticeable in *Uroleptus* is not universal among ciliates.

As Maupas found for other hypotrichous ciliates, the condition of physiological depression is accompanied by morphological changes. During the period of active metabolism the cell rapidly grows to full size after division ($140\ \mu$ to $165\ \mu$). The macronuclei are eight in number, with from four to six micronuclei. In preparation for division a portion of each macronucleus is thrown off and is absorbed in the cytoplasm, while the remaining portions fuse to form a single division nucleus. All but two of the micronuclei are likewise absorbed. In the late individuals of a cycle, the macronuclei lose their characteristic nuclear clefts and in some cases show a tendency to fuse, while in other cases the number is increased from eight to as many as sixteen smaller and irregularly shaped nuclei. The micronuclei do not increase in number, but undergo degeneration by hypertrophy or by granular degeneration, and finally disappear in the cytoplasm. In the last individuals of a series, the chromatin of each macronucleus collects in a single large, highly refractile, and densely staining granule. The size of the cell is greatly reduced and it is unable to divide. The cytoplasm is probably as much changed as the nuclei, but morphological evidence of such change is difficult to detect. In general there is a tendency to increased vacuolization, while the mitochondria, which form a cortical layer in the normal individual, are rare and irregularly distributed. Some individuals in this final stage of depression live without dividing for thirty-six (C series) and forty (B series) days.

In conjugation, apart from the processes of maturation and reduction in number of chromosomes from eight to four, and union of the gametic nuclei, the most significant phenomenon is the granular disintegration of the old macronuclei, and absorption of the relatively large quantity of nuclear substance in the cytoplasm. Not only do the macronuclei thus furnish nucleoproteins to the cytoplasm, but the micronuclei also contribute no small part. Thus, if an individual goes into conjugation with

four micronuclei, all four of them may undergo the first maturation division. Of the eight possible micronuclei thus formed, only two undergo the second maturation division, while six are absorbed. All of the four products of the second maturation division may undergo the third division, and of the eight products of this phase, only two become pronuclei, the other six being absorbed. Finally, in the second division of the amphinucleus, two of the four products form the new micronuclei, one forms the new macronucleus, while one, the sister nucleus of the new macronucleus, is absorbed in the cytoplasm (Calkins, loc. cit., pp. 316-326).

The cytological details of encystment are not yet worked out. Anticipating the description of the process, it may be briefly stated here that the macronuclei break up into granules as they do after conjugation, and these granules are absorbed in the cytoplasm.

One phenomenon, therefore, common to division, conjugation, and encystment, is the absorption of variable quantities of nuclear substance in the cytoplasm. That the physical and chemical consequences of such absorption are connected with the phenomenon of rejuvenescence seems probable. That the relation between the new amphinucleus after conjugation, or the new nuclear complex after encystment, and this reorganized cytoplasm is likewise connected with the phenomenon of rejuvenescence is equally probable. The nature of such connections and of such relations is a matter of speculation for which we are not yet prepared.