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The effect of sodium chloride upon the fission rate within a single clone of Paramecium caudatum and the heritability of that effect.

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THE EFFECT OF SODIUM CHLORIDE UPON THE FISSION RATE WITHIN A SINGLE CLONE OF PARAMECIUM CAUDATUM AND THE HERITABILITY OF THAT EFFECT

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My deep gratitude is due Dr. Austin R. Middleton, for his gracious guidance and constructive criticism of this work. Dr. Middleton has given unstintingly of the results of his numerous researches done within the clone of *Paramecium caudatum*, and this work is hereby dedicated to him.

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INTRODUCTION

Inherited characteristics have been found remarkably constant in organisms multiplying without admixture of two parents that differ in hereditary constitution. Most of the recent work agrees that in such uniparental reproduction, inherited variations occur rarely or not at all, and that selection has practically no effect in altering racial characteristics.

A certain number of observations have been made which bear on the inheritance of the fission rate and on the question whether there are differences among the progeny of a single individual in respect to these. Maupas (15) made extensive studies of the effects of temperature and of other conditions on fission rates of Paramecium caudatum. He was convinced from the result of his studies, that under given conditions the fission rate is uniform in all progeny of a given individual, and that inherited variations in the rate do not occur in such a pure race or within a single clone. On this subject, his conclusions are in complete harmony with those of the 'pure line' workers. He summarizes his results as follows:

"In all my cultures I have always seen all the normal descendants of the same ancestor, grow and multiply with the most perfect uniformity. I have become convinced of the integral transmission of the faculty of development from one generation to another, and the most complete physiological equivalence must exist among all the normal individuals produced in the successive generations.

In long and numerous experiments on fifteen to twenty species, I have never observed anything which permits belief in the existence of morphological and physiological differences, not merely between the two products of a given fission, but even among all those which have descended from such a fission by regular and continuous generations."

A paper of Jennings (11), likewise deals to a certain extent with this subject. He states that in a wild population, many strains differing in rate of fission (under the same conditions) were found to occur. Futhermore, it was demonstrated that even in a population derived by fission from a single individual in a pure strain, conjugation produced inherited differences in the fission rate. After conjugation there were present strains showing constant differences in fission rate. On the other hand, if no conjugation has occurred among the progeny of a single individual, the fission rate was found to be nearly uniform.

Calkins and Gregory (3) state that there are in many cases differences in the fission rate among the four sets of progeny resulting from the first two divisions of an individual that has just conjugated.

When conditions are not uniform, the rate of fission will not be uniform in all the progeny of a given individual. When the progeny of a single paramecium are put into different culture media, under constant conditions, the fission rate of the paramecia will differ according to the medium used.

THE SPECIFIC PROBLEM

When a single paramecium divides, often one of its two progeny again divides before the other does. In successive generations this same thing may be repeated. Figure 1 shows that among the progeny of a single individual at a given moment, there may occur animals that are the products of four and others that are the products of two fissions. Hence there are differences of fission rate among the descendants of a single individual. Under these circumstances, selection for either fast or slow rate of fission is unavoidable. Middleton (16) by his device of 'balanced selection', (which will be explained in the discussion of technique) eliminated this selection. This opened the possibility of more accurately testing the effects of environmental factors upon the hereditary fission rate. The first of these studies was that of Middleton in which it was shown that temperature differences produce heritable differences in the fission rate. He has shown also that organic and inorganic substances modify hereditarily the fission rate.

This study considers the effect of sodium chloride upon the fission rate within a single clone of <u>Paramecium</u>

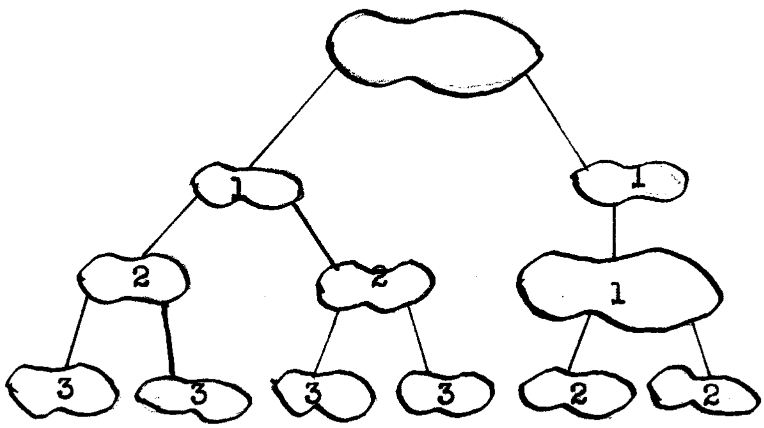

Figure 1 shows the successive fissions among the progeny of two individuals, that at a given moment we may have, among the progeny of these two parents, individuals of the second and the third generations which are indistinguishable. The numbers within the outlines of the animals indicate the number of fissions which have intervened between it and its original parent.

caudatum, and the heritability of that effect.

The present paper is a statement of the results of a series of experiments designed to throw light upon the question of the possibility of modifying living systems by environmental factors so that the modifications persist in later generations in the absence of the factor which brought forth the modification. Can the individual, by subjecting it to the sodium chloride culture medium, become so changed that the changes persist through later generations?

TECHNIQUE

On September 21, 1931, a single *Paramecium caudatum* was isolated. On September 25, 1931, forty individuals, all of the eighth generation, were obtained.

The animals were cultivated on ground-glass slides having each two circular depressions capable of holding four or five drops of culture medium. These slides were kept in moist chambers. Five drops of culture medium were used in each depression and no cover-glasses were employed.

Table 1 shows how the paramecia were selected daily in order to obtain a clone all of the same generation.

Twenty two-groove ground-glass slides were employed. One paramecium was put into each groove, thus making forty individuals. The following day, a single paramecium was selected from among the progeny of each of these and put into each groove of the twenty clean slides containing freshly made culture media.

On September 25, 1931, individuals all of the eighth generation were secured.

Table 1

The daily selection of <u>Paramecia caudatum</u> for the securing of a clone. On Sept. 21 a single paramecium was isolated. Lines A, B, C, D, E, F, G, H, and I were established on Sept. 22. On Sept. 23 paramecia were selected from line D. Through the selection of paramecia from line D, a clone of the eighth generation was established on Sept. 24, 1931.

Sept. 22, 1931.

Row 1. (Slides 1-10)	*	Line	
	3-4	A	3-4
	3-4	A	3-4
	3-4	B	3-8
	6-4	C	6-4
	6-8	C	6-8

Row 2 (Slides 11-20)		Line	
	6-8	C	6-8
	7-8	D	7-8
	7-4	D	7-8
	7-8	E	7-8
	7-8	E	7-8

Sept. 22, 1931.

Row 3. Slides (21-30)		Line	
	5-4	F	5-4
	5-8	G	5-8
	5-8	G	4-1
	4-1	G	4-1
	4-1	H	6-8

Row 4. (Slides 31-40)		Line	
	6-8	H	6-8
	6-8	H	6-4
	6-4	H	-
	6-8	I	6-8
	6-4	I	6-4

Sept. 23, 1931.

Row 1. (Slides 1-10)		Line	
	32-1	D	32-1
	32-1	D	32-1
	32-1	D	32-1
	32-1	D	32-1
	32-1	D	32-1

Row 2. (Slides 11-20)		Line	
	32-1	D	32-1
	32-1	D	32-1
	32-1	D	32-1
	32-1	D	32-1
	32-1	D	32-1

Sept. 24, 1931.

Row 1. (Slides 1-10)		Line	
	128-1	D	128-1
	128-1	D	128-1
	128-1	D	128-1
	128-1	D	128-1
	128-1	D	128-1

Row 2. (Slides 11-20)		Line	
	128-1	D	128-1
	128-1	D	128-1
	128-1	D	128-1
	128-1	D	128-1
	128-1	D	128-1

*Fission rate of paramecia

Of the forty individuals thus obtained, twenty were placed in a 1/16% solution of Horlick's Malted Milk culture. This culture medium was made by dissolving one-half gram of the malted milk powder in 50 c.c. of boiling distilled water. Six and two-thirds c.c. of this solution were next diluted to 100 c.c. with boiling distilled water, making a 1/16% solution. This solution was cooled before using. This culture medium was used for the control and duplicate-experimental lines. The remaining twenty paramecia were isolated similarly in a 1/16% Horlick's Malted Milk solution. But in the preparation of this medium, a 1/10% distilled water solution of chemically pure sodium chloride was used as the solvent of the malted milk powder instead of plain distilled water. This sodium chloride solution was made by dissolving one Parke Davis sodium chloride tablet in four fluid ounces of distilled water. Twenty-five c.c. of this saline solution was diluted to 150 c.c. with distilled water, making a 1/10% solution which was used for the experimental lines. Each day a fresh 1/10% malted milk sodium chloride solution was made.

The slides of the control and experimental lines were so arranged, that slide No. 1 of the experimental set corresponded with slide No. 1 of the control set, and so on throughout the ten slides of the two sets.

Figure 2 illustrates the correspondence of the slides of the two sets.

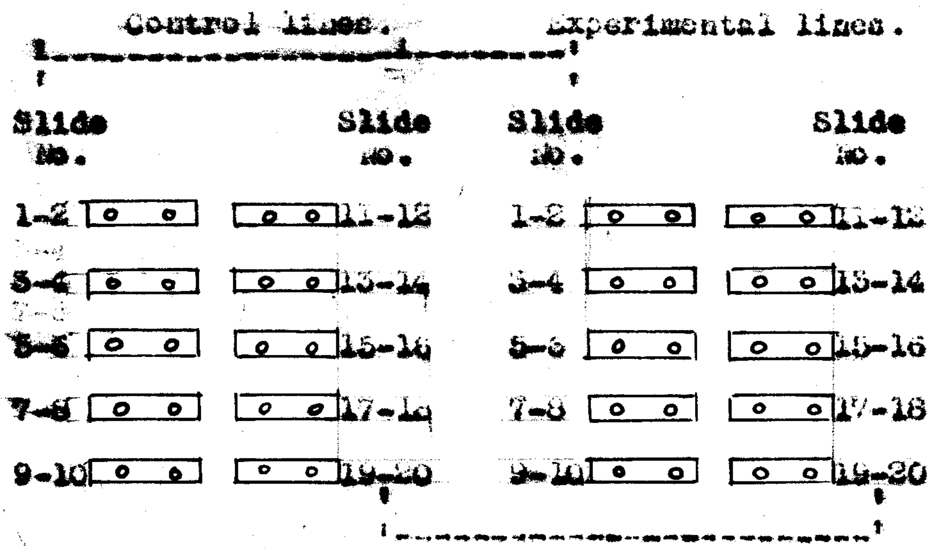

Figure 2 shows how the slides of the control and experimental set were arranged so that slide No. 1 of the experimental set corresponded with slide No. 1 of the control set, and so on throughout the ten slides of the two sets.

There were twenty corresponding lines established in the two sets.

Each slide contained two lines, since there was one line in each of the two grooves.

An individual of the same line was put into slide No. 1 of the experimental set as into slide No. 1 of the control set. Likewise with slides 2-3-4 etc.

Likewise the paramecia of the experimental and later of the duplicate experimental sets were changed daily to fresh culture slides of their own sets.

The duplicates of the experimental lines are referred to throughout this study as the duplicate-experimental lines.

The control and experimental set ran for a period of twenty-five days. At the end of the fifth day, the experimental set was duplicated. This set was termed the duplicate experimental set. The paramecia which were transferred from the sodium chloride culture medium of the experimental set, were put into the 1/16% malted milk culture medium.

One paramecium from groove No. 1 was put into corresponding groove No. 1 of the fresh slide. If there were two individuals found in a single groove, the slide was numbered 2-1. This record meaning that there were two individuals resulting from one fission. If there were four paramecia of the same size in the groove, the slide was labelled 4-2, meaning that there were four individuals resulting from two fissions. If there were three paramecia, one large and two small individuals in the groove, it meant that there had been two fissions, the original individual dividing once, making two individuals, and then the dividing of only one of these two paramecia, making the three individuals. If one of the smaller individuals was selected, the slide was marked 3-4 S., meaning that a slow selection was to be made for that line at the first opportunity. On the other hand, had the large individual been selected, the slide would have been marked 3-2 F., designating that the next selection must be a fast one.

Through this means 'balanced selection' was assured throughout all lines. 'Balanced selection' is the process of compensating for the effect of every selection that one is compelled to make by making the reverse selection at the next opportunity; in other words, one makes the same number of plus and minus selections in any given line during each successive time interval adopted.

The most obvious method without 'balanced selection' might have been to select all lines, both experimental and control for fast rate. But, under such a procedure, if there was an effect of the sodium chloride slowing the fission rate of the experimental lines, there would be an opportunity for more frequent selections in the control lines. On the other hand, should the sodium chloride increase the fission rate of the experimental lines, then the reverse would be the case. In either case, this procedure would not reveal the true effect of the sodium chloride on the fission rate. The paramecia might have been selected at random, resulting in an untrue balance at the end of the test period.

In order that results may be of value, conditions must be uniform throughout. In adding to the uniformity of conditions, temperature and bacterial content should not vary. A rise in temperature increases the fission rate; in very warm weather fission rate is considerably accelerated. On the other hand, a drop in temperature, decreases the fission rate.

If the bacterial content becomes too great, the fission rate decreases. One-celled animals or plants sometimes destroy the paramecia. Uniformity of bacterial content in the culture media was secured by washing the paramecia, at certain intervals, in fresh culture medium before transferring them to a new slide. The paramecia were allowed to remain (usually ten minutes) in the fresh medium in order to wash themselves as free as possible of bacteria. They were then transferred to new culture media on the definitive slide. The pipette used in transferring the individuals was invariably sterilized in boiling distilled water after each transfer, thus preventing the unintentional introduction of any individual which might cling to the pipette. The slides were labelled in soft lead pencil; the number of individuals and fissions at each examination were likewise recorded on them, to be later transferred to permanent records.

The slides were examined daily at approximately the same time so that there would not be a longer period one day and a shorter one on the following day.

EXPERIMENT I

EXPERIMENT I

A comparison of the fission rate of the control set with that of the experimental set for a twenty-five day test period.
(Sept. 25-- Oct. 19,1931)

On September 25, 1931, forty Paramecia caudatum, all of the eighth generation, were isolated on ground glass slides, one paramecium to each concavity, thus dividing the forty paramecia into two groups of twenty each. One group was kept as the control set in a 1/16% Horlick's malted milk culture medium, the other to be designated as an experimental set, in a 1/10% sodium chloride culture medium. For twenty-five days, these twenty control lines and twenty experimental lines were propagated with 'balanced selection'.

Table 2 shows the actual number of fissions per five-day period per line in the control and experimental sets. When the differences per line per five-day period of the forty lines are averaged, it shows that the control lines produced .35 fission more than the average for the experimental lines in the first five-day period, .65 generations more in the second five-day period, 3.65 generations more in the third five-day period, 3.15 generations more in the fourth five-day period, and 5.15 generations

Table 2

Actual number of fissions per five day period per line of the 'balanced selection' in the Control and Experimental lines of Paramecium Caudatum. The differences in number of fissions in favor of the Control lines are also given.

The average difference in favor of the Control line, per five-day period during this experiment, is 2.59 fissions per line.

Lines Number	1	2	3	4	5	6	7	8	9	10	11	12	13
First Five day period:													
Control	3	4	3	7	4	4	4	6	3	3	4	4	5
Experimental	6	4	0	1	6	4	3	3	2	4	5	5	4
Difference	-3	0	3	6	-2	0	1	3	1	-1	-1	-1	1
Second 5-day period :													
Control	0	4	1	3	4	7	5	4	1	5	7	6	5
Experimental	6	5	1	4	3	3	4	0	3	4	4	3	4
Difference	-6	-1	0	-1	1	4	1	4	-2	1	3	3	1
Third Five day period:													
Control	6	6	6	7	2	5	7	7	2	5	7	9	6
Experimental	3	3	4	1	3	5	2	3	2	3	2	1	1
Difference	3	3	2	6	-1	0	5	4	0	2	5	8	5
Fourth 5-day period :													
Control	5	7	6	6	4	6	7	8	8	3	5	4	7
Experimental	3	3	4	4	2	2	3	2	1	1	2	3	3
Difference	2	4	2	2	2	4	4	6	7	2	3	1	4
Fifth Five day period:													
Control	8	7	8	8	6	5	8	6	8	7	5	1	8
Experimental	2	3	1	1	2	2	2	2	1	1	2	2	3
Difference	6	4	7	7	4	3	6	4	7	6	3	-1	5
Differences for the whole 25 days.	2	10	14	20	4	11	17	21	13	10	13	10	18

Table 2

In this table, the numbers in the 'difference' columns indicate that the Control line has produced so many more fissions than the corresponding Experimental line; thus during the first five-day period Control line No. 4, produced 6 more fissions than Experimental line No. 4. When the number in the difference column is negative, the Experimental line has produced more fissions than the corresponding Control line during the five-day period; thus, during the first five-day periodExperimental line No.1 produced 3 more fissions than corresponding Control line No. 1.

14	15	16	17	18	19	20	Total	Average	% That The Difference Is Of the Total In All
4	1	4	3	3	4	3	76	3.80	
4	4	5	2	3	1	3	69	3.45	
0	-3	-1	1	0	3	0	7	.35	.048
4	5	5	7	5	5	6	89	4.45	
6	3	6	4	5	5	3	76	3.80	
-2	2	-1	3	0	0	3	13	.65	.078
8	7	3	8	6	9	8	124	6.20	
1	1	2	3	4	4	5	51	2.55	
7	6	1	5	2	5	3	73	3.65	.417
6	6	5	6	5	6	7	117	5.85	
3	3	5	3	4	2	1	54	2.70	
3	3	0	3	1	4	6	63	3.15	.368
8	6	8	10	8	10	7	142	7.10	
4	2	2	3	1	1	2	39	1.95	
4	4	6	7	7	9	5	103	5.15	.567
12	12	5	19	10	21	19	259	12.95	

more in the fifth five-day period. The average difference in favor of the control lines, per five-day period during the twenty-five day experiment, is 2.59 fissions per line. The actual total difference per twenty lines per five-day period in favor of the control lines was 7 fissions in the first five-day period, 13 fissions in the second five-day period, 73 fissions in the third five-day period, 63 fissions in the fourth five-day period, and 103 fissions in the fifth five-day period. There was a total difference of 259 fissions for twenty-five days.

This table shows that the difference in fission rate in favor of the control set was not great at the beginning of the experiment, but as the experiment progressed, the fission rate decreased, showing a large difference in the fission rate of the two sets.

Figure 3 is a polygon showing the average fission rates for five-day periods of the control and experimental sets. The largest fission rate for the experimental set was 15.50 for the second five-day period, while the largest fission rate for the control set was 28.25 for the fifth five-day period.

Figure 4 is a polygon showing the five-day dif-

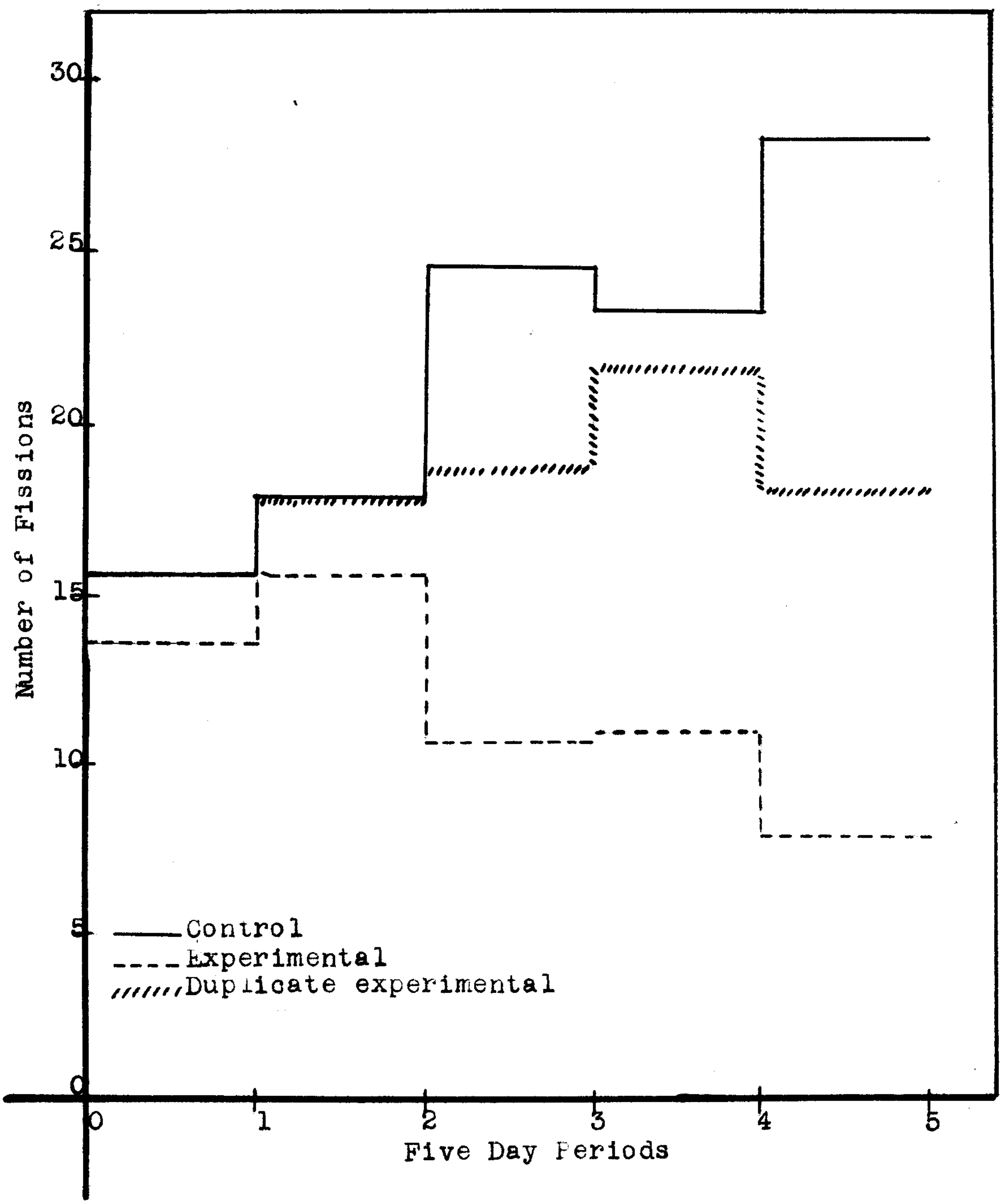

Figure 3. Polygon showing the average number of fissions for five day periods.

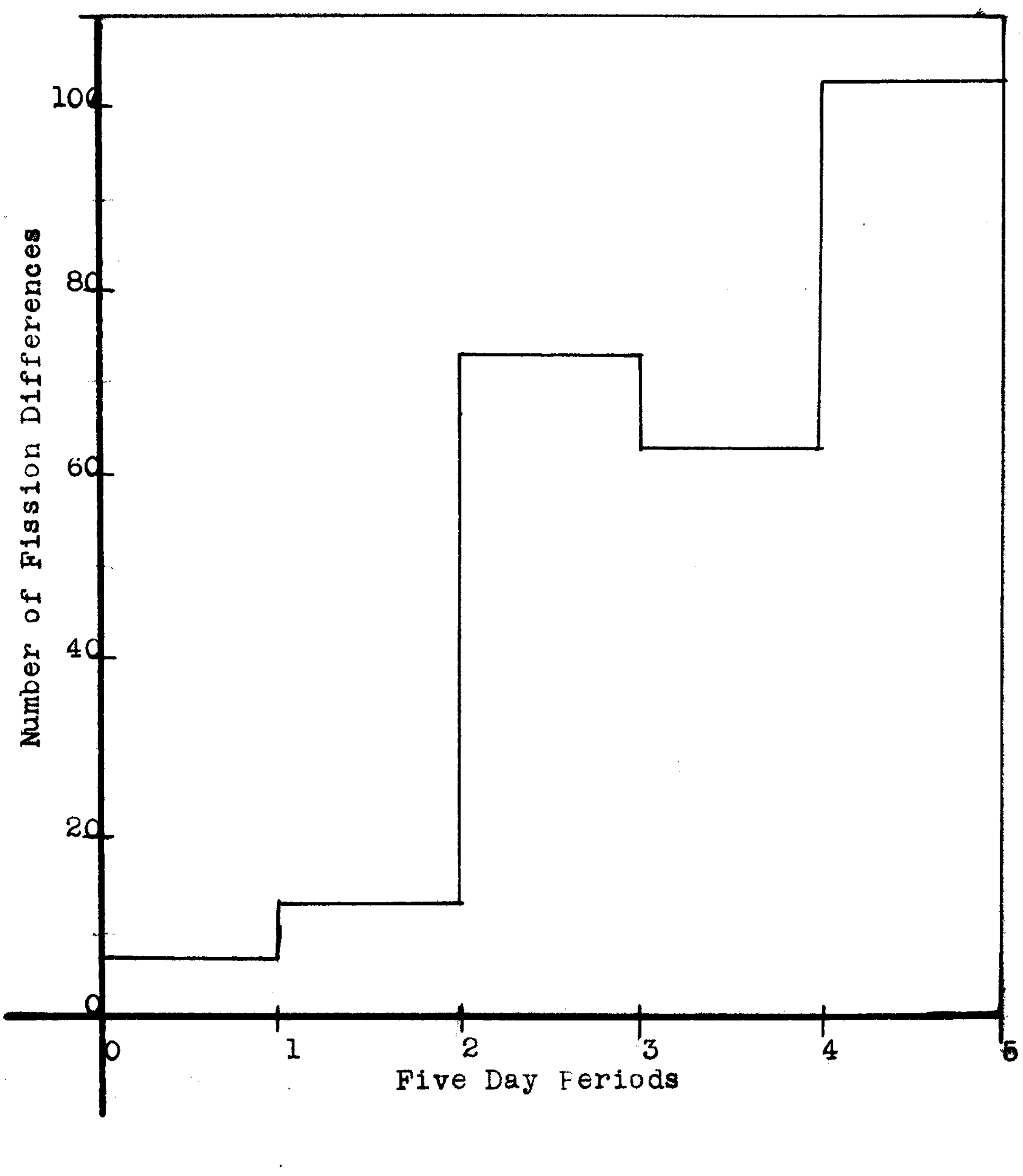

Figure 4. Polygon showing the five-day differences in fission between the control and experimental lines of Paramecium caudatum.

Excess favors control lines.

ferences in fission rate between the control and experimental sets for five-day periods. The fission rate is in favor of the control set. The fission difference during the first period was 7, the second period 13, the third period 73, the fourth period 63, and the fifth period 103. The results for Figure 4 was given on Table 2.

Table 3 shows the average daily fission in the control and experimental sets for a period of twenty-five days. The difference between the averages in the fission rate of the two sets, is in favor of the control set. On the third, fourth, and seventh days, the fission rate of the experimental set averaged more than that of the control. The respective differences on these three days are 0.25, 0.10, and 0.25 fissions in favor of the experimental set. On the remaining twenty-two days, the averaged daily fission rate of the control set is in excess of that of the experimental set.

Figure 5 shows clearly the curve of the daily differences between the average number of fissions produced by the control and experimental sets. Figure 5 supplements Table 3. The curve designating the daily differences in fission rate in favor of the control set, increased steadily upward as the period progressed.

Table 3

Average daily fission in Control and Experimental lines of Paramecium Caudatum while balanced selection was in progress for a period of twenty-five days. The difference between the averages in fissions of the two lines is given, the excess being in favor of the Control line.

(Differences given the minus sign show an excess of the Experimental line.

Number of days	1	2	3	4	5	6	7	8	9
Average fissions:									
Control	0	1.15	.80	.90	.95	.75	.60	1.20	.75
Experimental	0	.80	1.05	1.00	.60	.55	.85	.95	.60
Difference	0	.35	-.25	-.10	.35	.20	-.25	.25	.15

Number of days	10	11	12	13	14	15
Average fissions:						
Control	1.15	1.75	1.20	1.30	1.15	.80
Experimental	.85	1.05	.60	.40	.50	.00
Difference	.30	.70	.60	.90	.65	.80

Number of days	16	17	18	19	20	21
Average fissions:						
Control	.75	1.30	1.70	.90	1.20	1.40
Experimental	.05	.60	1.10	.05	.90	.35
Difference	.70	.70	.60	.85	.30	1.05

Number of days	22	23	24	25
Average fissions:				
Control	1.15	1.05	1.85	1.65
Experimental	.35	.20	135	.70
Difference	.80	.85	1.50	.95

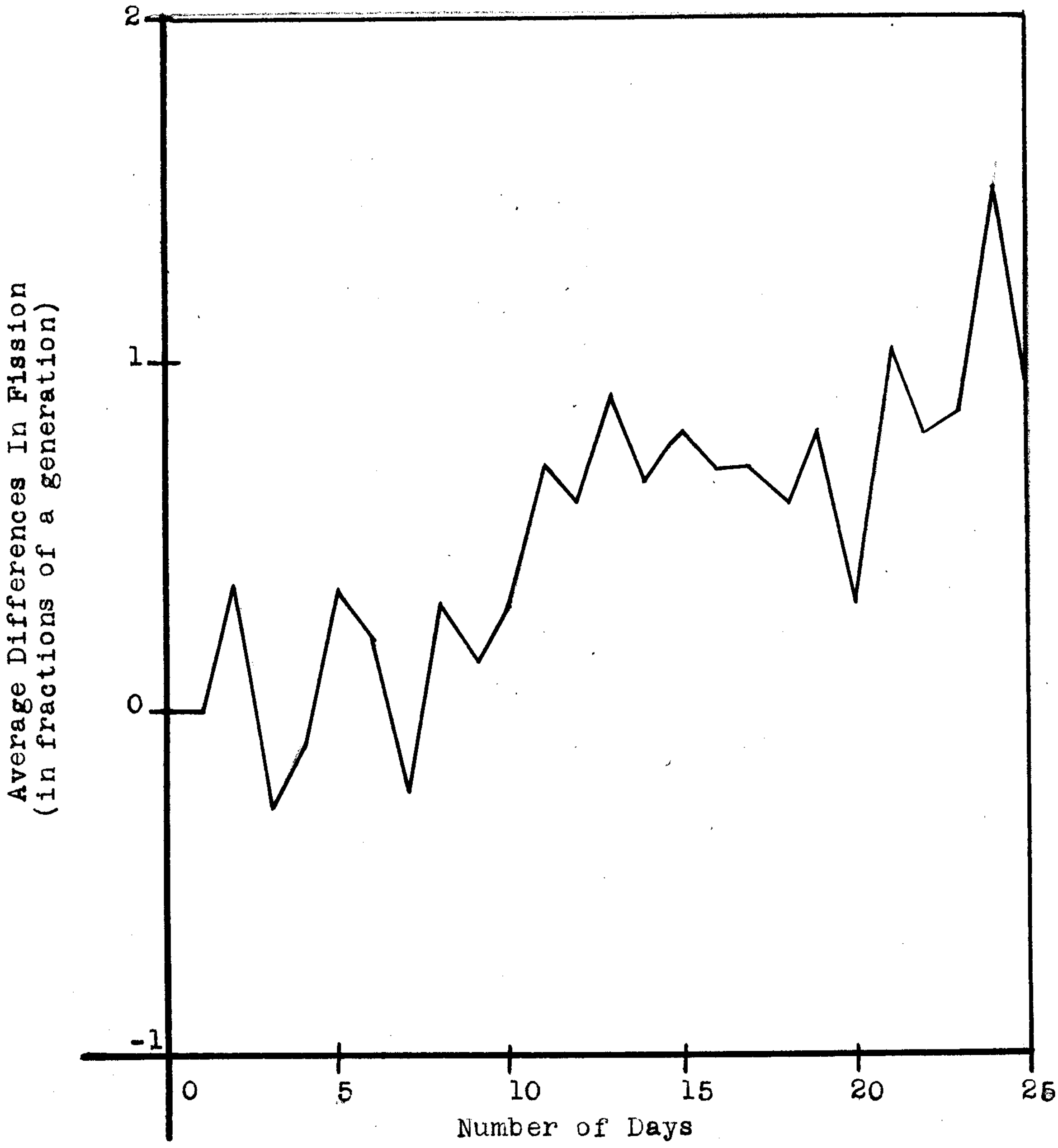

Figure 5. Showing curve of the daily differences between the average number of fissions produced by the control and experimental lines.

Excess is in favor of control lines.

Table 4 shows the distribution of the actual number of fissions per five-day period for the twenty lines of the control and experimental sets. The fission rate for the twenty-five day period ranged from 4 to 6.8 for the control set. The total fission rate was 104.74. The fission rate for the same period ranged from 1.8 to 4 for the experimental set, with a total of 51.66 fissions. The fission range for the twenty lines in the control set was above the fission range of the experimental set. The two sets overlapped at 4 fissions. The largest fission rate in the experimental set was 4 fissions, (two lines having 4 fissions). The lowest fission rate in the control set was 4 fissions, (one line having 4 fissions).

Figure 6 shows the daily differences in the fission rate of the control and experimental sets for the twenty-five day test period. On only three days, the third, fourth, and seventh day was the fission rate of the experimental set greater than that of the control set. On the third day, the experimental set had 21 fissions, while the control set had only 18 fissions; on the fourth day the experimental set had 20 fissions, while the control set had only 18 fissions; and on the seventh day

Table 4

Distribution of lines, as regards average number of fissions in the control and experimental lines during the twenty-five day test period. The fissions are averaged for five-day periods.

No. Fissions	1.8	2	2.2	2.4	2.6	2.8	3	3.2
Control (No. lines)								
Experimental (No. lines)	1	2	1	1	3	2	3	2

No. Fissions	3.4	3.6	4	4.4	4.6	4.8	5	5.4
Control (No. lines)			1	2	1	2	2	2
Experimental (No. lines)	1	2	2					

No. Fissions	5.6	6	6.2	6.8	Total
Control (No. lines)	2	1	5	2	104.74
Experimental (No. lines)					51.66

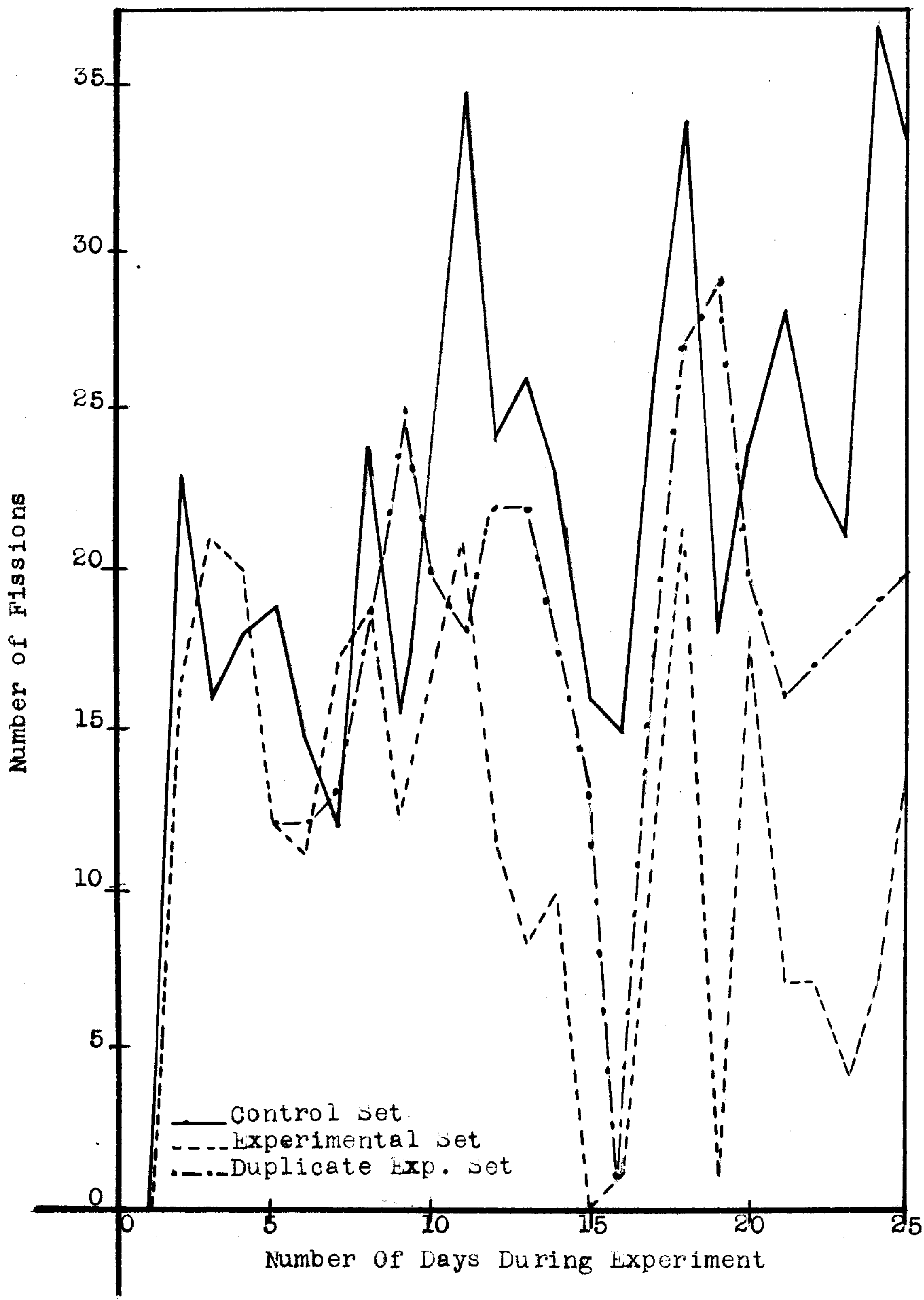

Figure 6. Curves showing daily fission rates in the control, experimental, and duplicate experimental sets.

the experimental set had 17 fissions, while the control set had only 12 fissions. There was a difference in fission rate between these two sets of 5, 2, and 5 fissions respectively in favor of the experimental set. On the remaining twenty-two days, the fission rate in the control set was greatly in excess of that of the experimental set. For the first ten days of the test, the difference in fission rate for the two sets was not so noticeable as it was for the next fifteen days. There was as steady an increase in the fission rate of the control set as there was a decrease in the fission rate of the experimental set. The figure shows a great divergence in the lines which represent the daily fission rate of the two sets. On the twenty-fifth day, the fission rate was 33 fissions for the control set and 14 fissions for the experimental set.

Figure 7 shows the variation in average five-day fissions of the control and experimental lines during the twenty-five day test period. This figure supplements Table 4, in which is given the number of lines and their five-day fission averages. This figure plainly demonstrated the meeting of the lines

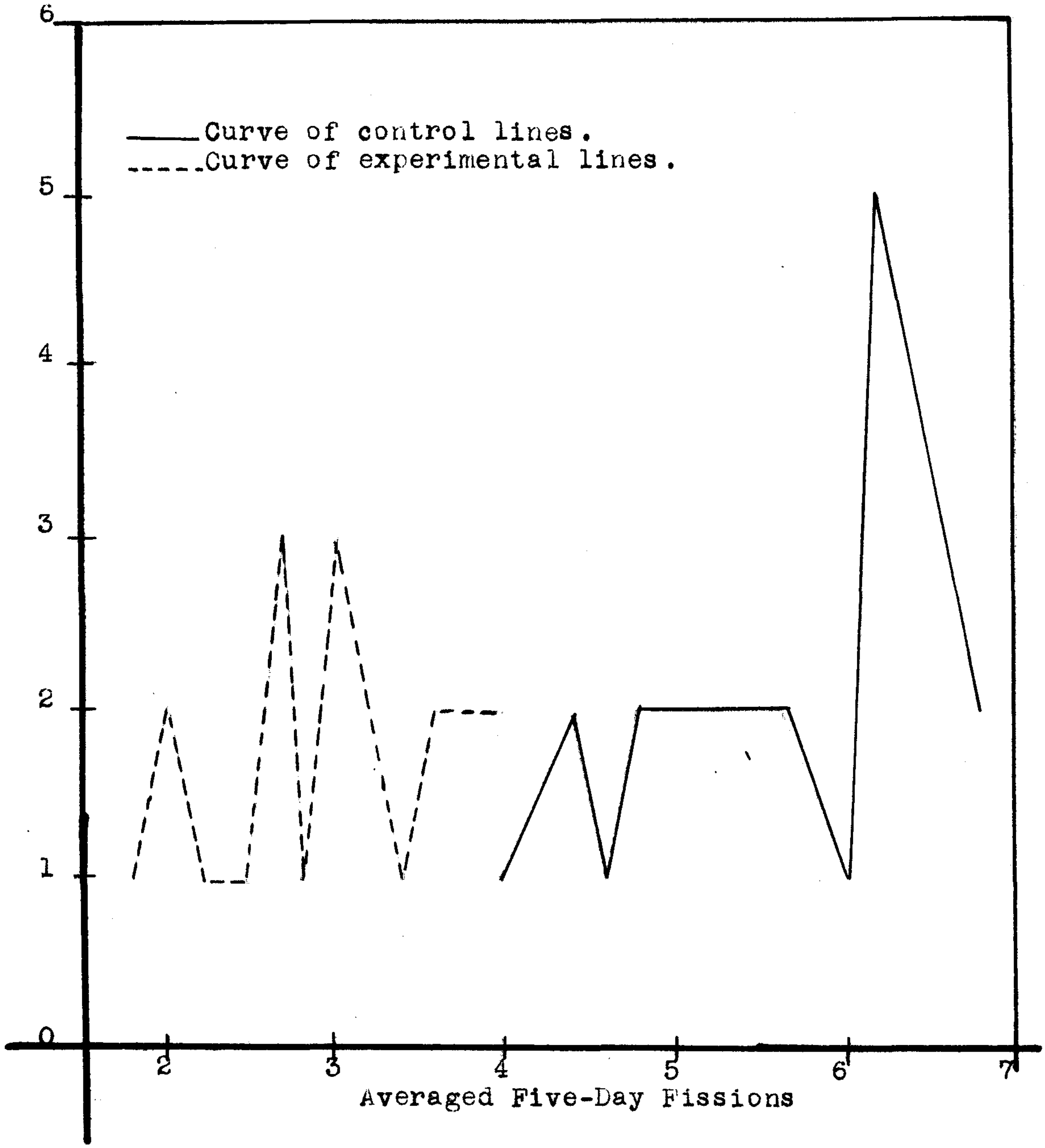

Figure 7. Curve showing the average fission rate for five-day periods in the control and experimental lines during the twenty-five day test period.

of the control and experimental sets. The two lines meet at 4 fissions. All fissions in the experimental set are below the 4 fissions mark, while all fissions in the control set are above the 4 fissions mark.

RESULTS

Throughout the twenty-five day period, the fission rate of the control set was so much greater than that of the experimental set, that several important facts present themselves upon studying the figures pertaining to the two sets. First--that the sodium chloride had a definite effect on the reduction of fission rate among the experimental lines, throughout the period. Second--that the reduced fission rate persisted throughout the period in the experimental set.

EXPERIMENT II

EXPERIMENT II

A comparison of the fission rate of the control set with that of the Duplicate experimental set for a twenty-day test period.
(Sept. 30--Oct. 19, 1931)

On September 30, 1931, five days after the beginning of the experiment between the control and experimental sets were started, the experimental lines were duplicated. A single paramecium from each of the twenty lines in the experimental set was isolated in each of twenty correspondingly numbered ground-glass slides. This new set was termed the duplicate experimental set. The paramecia in this set were transferred daily to fresh slides containing the 1/16% malted milk culture medium. The paramecia in the experimental set were transferred daily to fresh slides containing the sodium chloride culture medium. The three sets were in progress at the same time for a period of twenty days. All conditions as temperature, light, bacterial content, and cleanliness, were uniformly maintained for the three sets.

Figure 3 (see page 26) is a polygon of the fissions averaged for each of the four five-day periods. In the control set, there was a daily average of 17.75 fissions during the first five-day period, and a daily average of 17.50 fissions in the duplicate experimental set, leaving a daily difference of .25 fission during the first five-day period in favor of the control set. During the second five-day period, there was an average daily fission rate of 24.50 fissions in the control set, and 18.50 fissions in the duplicate experimental set, leaving a difference of 6 fissions. During the third five-day period, there was an average daily fission rate of 23.25 fissions in the control set, and 21.50 fissions in the duplicate experimental set, leaving a daily difference of 2 fissions. The fourth five-day period shows an average daily fission rate of 28.25 fissions in the control set, and 18.00 fissions in the duplicate experimental set, leaving a daily difference of 10.25 fissions. There was a marked difference in fission rate between the control and experimental sets during each of the four five-day periods. The rate of fission in the control set increased as the period lengthened.

Figure 8 is a polygon showing the five-day fission differences between the control and duplicate experimental lines. All fission differences for the four five-day periods favor the control set. During

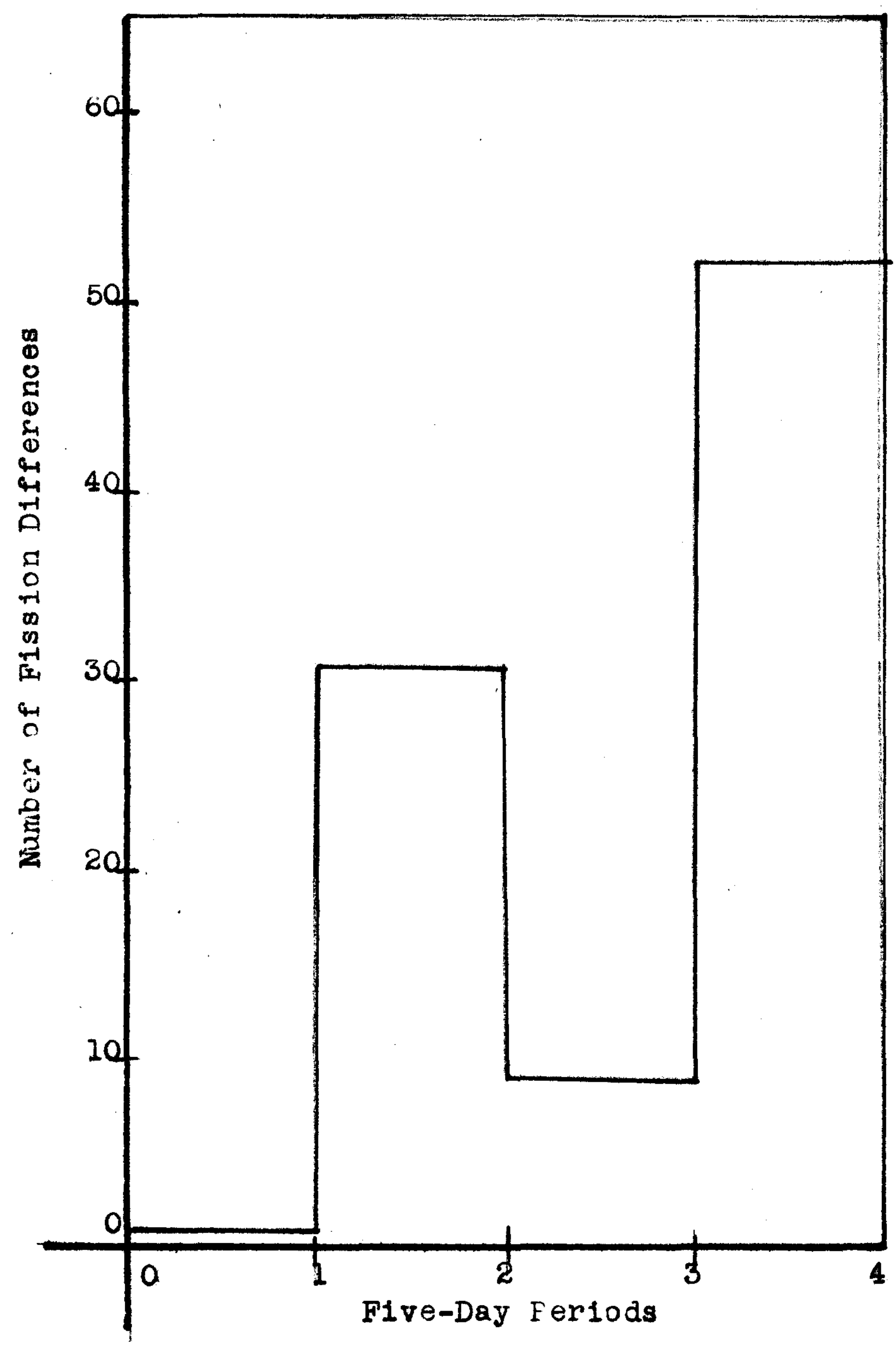

Figure 8. Polygon showing the five-day fission differences between the control and duplicate experimental lines.

Excess favors control lines.

the first period, there was a difference between the two sets of 1 fission. In the second period, there was a difference of thirty-one fissions. In the third period, there was a difference of 9 fissions. During the fourth five-day period, there was a difference of 52 fissions. There was a total difference in favor of the control set for the entire twenty days of 93 fissions. The fission difference in favor of the control set was greatest during the last five-day period. The fission rate of the paramecia of the duplicate experimental set, released from sodium chloride medium, after five days tended to increase somewhat upon the introduction of the malted milk culture medium.

Figure 9 shows the daily differences between the average number of fissions produced by the control and duplicate experimental sets for a period of twenty days. The fission differences for the twenty days are as follows: first day, .15 fissions in favor of the control set; second day, .05 fission in favor of the duplicate experimental set; third day, .3 fission in favor of the control set; fourth day, .5 fission in favor of the duplicate experimental set; fifth day, .15 fission in favor of the control set; sixth day,

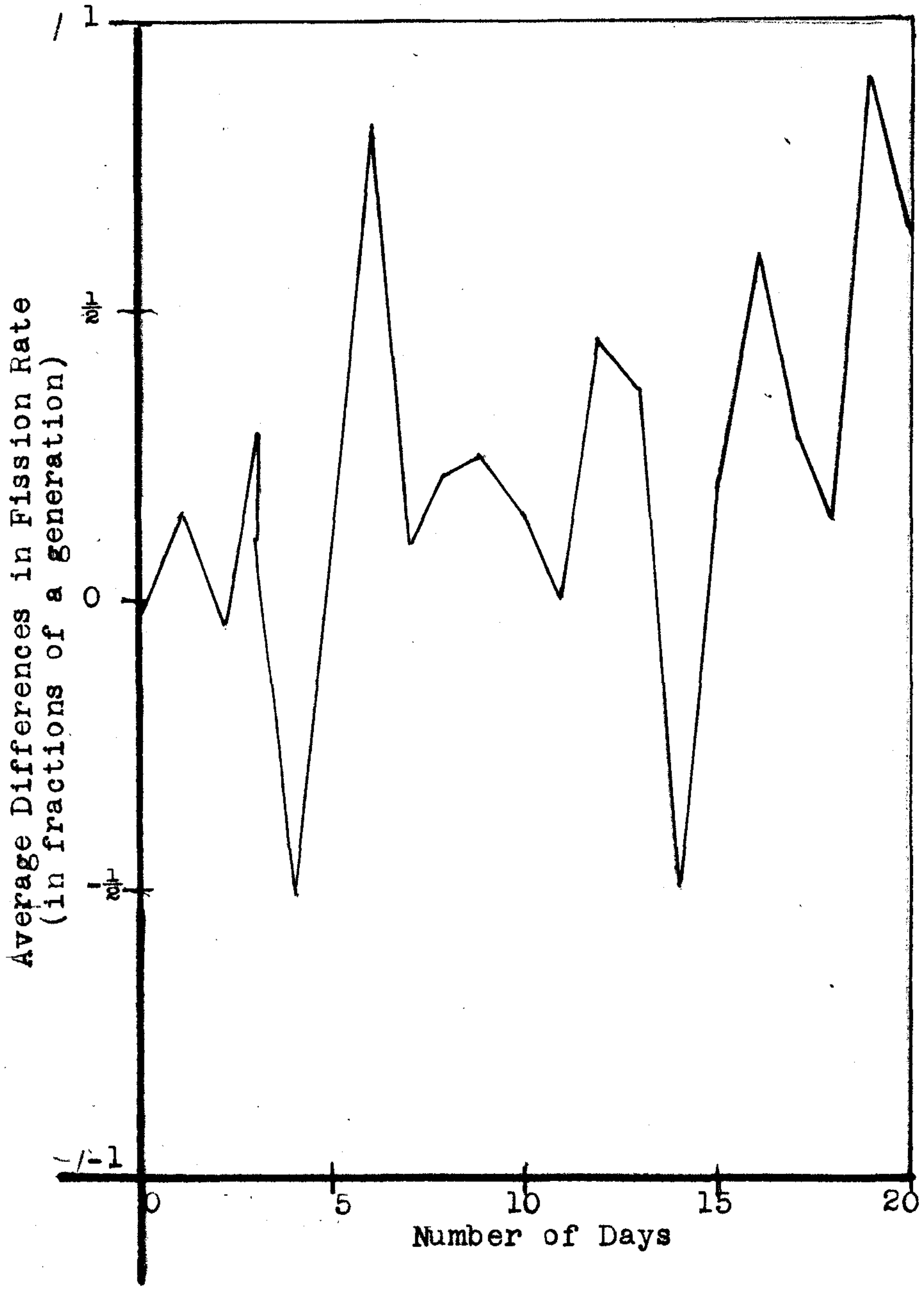

Figure 9. Showing the curve of daily differences between the average number of the fissions produced by the control and experimental lines.

Excess in fission rate is in favor of the control lines.

.85 fission in favor of the control set; seventh day, .1 fission in favor of the control set; eighth day, .2 fission in favor of the control set; ninth day, .25 fission in favor of the control set; tenth day, .15 fission in favor of the control set; eleventh day, fission rate was equal; twelfth day, .45 fission in favor of the control set; thirteenth day, .35 fission in favor of the control set; fourteenth day, .55 fission in favor of the duplicate experimental set; fifteenth day, .2 fission in favor of the control set; sixteenth day, .6 fission in favor of the control set; seventeenth day, .3 fission in favor of the control set; eighteenth day, .15 fission in favor of the control set; nineteenth day, .9 fission in favor of the control set; and twentieth day, .65 fission in favor of the control set. On only the second, fourth, and fourteenth days did the average daily fission rate of the duplicate experimental set exceed that of the control set.

Table 8 (see page 44) gives the distribution of the twenty lines as regards actual number of fissions in the control and duplicate experimental sets for the twenty-day test period. The fissions were taken per five day periods. The range for the twenty lines

Table 5

Distribution of lines, as regards average number of fissions in the control and duplicate experimental lines during the twenty-day test period.

The fissions are averaged for five-day periods.

No. Fissions	4	4.25	4.50	4.75	5	5.25	5.50
Control (No. lines)	1			2	2	2	
Duplicate experimental (No. lines)	2	4	2	3	6	1	1

No. Fissions	5.75	6	6.25	6.50	6.75	7	7.25
Control (No. lines)	1	5	1	2	1	1	
Duplicate experimental (No. lines)	1						

No. Fissions	7.50	7.75	Total
Control (No. lines)	1	1	118.00
Duplicate experimental (No. lines)			94.75

in the control set was from 4 to 7.75 fissions, with a total of 118.00 fissions for the twenty days. One line had 4 fissions, two lines had 4.75 fissions, two lines had 5.00 fissions, two lines had 5.50 fissions, one line had 6.25 fissions, two lines had 6.50 fissions, one line had 6.75 fissions, one line had 7 fissions, one line had 7.50 fissions, and one line had 7.75 fissions.

The range for the twenty lines in the duplicate experimental set was from 4 to 5.75 fissions, two fissions less than that of the range for the control set. Two lines in the duplicate experimental set had 4 fissions, four lines had 4.25 fissions, two lines had 4.50 fissions, three lines had 4.75 fissions, six lines had 5 fissions, one line had 5.25 fissions, one line had 5.50 fissions, and one line had 5.75 fissions, making a total of 94.75 fissions in the twenty lines for the twenty days. The greatest number of fissions in the control set fell at 6 fissions, while the greatest number of fissions in the duplicate experimental set fell at 5 fissions. The mean for the fission rate in the control set fell at 6 fissions, while the mean for the fission rate in the duplicate experimental set fell at 4.75 fissions, the difference between the two means being 1.25 fissions.

Figure 6 (see page 33) shows the daily differences in the fission rate of the control and the duplicate experimental sets. The daily differences in fission rate between the two sets are as follows: first day, 7 fissions favoring the control set; second day, 1 fission favoring the duplicate experimental set; third day, 6 fissions favoring the control set; fourth day, 10 fissions favoring the duplicate experimental set; fifth day, 3 fissions favoring the control set; sixth day, 17 fissions favoring the control set; seventh day, 2 fissions favoring the control set; eighth day, 4 fissions favoring the control set; ninth day, 5 fissions favoring the control set; tenth day, 3 fissions favoring the control set; eleventh day, fissions were equal; twelfth day, 9 fissions favoring the control set; thirteenth day, 7 fissions favoring the control set; fourteenth day, 11 fissions favoring the duplicate experimental set; fifteenth day, 4 fissions favoring the control set; sixteenth day, 12 fissions favoring the control set; seventeenth day, 6 fissions favoring the control set; eighteenth day, 3 fissions favoring the control set; nineteenth day, 13 fissions favoring the control set; and twentieth day, 19 fissions favoring the control set. The total difference of fissions in favor of the control set for the test period was 125 fissions, while in the duplicate experimental set the total difference was 22 fissions. This made a fission difference for the entire period of 103 fissions in favor of the control set.

Figure 10 shows the variation in average 5 day fissions of the control and duplicate experimental sets. This figure supplements Table 5 (see page 44). The line which represents the average five-day fissions in the duplicate experimental set shows the twenty lines within the fission range of 4 to 5.75 fissions, while the line which represents the average five-day fissions in the control set shows the twenty lines having the greater fission range of 4 to 7.75 fissions.

Table 7 shows the number of fissions per five-day period per line in the control and duplicate experimental sets.

During the first five-day period, the twenty lines of the control set averaged .05 fissions more than the twenty lines of the control set. During the second five-day period, the twenty lines of the control set averaged 1.55 fissions more than did the duplicate experimental set. In the third five-day

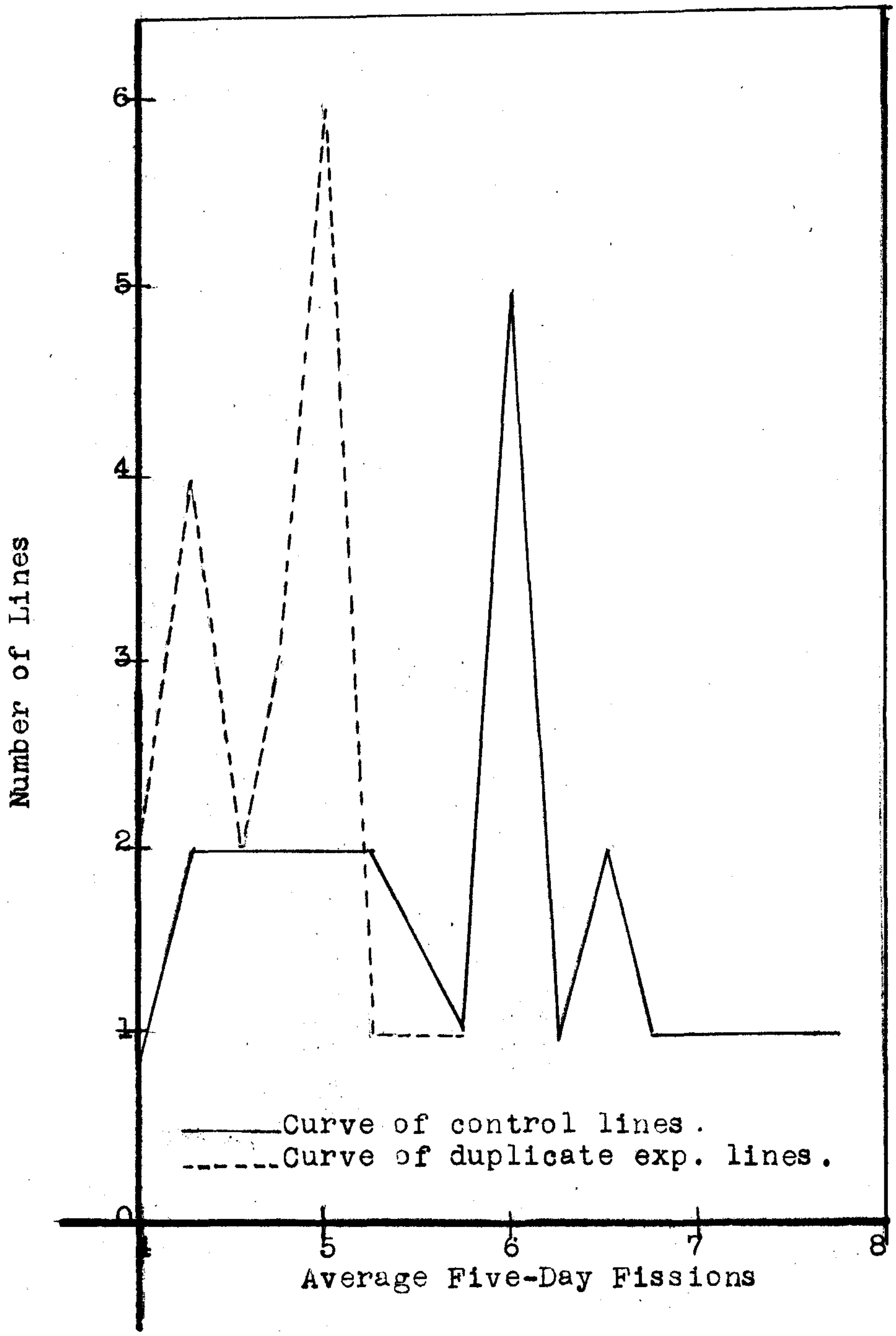

Figure 10. Curve showing the average fission rate for five-day periods in the duplicate experimental and control lines during the twenty-day test period.

Table 7

Actual number of fissions per five-day period per line of the 'balanced selection' in the Control and Experimental (Duplicate) lines of Paramecium Caudatum. The differences in number of fissions in favor of the Control lines are also given.

The average difference in favor of the Control lines per five-day period during the twenty-day test period, is 1.16 fissions per line.

Lines Number	1	2	3	4	5	6	7	8	9	10	11
First 5-day period:											
Control	0	4	1	3	4	7	5	4	1	5	7
Dup. Experimental	4	5	4	4	5	5	4	3	4	4	6
Difference	-4	-1	-3	-1	-1	2	1	1	-3	1	1
Second 5-day period:											
Control	6	6	6	7	2	5	7	7	2	5	7
Dup. Experimental	3	5	5	5	5	5	4	4	6	5	3
Difference	3	1	1	2	-3	0	3	3	-4	0	4
Third 5-day period:											
Control	5	7	6	6	4	6	7	8	8	3	5
Dup. Experimental	4	6	5	6	5	5	5	5	6	6	6
Difference	1	1	1	0	-1	1	2	3	2	-3	-1
Fourth 5-day period:											
Control	8	7	8	8	6	5	8	6	8	7	5
Dup. Experimental	5	5	4	5	5	4	4	5	4	4	5
Difference	3	2	4	3	1	1	4	1	4	3	0
Difference for the whole 20 days.	3	3	3	4	-4	4	10	8	-1	1	4

Table 7.

In this table, the numbers in the 'differences' columns indicate that the Control line has produced so many more fissions than the corresponding Duplicate Experimental line; thus, during the first five-day period, Control line No. 17 produced four more fissions than Duplicate Experimental line No. 17. When the number in the difference column is negative, the Duplicate Experimental line has produced more fissions than the corresponding Control line during the five-day period; thus, during the first five-day period, Duplicate Experimental line No. 1 produced Four more fissions than corresponding Control line No. 1.

12	13	14	15	16	17	18	19	20	Total	Average	% Difference Is of Total in All
6	5	4	5	5	7	5	5	6	89	4.45	
6	5	5	3	4	3	4	5	5	88	4.40	
0	0	-1	2	1	4	1	0	1	1	.05	.01
9	6	8	7	3	8	6	9	8	124	6.20	
7	5	4	4	5	4	4	7	3	93	4.65	
2	1	4	3	-2	4	2	2	5	31	1.55	.14
4	7	6	6	5	6	5	6	7	117	5.85	
6	6	6	6	5	5	5	6	4	108	5.40	
-2	1	0	0	0	1	0	0	3	9	.45	.04
1	8	8	6	8	10	8	10	7	142	7.10	
4	4	5	4	5	4	5	4	5	90	4.50	
-3	4	3	2	3	6	3	6	2	52	2.60	.22
-3	6	6	7	2	15	6	8	11	93	4.65	

period, there was a difference of 9 fissions in favor of the control set, making an average of .45 fissions for the third period in favor of the control set. During the fourth five-day period, the control set produced 52 fissions more than did the duplicate experimental set, so that the control set averaged 2.60 fissions more than did the duplicate experimental set.

The difference in fission rate in favor of the control set for the twenty days was 93 fissions. The average difference in fission rate in favor of the control set for the twenty days was 4.65 fissions.

RESULTS

Throughout the period, the lines in the control set have persistently shown a greater fission rate than the lines in the duplicate experimental set. When the paramecia were started in the control set in the malted milk culture medium, they tended to steadily increase their fission rate as the period progressed. The fission rate of the control set was at a much higher mark all throughout the period than was the fission rate of the duplicate experimental set. When the paramecia were duplicated from the experimental set and cultivated in the malted milk culture medium, a pronounced effect in a low fission rate is shown, as a result of the paramecia having been five days in the sodium chloride culture medium. The five days in the sodium chloride culture medium had so affected the fission rate of the paramecia that throughout the next twenty days in the malted milk culture medium, these paramecia were never able to reproduce as were those which had been in the malted milk culture medium throughout the whole experiment. Thus the heritable effect of the decreased fission rate caused by the sodium chloride was demonstrated throughout the twenty-day test period.

EXPERIMENT III

EXPERIMENT III

A comparison of the Fission Rate of the Duplicate Experimental Set with That of the Experimental set for a Twenty-Day Test Period.
(Sept. 30--Oct. 19, 1931)

In this experiment, the fission rate of the duplicate experimental set and of the experimental set continued for a period of twenty days. The paramecia in the experimental set had been running for a period of five days before the time of this test period. Likewise, the paramecia in the duplicate experimental set had been held in the sodium chloride culture medium five days previous to their transferance into the malted milk culture medium. The effect of maintaining the paramecia in the sodium chloride culture medium before releasing them into the malted milk culture medium, will be considered in this experiment. Also, the effect of the sodium chloride on the fission rate of the experimental set and the heritability of that effect will herein be considered. Thus, paramecia all of the same clone in sodium chloride culture medium, will be tested for the effect of the culture medium on the fission rate.

Figure 11 shows the five-day fission differences between the duplicate experimental and experimental sets for the four five-day periods. In the first period, there was a difference of 12 fissions; in the second period, there was a difference of 42 fissions; in the third period there was a difference of 54 fissions; and in the fourth period, there was a difference of 51 fissions. All fissions differences were in favor of the duplicate experimental set. There was a total excess of 159 fissions in the duplicate experimental set. During the first period, there was not such a marked increase in the fission rate of the duplicate experimental set upon its release from the sodium chloride culture medium, but during the next three periods there was a great increase in fission rate. The fission rate in the experimental set tended to steadily decrease as the periods progressed, resulting in a slower fission rate.

Table 8 gives the distribution of the twenty lines, as to actual number of fissions per five-day periods, during the twenty-day test period between the dupli-

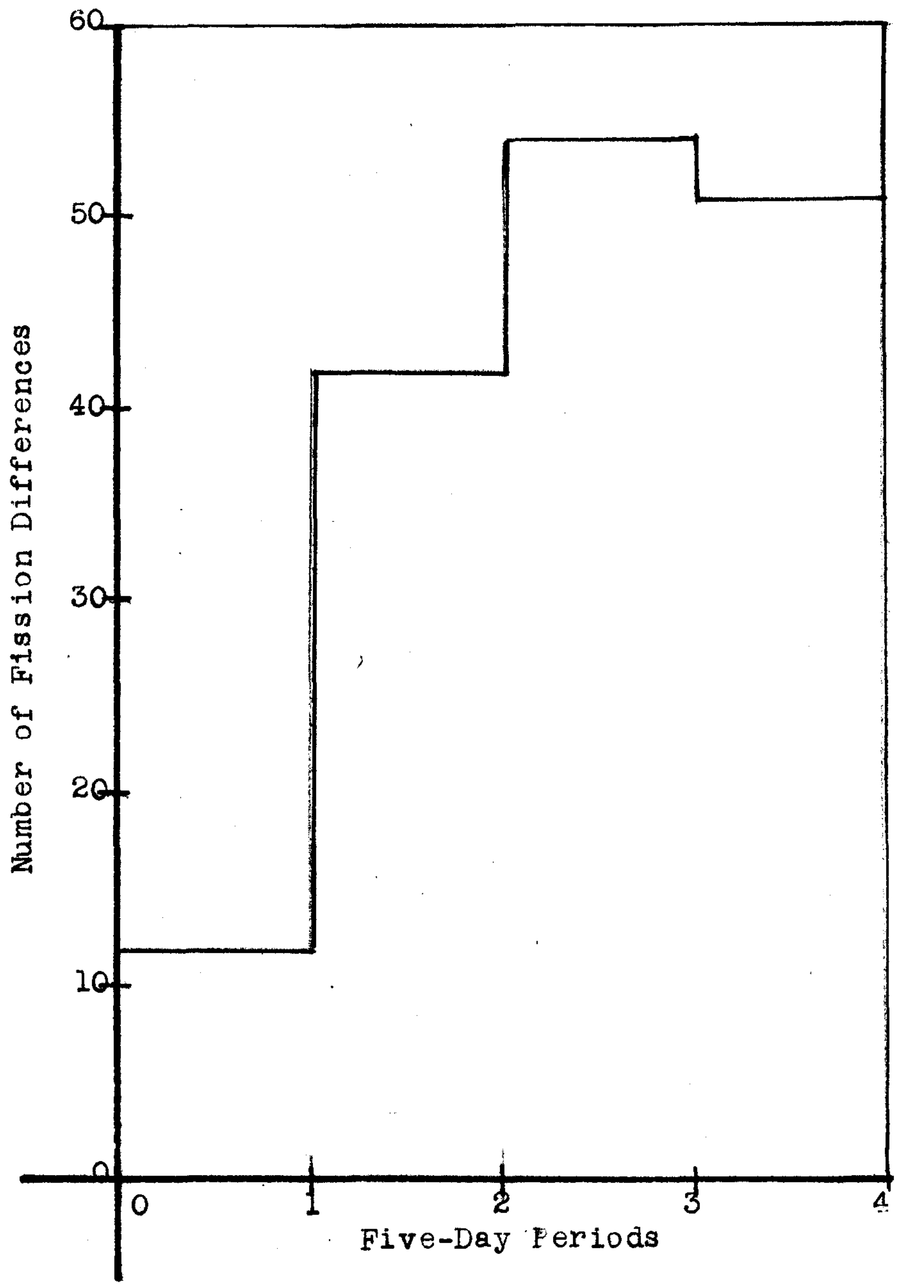

Figure 11. Polygon showing the five-day fission differences between the duplicate experimental and experimental sets.

Excess favors duplicate experimental set.

Table 8

Actual number of fissions per five-day period per line of the 'balanced selection' in the Duplicate Experimental and Experimental lines of Paramecium caudatum. The differences in number of fissions in favor of the Duplicate Experimental Lines are also given.

The average difference in favor of the Duplicate Experimental line, per five-day period during the twenty-day test period, is 1.99 fissions.

Lines number	1	2	3	4	5	6	7	8	9	10	10
First 5-day period:											
Dup. Experimental.	4	5	4	4	5	5	4	3	4	4	6
Experimental	6	5	1	4	3	3	4	0	3	4	4
Difference	-2	0	3	0	2	2	0	3	1	0	2
Second 5-day period:											
Dup. Experimental.	3	5	5	5	5	5	4	4	6	5	3
Experimental	3	3	4	1	3	5	2	3	2	3	2
Difference	0	2	1	4	2	0	2	1	4	2	1
Third 5-day period:											
Dup. Experimental	4	6	5	6	5	5	5	5	6	6	6
Experimental	3	3	4	4	2	2	3	2	1	1	2
Difference	1	3	1	2	3	3	2	3	5	5	4
Fourth 5-day period:											
Dup. Experimental.	5	5	4	5	5	4	5	5	4	4	5
Experimental	2	3	1	1	2	2	2	2	1	1	2
Difference	3	2	3	4	3	2	3	3	3	3	3
Differences for the whole 20-days.	2	7	8	10	10	7	6	10	13	10	10

Table 8

In this table, the numbers in the 'differences' columns indicate that the Duplicate Experimental line has produced so many more fissions than the corresponding Experimental line; thus, during the first five-day period, Duplicate Experimental line NO. 3 has produced three more fissions than Experimental line No.3. When the number in the difference column is negative, the Experimental line has produced more fissions than the corresponding Duplicate Experimental line during the five-day period; thus, during the first five-day prriod , Experimental line NO.1 has produced two more fissions than corresponding Duplicate Experimental line No.1.

12	13	14	15	16	17	18	19	20	Total	Average	% Difference is of Total in All
6	5	5	3	4	3	4	5	5	88	4.40	
3	4	6	3	6	4	5	5	3	76	3.80	
3	1	-1	0	-2	-1	-1	0	2	12	.60	.07
7	5	4	4	5	4	4	7	3	93	4.65	
1	1	1	1	2	3	4	4	3	51	2.55	
6	4	3	3	3	1	0	3	0	42	2.10	.28
6	6	6	6	5	5	5	6	4	108	5.40	
3	3	3	3	5	3	4	2	1	54	2.70	
3	3	3	3	0	2	1	4	3	54	2.70	.35
4	4	5	4	5	4	5	4	5	90	4.50	
2	3	4	2	2	3	1	1	2	39	1.95	
2	1	1	2	3	1	4	3	3	51	2.55	.39
14	9	6	8	8	5	6	10	8	167	8.35	

cate experimental set and the experimental set. In the duplicate experimental set, there were two lines having 4 fissions, four lines having 4.25 fissions, two lines having 4.50 fissions, three lines having 4.75 fissions, six lines having 5 fissions, one line having 5.25 fissions, one line having 5.50 fissions, and one line having 5.75 fissions per five-day period, making a total of 94.75 fissions.

In the experimental set, there were two lines having 1.75 fissions, five lines having 2.25 fissions, four lines having 2.50 fissions, two lines having 2.75 fissions, two lines having 3 fissions, one line having 3.25 fissions, three lines having 3.50 fissions, and one line having 3.75 fissions, making a total of 53.75 fissions.

Figure 3 (see page 26) is a polygon showing the average number of fissions (averaged for five-day periods) for the duplicate experimental and experimental sets. During the first five-day period, the duplicate experimental set averaged 17.50 fissions, while the experimental set averaged 15.50 fissions, leaving a difference of 2 fissions in favor of the duplicate experimental set. The duplicate experimental set averaged 18.50 fissions during the second period, while the experimental set averaged 10.50 fissions, showing an excess of 8 fissions for the duplicate experimental set. In the third period, the duplicate experimental set averaged 21.50 fissions and the experimental set averaged 11.75 fissions, making a difference of 9.75 fissions in favor of the duplicate experimental set. The duplicate experimental set during the fourth period averaged 18 fissions, while the experimental set averaged 7.75 fissions, leaving a difference in favor of the duplicate experimental set of 10.25 fissions. The excess in fission rate averaged for five-day periods was 30 fissions.

Figure 6 (see page 33) shows the daily differences in fission rate in the duplicate experimental and experimental sets. The duplicate experimental set shall be called set 1, and the experimental set shall be called set 2. On the first day, set 1 had 12 fissions, set 2 had 11 fissions; on the second day, set 1 had 13 fissions, set 2 had 17 fissions; on the third day, set 1 had 18 fissions, set 2 had 19 fissions; on the fourth day, set 1 had 25 fissions set 2 had 12 fissions; on the fifth day, set 1 had 20 fissions, set 2 had 17 fissions; on the sixth day, set 1 had 18 fissions, set 2 had 21 fissions; on the seventh day, set 1 had 22 fissions, set 2 had 12 fissions; on the eighth day, set 1 had 22 fissions, set 2 had 8 fissions; on the ninth day, set 1 had 18 fissions, set 2 had 10 fissions; on the tenth day, set 1 had 13 fissions, set 2 had no fissions; on the eleventh day, set 1 had 15 fissions, set 2 had 1 fission; on the twelfth day, set 1 had 17 fissions, set 2 had 12 fissions; on the thirteenth day, set 1 had 27 fissions, set 2 had 22 fissions; on the fourteenth day, set 1 had 29 fissions, set 2 had 1 fission; on the fifteenth day, set 1 had 20 fissions, set 2 had 18 fissions; on the sixteenth day, set 1 had 16 fissions, set 2 had 7 fissions; on the seventeenth day, set 1 had 17 fissions, set 2 had 7 fissions; on the eighteenth day, set 1 had 18 fissions, set 2 had 4 fissions; on the nineteenth day, set 1 had 19 fissions, set 2 had 7 fissions; on the twentieth day, set 1 had 20 fissions, set 2 had 14 fissions.

On the second, third, and sixth day, the fission rate of set 2 exceeded that of set 1 by 4, 1, and 3 fissions respectively. On the remaining seventeen days, the fission rate of set 1 exceeded that of set 2.

Figure 12 is a curve of the daily differences between the average number of fissions produced by the duplicate experimental and experimental sets. The average differences in fractions of a generation, in favor of the duplicate experimental set, are shown. On the first day there was a difference between the average number of fissions of .05 fissions in favor of the duplicate experimental set, .2 fissions difference on the second day in favor of the experimental set, .05 fissions difference on the third day in favor of the experimental set, .65 fissions difference on the fourth day in favor of the duplicate experimental set, .15 fission difference on the fifth day in favor of the duplicate experimental set, and .15 fissions difference on the sixth day in favor of the experimental set. For the remainder of the twenty-day period, the difference in fission rate between the average number of fissions of the two sets is in favor of the duplicate experimental set. These differences are as follows: seventh day, .5 fissions; eighth day, .7 fission; ninth day, .4 fission; tenth day, .65 fission; thirteenth day, .25 fission; fourteenth day, 1.4 fission; fifteenth day, 1. fission; sixteenth day, .45 fission; seventeenth day, .5 fission; eighteenth day, .7 fission; nineteenth day, .6 fission; and twentieth

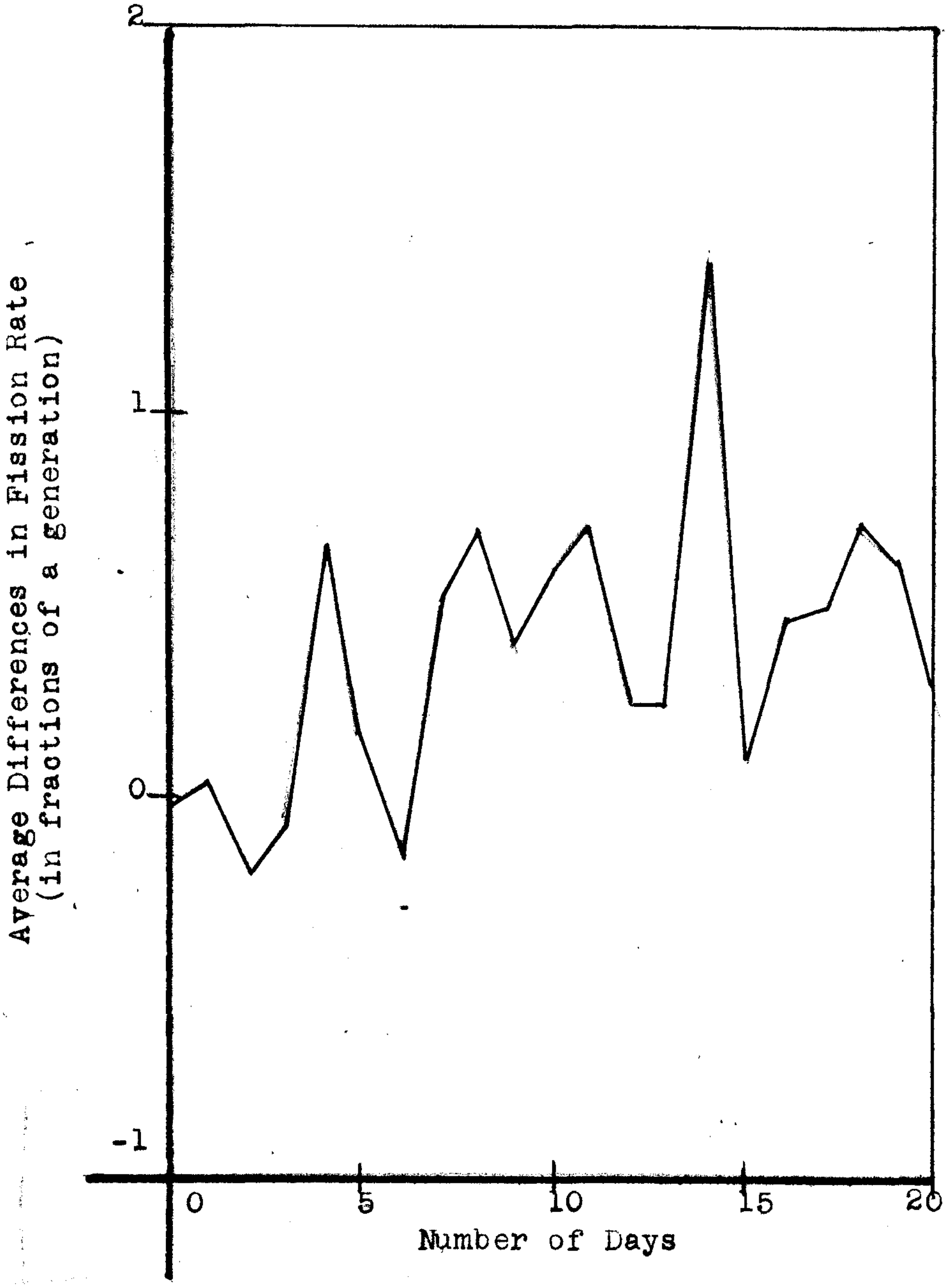

Figure 12. Showing the curve of the daily differences between the average number of fissions produced by the duplicate experimental and experimental lines.

Excess in fission rate is in favor of the duplicate experimental lines.

day, .3 fission.

Figure 13 is the curve of variation in average five-day fissions of the twenty duplicate experimental and the twenty experimental lines during the twenty-day test period.

The curve of variation in average five-day fissions for the duplicate experimental lines ranges from 4 to 5.7 fissions. There were two lines having 4 fissions, four lines having 4.25 fissions, two lines having 4.5 fissions, three lines having 4.75 fissions, six lines having 5 fissions, one line having 5.25 fissions, one line having 5.5 fissions, one line having 5.75 fissions.

The curve of variation in average five-day fissions for the experimental lines ranges from 1.75 to 3.75 fissions. There were two lines having 1.75 fissions, five lines having 2.25 fissions, four lines having 2.50 fissions, two lines having 2.75 fissions, two lines having 3 fissions, one line having 3.25 fissions, three lines having 3.50 fissions, and one line having 3.75 fissions.

The fission range for the duplicate experimental set was much lower than that for the experimental set. In the duplicate experimental set, there were six lines having in the average five-day rate, 5 fissions.

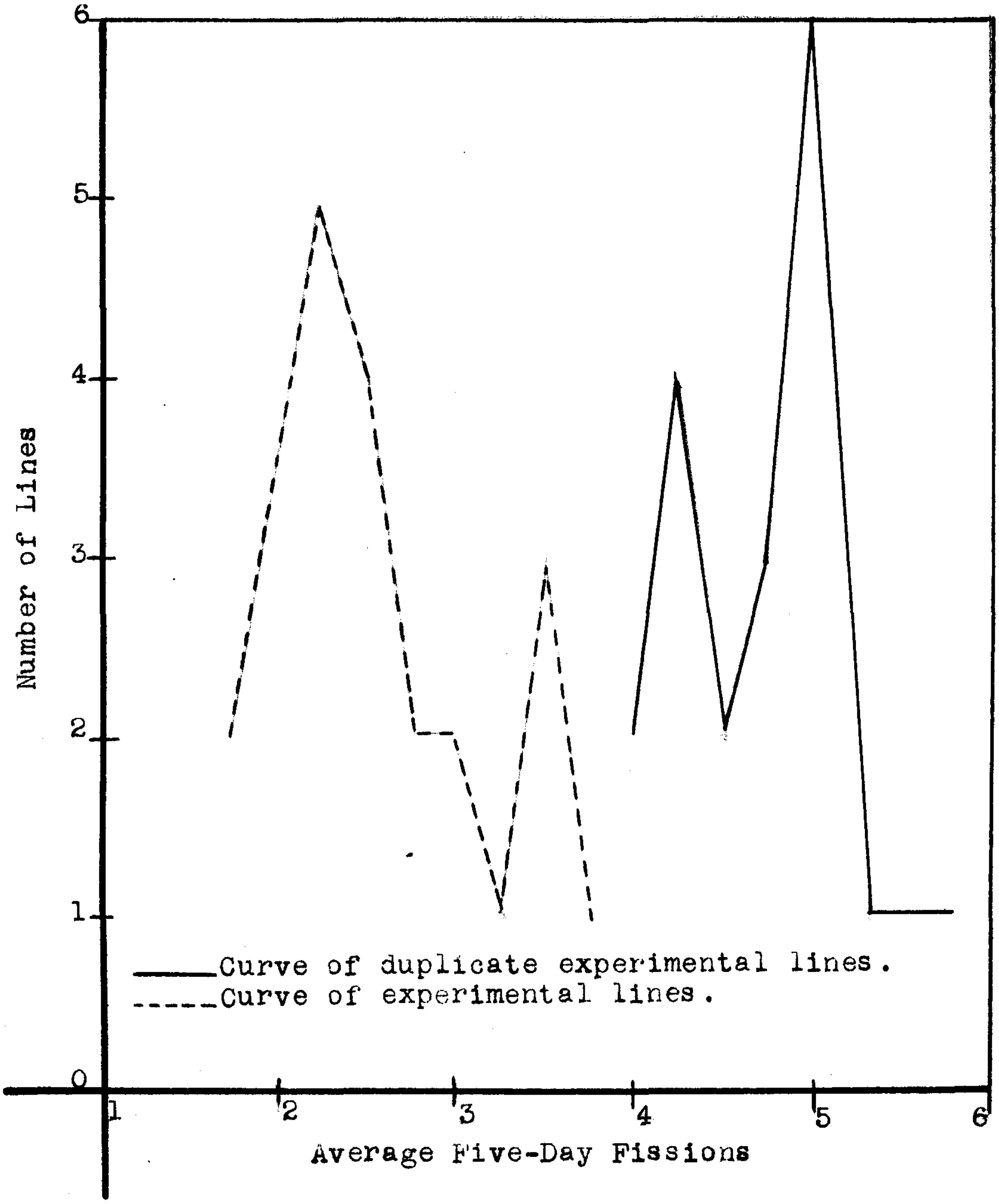

Figure 13. Showing the curve of variation in average five-day fissions of the duplicate experimental and experimental lines during the twenty-day test period.

In the experimental set, there were five lines with 2.25 fissions in the average five-day fission rate.

Table 6 (see page 67) gives the actual number of fissions per five-day period per line in the duplicate experimental and experimental sets. The differences in number of fissions in favor of the duplicate experimental lines are also given.

During the first five-day period, there was a total for the twenty lines in the duplicate experimental set of 88 fissions, and a total for the twenty lines in the experimental set of 76 fissions, leaving a difference of 12 fissions in favor of the duplicate experimental set. During the first five-day period, the twenty lines in the duplicate experimental set averaged 4.40 fissions, while the twenty lines in the experimental set averaged 3.80 fissions, leaving a difference of .60 fission in favor of the duplicate experimental set.

During the second five-day period, there was a total for the twenty lines in the duplicate experimental set of 93 fissions, and a total for the twenty lines in the experimental set of 51 fissions, leaving a difference of 42 fissions. During this second period, the twenty lines in the duplicate experimental

Table 6

Distribution of lines, as regards average number of fissions in the duplicate experimental and experimental lines during the twenty-day test period.
The fissions are averaged for five-day periods.

No. Fissions	1.75	2.25	2.50	2.75	3
Duplicate experimental (No. lines)					
Experimental (No. lines)	2	5	4	2	2

No. Fissions	3.25	3.50	3.75	4	4.25
Duplicate experimental (No. lines)				2	4
Experimental (No. lines)	1	3	1		

No. Fissions	4.50	4.75	5	5.25	5.50
Duplicate experimental (No. lines)	2	3	6	1	1
Experimental (No. lines)					

No. Fissions	5.75	Total
Duplicate experimental (No. lines)	1	94.75
Experimental (No. lines)		53.75

set averaged 4.65 fissions, while the twenty lines in the experimental set averaged 2.55 fissions, leaving a difference of 2.10 fissions in favor of the duplicate experimental set.

During the third five-day period, there was a total for the twenty lines in the duplicate experimental set of 108 fissions, and a total for the twenty lines in the experimental set of 54 fissions, leaving a difference of 54 fissions. During this period, the duplicate experimental set averaged 5.40 fissions, while the experimental set averaged 2.70 fissions, leaving a difference of 2.70 fissions in favor of the duplicate experimental set.

During the fourth five-day period, there was a total for the twenty lines in the duplicate experimental set of 90 fissions, and a total for the twenty lines in the experimental set of 39 fissions, leaving a difference of 51 fissions. The duplicate experimental set averaged 4.50 fissions, while the experimental set averaged 1.95 fissions, leaving a difference of 2.55 fissions in favor of the duplicate experimental set.

For the four five-day periods, there was a total difference of 167 fissions in favor of the duplicate experimental set and an average difference of 8.35 fissions, also in favor of the duplicate experimental set.

The average difference in fission rate in favor of the duplicate experimental set, per five-day period during the twenty-day test period, was 1.99.

SUMMARY

In *Paramecium caudatum*, by 'balanced selection' through many generations resulting from a single individual, it was possible through the use of the two different culture media, to produce two sets differing hereditarily in rate of fission. During this 'balanced selection', there was a gradual increase in average heritable results in the control set of *Paramecia caudatum*. The sodium chloride had a certain definite effect on maintaining at a lowered rate, the fission among the paramecia of the experimental set.

This hereditary trait of a decreased fission rate due to the environmental factor of the sodium chloride persisted throughout the period, and even persisted when the environmental factor was removed, and the control culture medium substituted.

The hereditary differences of the 'malted milk' and 'sodium chloride' sets continued throughout the entire period of 'balanced selection'; the hereditary differences of the 'sodium chloride' set did not disappear when these paramecia were changed to a malted milk culture medium.

Thus in _Paramecia caudatum_, from a single clone of given genotype, it is possible to obtain through a long period of 'balanced selection' during reproduction by fission, two sets of diverse genotype, differing characteristically from each other in rate of fission, under dissimilar culture media, and retaining these differences from generation to generation.

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