U. of M.

REPORT

of

COMMITTEE ON EXAMINATION

This is to certify that we the undersigned, as a Committee of the Graduate School, have given Barbara Lee Lund final oral examination for the degree of Master of Arts. We recommend that the degree of Master of Arts be conferred upon the candidate.

Minneapolis, Minnesota

June 4 1916

Chairman

F. H. MacDougall

E. J. Lund.

Henry F. Nachtrieb

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by

Barbara Lee Lund

for the degree of Master of Arts

They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts.

E. J. Lund
Chairman

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June 4 1918

THE TOXIC ACTION OF KNC IN ITS RELATION TO THE STATE OF NUTRITION AND AGE OF THE CELL AS SHOWN BY PARAMECIUM AND DIDINIUM

A THESIS

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF MINNESOTA

BY

BARBARA LEE LUND

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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MASTER OF ARTS

INTRODUCTION.

Many results from studies on behavior and inheritance in the unicellular organisms show that the same cell is different at different times. The precise conditions under which these differences arise have been only incompletely determined, and in relatively few instances do we know enough about the conditions of their occurrence to be able to repeat exactly the results. In short, physiology knows that differences of this or that kind occur among cells of the same cell species (i.e., cells which are apparently identical in outward appearances and history), but knows very little about how they occur and what the changes are in the cell that cause <u>difference in response to identical external conditions at different times</u>.[1]

One of the striking instances of this variability is commonly observed when individuals from a pure line of Paramecium or other protozoa are taken from the same culture and placed in solutions of various kinds of chemicals. Some of the cells die quickly, others survive for long periods of time. What are the origin and causes of such differences among individuals of a pure line population? The following is an attempt to solve some of the features of this problem. In order to increase the general significance of the results the experiments have been carried out on Didinium as well as on Paramecium caudatum. Preliminary experiments on Paramecium indicated that the state of nutrition played an important role in determining the results, so that Didinium was selected because its food (Paramecium) can be controlled both as to quality

[1]For discussion of certain phases of this problem, see Jennings, "Behavior of the Lower Organisms," chap. XVI.

(a pure line of Paramecium) and as to quantity if necessary. Later it was discovered that an equally good control of the food of Paramecium could be obtained by feeding yeast. A special study of the possibilities and limits of the use of pure yeast as a food for Paramecium is in progress.

The problem of the cause of the differences in resistance to the toxic action of potassium cyanide which Paramecium and Didinium individuals show was chosen because it has very generally been assumed that the toxic action of the cyanides on other organisms is specifically due to their ability to inhibit intracellular oxidations, and that differences in survival time in cyanide solutions were directly due to the difference in the rate of oxidations; or, as more generally expressed, "rate of metabolism" in the organisms studied. Child, '15. Does difference in resistance of Paramecia or Didinia to KNC depend upon the rate of oxidations in the cell?

The variety of types of cell suitable for studies on cell individuals under normal conditions is very limited. Most of the information along this line has been obtained from blood cells, yeast cells, Echinoderm eggs and protozoa. The most extensive and accurate physiological data on such questions as the nature of the toxic action of the cyanides, rate of intracellular oxidations and its changes in the cell during the period of cell division and during the time between successive cell divisions, etc., have been obtained from experiments on Echinoderm eggs. It is interesting therefore to see to what extent a comparison can be made of the results on Echinoderm eggs with those on unicellular organisms during division and the changes which occur in a protozoan cell

between two successive divisions. For this reason I have given below a brief review of the literature on the changes that occur in the Echinoderm egg during cleavage.

It is important to note that the following results on survival time in the solutions of KNC were obtained not by observing large numbers of cells at a time, as is usual in this type of experiment, but instead by observing one or two individuals at a time and recording the survival time of each individual, so that the averages given are averages of the survival times of individual cells with a definitely known history. This necessitated a great deal more labor, but was necessary in order to know exactly the age and history of each cell that was used.

EXPERIMENTAL.

The first two experiments were preliminary and served to orient the problem.[1]

Experiment I. Suspecting that starvation of Paramecia had some relation to its power of survival in KNC solutions, a quantity of Paramecia from a pure line hay infusion culture was centrifuged and placed in one-half native medium and one-half tap water. This was used as a control. Twenty cc. of this was added to a jar containing 608 cc. tap water, on March 18th; and the same to another jar, on March 19th. I made no counts, but there were not over seven or eight hundred animals in each jar, so that there was a large volume for each individual. Forty-three of the control animals were tested immediately with the KNC. About

[1] They were carried out in 1915 in the Zoological Laboratory of the University of Pennsylvania.

twenty of those added to the pure tap water were tested as soon as added, and about the same number on each successive day following. The animal to be tested was transferred from the tap water to about 2 cc. N/50 KNC solution in a watch glass by means of a capillary pipette. As small an amount of water as possible was transferred with the animal. It was then watched, using a binocular microscope, until all movement ceased, cessation of movement being considered the death point. Soon after this, as a rule, the animal completely disintegrated. The average time of survival is given in Table I.

As can be seen by glancing at the table the survival time varies greatly in both lots, and apparently chaotically. The standard deviation' each day was large, varying from 0.63, lot I, for the seventh day to 2.73 on the fifth day; and similarly for lot II. This did not decrease as time went on.

Before proceeding further, the question arose as to whether the time of beginning cytolysis might be a more unvarying death point than the time of cessation of movement. If the animals are in the same condition as regards food and age then the death point which is the most constant should be used. I obtained dividing animals in the usual way (described below) and used the daughter cells as soon as division had taken place. Thus the animals were all the same age. The two sisters were placed in a watch crystal with 1 cc. N/75 KNC solution, and the time of cessation of movement recorded. They were further watched and the time of beginning cytolysis recorded. Two experiments were carried out, 30 animals

'The standard deviation is a measure of the degree of variation. See C. B. Davenport, "Handbook of Statistical Methods."

Table I.

Several hundred Paramecia were transferred from a pure line mass culture to each of two jars of tap water, and about twenty of these animals were taken at random each day and treated with KNC. The average of these survival times is given in the vertical column headed "Average survival time in minutes". The average of the survival times of 43 control individuals from the hay infusion was 2.19 minutes.

	Lot I, March 18th.			Lot II, March 19th.		
	Number tested.	Average survival time in minutes.	Standard deviation.	Number tested.	Average survival time in minutes.	Standard deviation.
1st. day	23	3.289	1.11±.11	21	1.995	0.76±.07
2nd. day	25	1.100	0.72±.06	22	0.870	0.64±.06
3rd. day	25	1.132	1.09±.10	24	1.595	1.25±.12
4th. day	28	1.083	2.73±.24	20	0.717	1.54±.16
5th. day	22	3.190	0.94±.09	20	1.877	1.15±.12
6th. day	22	1.675	0.63±.06	21	0.954	0.65±.06
7th. day	22	1.279		20	0.902	
8th. day	21	0.966				
9th. day	19	1.157				
10th. day	20	1.257				

being tested in the first and 60 in the second. In order to obviate giving all the detailed data the standard deviation, which is a measure of the degree of variation, is given. In the first experiment the standard deviation for the cessation of movement was found to be 2.74, while for cytolysis it was 3.86. In the second the figures were 1.08 for cessation of movement, and 2.37 for cytolysis. Thus it is seen that the time of cessation of movement in KNC is less variable, and throughout this work I have used it as the "death point."

Experiment II. Since the animals in experiment I were taken at random from a pure line mass culture it is apparent that (1) some of them must have been old and some of them young; (2) some of them had also probably eaten more recently than others, containing therefore more food in vacuoles. Could these two factors account for the random variation from day to day, and the deviation in each day's experiment?

To answer this question I first attempted to see whether there was any difference in the survival time between old and young Paramecia when they were treated with N/85 KNC solution. A pure line of Paramecia was cultivated in Syracuse watch glasses in hay infusion. About eight fresh watch glasses were set and seeded with the same pure line every day so that a constant supply of dividing animals was at hand. The experiment consisted of four parts:

Part I. The watch glass cultures were looked over and animals with constrictions isolated in watch crystals. As soon as one divided one of the sisters was picked out with a capillary pipette, washed in one cc. tap water for about thirty seconds, and then placed in N/85 KNC solution and the death point noted;

while the other sister was left to age in tap water. Thirty pairs were so treated, the older sisters being killed at intervals varying from 25 to 215 minutes. The average survival time of the older sisters was less than that of the younger sisters.

Part II. Thinking that the tap water might have something to do with this result I repeated the experiment but eliminated tap water; that is, I diluted the KNC with distilled water and allowed the sisters to age in native medium (hay infusion). The average survival time of the older sisters was now greater than that of the younger.

Part III. Is this difference in result observed in parts I and II due to the fact that the KNC was diluted with tap water in one case and with distilled water in the other, or to the fact that the animals aged in tap water in one case and in native hay infusion in the other? To determine this I repeated the experiment, using KNC diluted with distilled water, and allowed the animals to age in tap water. The average survival time of the older sisters was again less than that of the younger, as in part I.

Part IV. I repeated the experiment, using KNC diluted with tap water, and allowed the animals to age in native hay infusion. The average survival time of the older sisters was greater than that of the younger. These results are summarized in Table II.

Conclusion. The older animals are evidently more resistant to KNC than the younger, and the apparent exceptions shown in parts I and III are due to the fact that the animals were left to age in tap water instead of in hay infusion. This was probably due to inanition and not to any deleterious effect of the tap water itself. See experiment VI below.

Table II.

Summary of results of experiment II.

	I (30 pairs)	II (30 pairs)	III (30 pairs)	IV (30 pairs)	
	KNC diluted with tap water. Animals left to age in tap water.	KNC diluted with distilled water. Animals left to age in native hay infusion.	KNC diluted with distilled water. Animals left to age in tap water.	KNC diluted with tap water. Animals left to age in native hay infusion.	
Average survival time of older sisters as compared to younger.	less	greater	less	greater	

Experiment III. In performing the preceding experiments the question arose as to whether the effects of ageing in native medium were cumulative; that is, whether the resistance increased regularly as age advanced. To answer this question the following experiment was performed. The procedure was the same as in experiment II except that N/75 KNC was used. One sister was killed in this immediately after division. The other sister was allowed to grow in the native medium (hay infusion) and killed at ages varying from 10 to 600 minutes. Thirty Paramecia were tested in each case; that is, thirty "young" and thirty "old" for each time interval. Averages of the survival times for the young and the old for each time interval were made, and in every case the <u>average</u> length of life after treatment with KNC was greater in the case of the old than of the young animals.

The results of this experiment are summarized by giving these averages and the difference between them. See Table III. They are plotted in curves (Figure I).

The difference between the averages increases as age of the surviving sister increases. The degree of variation in survival time is relatively small when compared to the average survival time, showing that the results are comparable and real; that is, that there are actual differences in survival time. This indicates so far as this experiment goes that there is an <u>absolute increase</u> in the survival time of the cells in hay infusion from one division to the next division, rather than that the increase is due to decrease in survival time of their sisters killed immediately after division; for the survival time of the latter tended to remain about the same throughout the experiment as

Table III. Paramecia.

A. Time between division of Paramecia and the killing of one sister in N/75 KNC.

B and C are self explanatory, each number representing an average of thirty individuals.

D. Average difference in survival time between the paramecia which were killed immediately after dividing and their sisters which were killed at the above intervals after living in native hay infusion. Each number represents the difference between the two preceding numbers in the same column.

Age of "old" in min.	A	10	20	30	40	60	120	180	300	480	600
Average survival time of "old" sisters in minutes.	B	2.22	2.62	3.30	3.35	2.85	2.90	3.00	3.11	3.55	3.71
Average survival time of "young" sisters in minutes.	C	2.09	2.10	2.90	2.74	2.30	2.16	2.07	2.09	2.24	2.55
Difference.	D	.13	.43	.40	.61	.55	.74	.93	1.02	1.31	1.16
Standard deviation of "old" sisters.	F	.51±.04	.34±.03	.36±.03	.72±.06	.64±.06	.47±.05	.48±.05	.40±.04	.85±.07	.67±.07

Figure I.

Survival time of "old" and "young" paramecia in KNO, See table III.

Curve Brepresents the average survival time of "old" sisters.

Curve C represents the average survival time of the "young" sisters of B.

Curve D represents the difference between the average survival times of the old and "young" sisters. That is, it is the difference between the curves B and C.

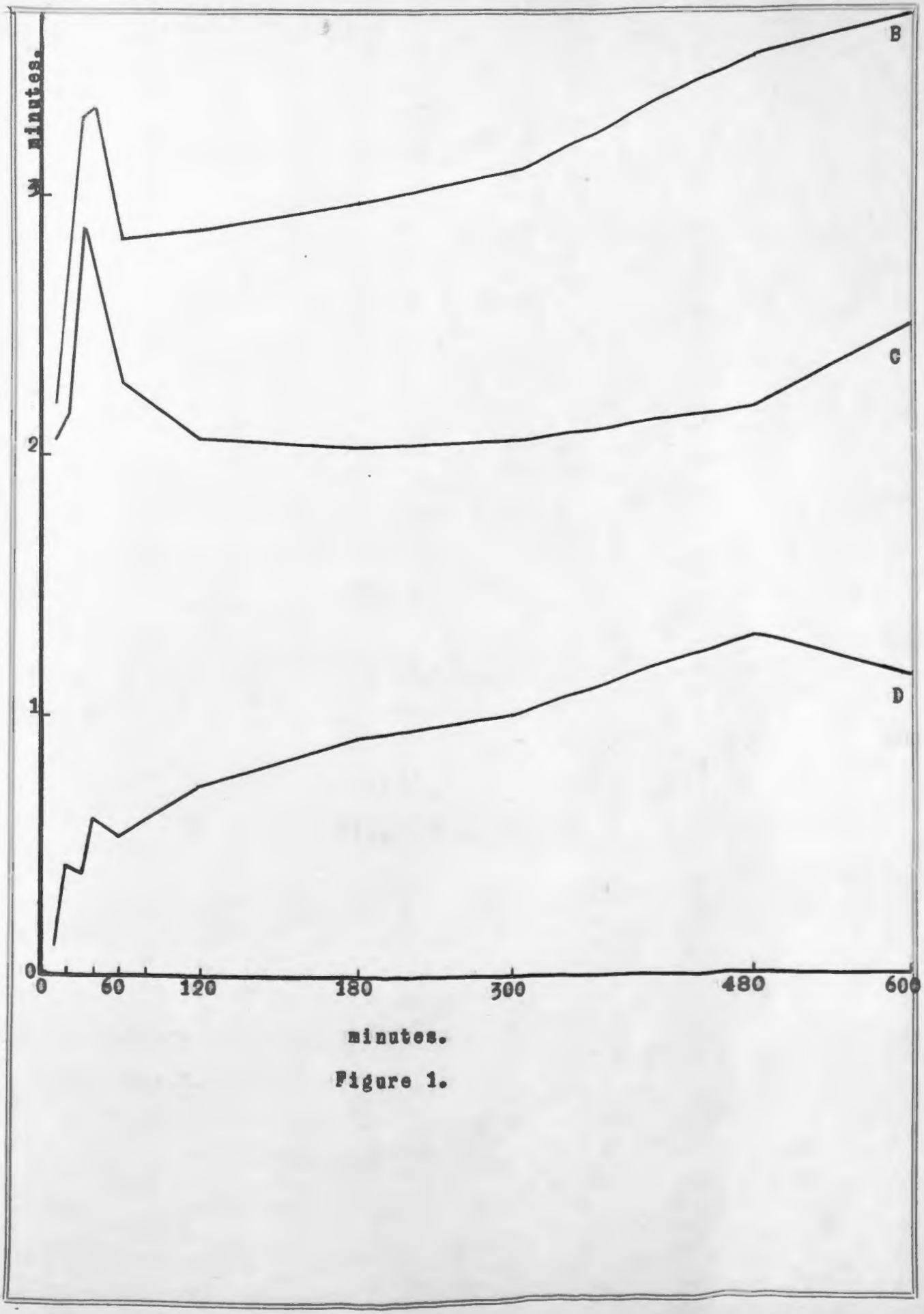

Figure 1.

shown by the horizontal trend of the curve, C.

We may then briefly summarize the results from the previous experiments on Paramecium by saying that these have clearly shown that beginning at division there is an increase in the resistance of Paramecium to KNC when it is permitted to live in hay infusion, while if placed in tap water the resistance decreases.

By using hay infusion it would be practically impossible to show in Paramecium that these results are due to the state of nutrition of the cell; for if we wished to show that it was due to the bacteria serving as food it would be necessary to provide the same medium (say, tap water) for both sisters, while only one sister was fed with bacteria. To control the bacterial food of Paramecia is very difficult, hence Didinia were used in the following experiments. A pure line of Paramecia served as food for the Didinia, and since the Paramecia could be washed free from hay infusion and placed in tap water it was possible to have all experimental conditions for the two sister cells of Didinia exactly the same, except presence or absence of food. This then supplied ideal conditions for answering the question as to whether the state of nutrition of the cell was the condition which determined the survival time of the cell in KNC.

Experiment IV. Didinia were cultivated in tap water in Syracuse watch glasses and fed on freshly washed Paramecia taken from a pure line mass culture. Animals with constrictions were isolated in watch crystals in tap water, and immediately on separation one sister was transferred to another watch glass in which was about one cc. tap water containing many Paramecia. The two dishes, one containing one of the pair in tap water, the

other containing the other sister in tap water and Paramecia, were then placed side by side in a moist chamber, and later the two sisters were killed at the same time in the same one cc. of N/20 KNC solution. A more concentrated solution of KNC was used here for convenience, to shorten the survival time, for Didinium is much more resistant to KNC than Paramecium. Occasionally it was impossible to tell the difference between the two sisters at the end of the desired period, the one placed with Paramecia evidently not having eaten; and in those cases they were killed separately but as near the same time as possible. By working with two binoculars it was possible to make the treatment of the two with KNC practically simultaneous. Thirty pairs were used in each case, so that sixty individuals were killed at the end of each period of 1/2, 1, 2, 3, 4, and 5 hours after division. The results are summarized in Table IV, and curves C, E, and D, Fig.II.

The average difference in survival time (row D) increases until the fourth hour, and in this lot three fed ones out of thirty-three had divided; while in the next lot, that of five hours, 37.5% or 18 out of the 48 individuals isolated had divided, and division of the remainder was apparently not far off. It was impossible to get a record of a six hour lot on account of division. Since the starved did not divide at the same rate as the fed it was possible to continue that part of the experiment beyond five hours. Table IV (B). It will be noted that the average survival time of these starving individuals shows a marked decrease. This is shown in Figure II, curve E. Is this increasing difference (Figure II, curve D) up to the fourth hour due entirely to the fact that one sister has eaten while the other has not; or do

Table IV. Didinia.

A. Time after division when the sisters were killed in N/20 KNC. One of them had been starving in tap water, the other feeding on Paramecia in tap water. The temperature for this experiment was between 23° and 24°C thruout, with the exception of the 12 hour lot, when the temperature went down to 19°C. This accounts for the longer survival time in that case.

B and C are self explanatory, each number representing an average of thirty individuals.

D. Difference between the two preceding figures in the same column. That is each number represents the average difference between 30 starved and 30 fed Didinia sisters.

Age of sisters in hrs.	A	1/2	1	2	3	4	5	6	7	8	9	12	15	19	24
Average survival time of starved in minutes.	B	5.9	4.268	3.45	3.743	2.64	2.28	1.67	1.19	1.00	0.87	1.32	0.80	0.72	0.50
Average survival time of fed in minutes.	C	6.38	8.524	8.776	9.766	6.11	5.090								
Difference.	D	0.483	4.256	5.326	6.023	3.464	2.804								

Figure II.

Survival time of Didinia in KNC. Summary of tables IV and V.

The abscissa represents time in hours between division and death of Didinia.

The ordinate represents survival time in minutes.

Curve C represents the average survival time of fed Didinia. See table IV, C.

Curve E represents the average survival time of the starved sisters of C. See table IV, E.

Curve D represents the difference between the average survival times of fed and starved sisters. That is, it is the difference between C and E. See table IV, D.

Curve A represents the difference between the average survival times of the less and more resistant sisters, when both are starved.

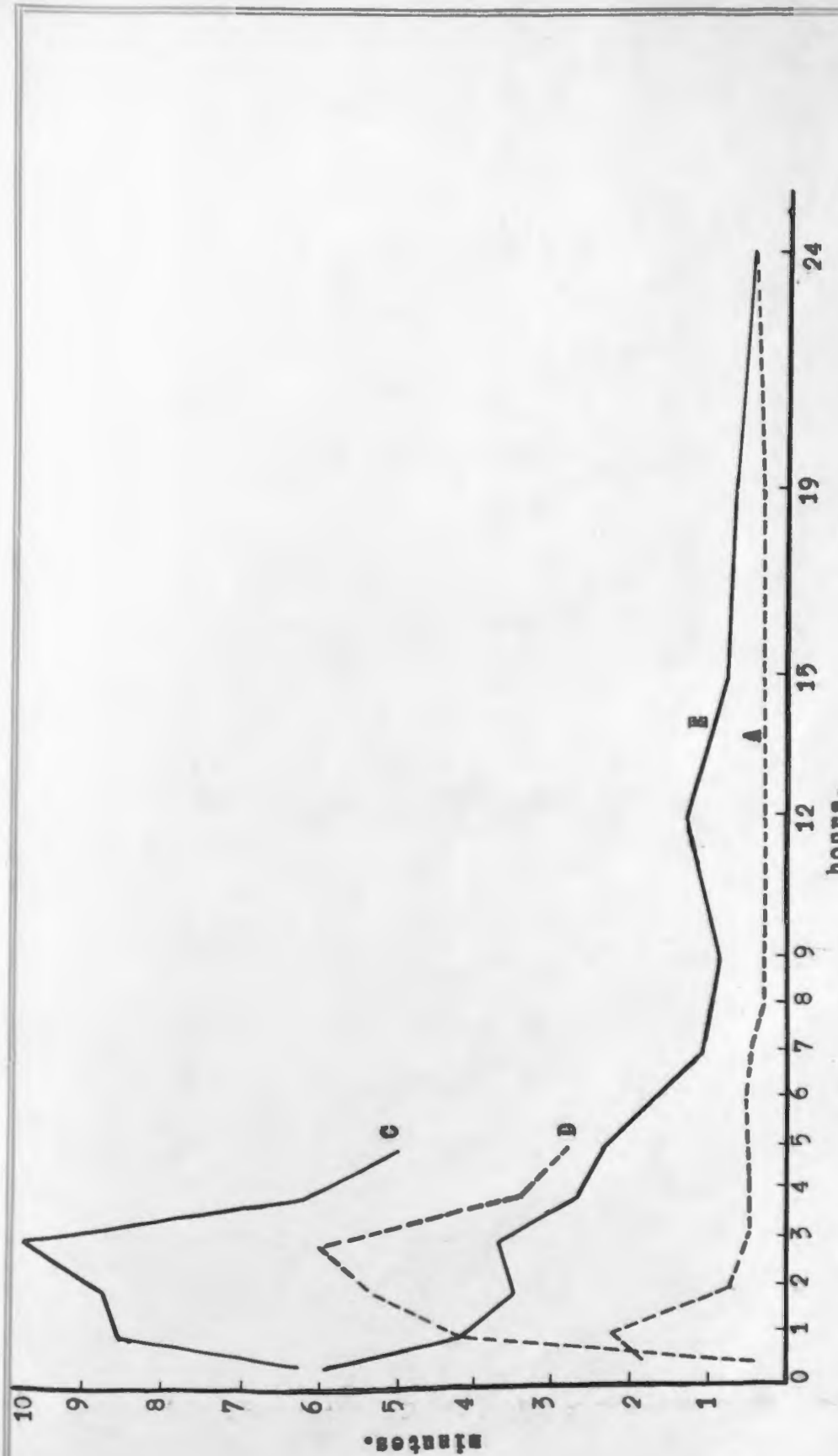

Figure II.

the sisters, presumably identical at the time of separation, gradually diverge as a result of differing rates of maturity, apart from effects of nutrition, so that even though subjected to the same conditions they would show different degrees of resistance as time went on?

Experiment V. To answer this question an experiment was carried out in the usual way, the dividing animals being isolated and killed with KNC. I found, however, that the concentration of KNC I had previously used did not bring out the small differences in survival time as well in this case where the sisters were in the same medium and both were without food as a weaker solution. Thirty pairs or sixty individuals were killed at the end of each period of 1/2, 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 19, and 24 hours after division. Since there was no difference in appearance or in previous treatment of the sisters I arbitrarily placed the survival times of the animals which died first in one column, and that of their more resistant sisters in another, took an average of the thirty individuals in each case, and have given in Table V the average survival times of each and the differences between these averages. The difference is also plotted in Figure II, curve A.

These results demonstrate that in the same environments the differences in survival time of the sisters do not increase but if anything decrease with time; i.e., the cells tend to become more alike in their resistance to KNC, and therefore the large difference shown in experiment IV must be due to the food one has eaten while the other has starved. Table V shows that the difference in survival time at one-half and one hour is greater than at any subsequent time. This is explained by the fact that division

Table V. Didinia.

D. Time after division at which the sisters, both of which had been subjected to the same treatment, were killed.

B. Each number represents the average survival time of 30 Didinia.

C. Each number represents the average survival time of the 30 sisters of the corresponding set of row B.

A. Difference between B and C.

Age of sisters in hrs.	D	1/2	1	2	3	4	5	6	7	8	9	12	19	24	
Average survival time in minutes of less resistant sisters.	B	3.57	3.30	2.93	2.49	2.60	2.18	1.45	1.53	1.80	1.59	1.69	2.22	2.51	
Average survival time in minutes of more resistant sisters.	C	5.37	5.50	3.61	3.02	3.12	2.69	2.04	1.97	2.12	2.42	2.02	2.56	3.10	
Difference.	A	1.80	2.20	0.68	0.53	0.52	0)51	0.59	0.44	0.32	0.83	0.33	0.34	0.59	

in Didinia is often unequal, one sister evidently containing more food in vacuoles than the other. As this food is used up by the cell the difference in survival time becomes less. See experiment VI below.

Two other experiments on Didinia were carried out which were duplicates of experiment IV, except that saponin was used in one case and phenylurethane in the other, in place of KNC. The survival time varied with individuals apparently irrespective of the state of nutrition of the cell. The death points were less definite than in the case of KNC, and hence further efforts to trace the causes of difference in survival time in these substances was abandoned for the time being.

Some time after the preceding results were obtained it was found possible to grow pure lines of Paramecia in tap water by feeding them yeast, thereby making it possible to duplicate experiments IV and V on Didinium.

Experiment VI. A pure line of Paramecia was raised in a suspension of fresh yeast and tap water. The Paramecia were centrifuged and washed thoroughly every morning, and added to a new suspension of fresh yeast to continue the culture. The cultures were kept in Syracuse watch glasses. If a culture is neglected and bacteria develop to any extent, the Paramecia become abnormal and finally die. If a starved Paramecium is put into a freshly made yeast suspension it eats greedily, and in a few hours increases greatly in size. From such yeast fed material, animals with constrictions were picked out with a capillary pipette and placed in tap water. As soon as they separated one was placed in a watch glass of fresh yeast suspension, while the other was left in tap water. Sets of thirty each of such pairs were killed in

N/75 KNC at intervals of 1, 2, 3, 4, 5, 6, 7, 8 and 9 hours. It was impossible to continue this experiment longer than nine hours, for a large percentage of those which were fed divided between the ninth and tenth hour. Sets of starved animals were continued however for 25 hours, being killed at intervals of 12, 15, 19 and 25 hours. The results are summarized in Table VI, and in the curves of Figure III.

The difference in survival time shows a general increase up to and including the ninth hour, the animals which were placed in the yeast suspension living longer than their sisters which were placed in clear tap water. The survival time of the latter showed a marked decrease with age. The experiment was continued; but instead of feeding one sister and starving the other, both were starved, as was done with Didinia in experiment V above. Thirty pairs were left to starve in tap water for each period of 15, 19, and 25 hours, and then tested with KNC. I arbitrarily placed the survival time of the sisters which died first in one column, and the more resistant sisters in another (though as a rule the survival time of sisters was the same), took an average of the two sets of sister cells of thirty individuals for each period and then found the differences between these averages, which I called the average difference between starved sisters, given in Table VI (E). This difference was very small, being .02, .03 and .02 minutes respectively for 15, 19 and 25 hours. This is practically the same as saying that there is no difference, since these numbers are within the limits of error in determining the death point. It is important to notice that the average difference in survival time of thirty pairs immediately after division, and the average difference after the sisters had been starving 25 hours, are

Table VI. Paramecia, yeast fed and starved.

A. Time between division and killing of the two sisters, one of which had lived in tap water, the other in tap water and fresh yeast suspension.

B. The averages of the survival times of 30 fed Paramecia.

C. Since all these left with food for 12 hours divided the experiment was continued without food for 15, 19, and 25 hour intervals. So that the numbers for 15, 19 and 25 hours, rows C and D, represent the average survival time of 30 starved sisters each.

D. The average survival time of the starved sisters of B from 0 to 12 hours and of C from 12 to and including 25 hours.

E. Differences between the rows B and D, and between C and D.

Age of sisters in hrs.	A	0	1	2	3	4	5	6	7	8	9	12	15	19	25	
Average survival time of fed.	B	2.42	2.64	2.58	2.52	2.51	2.50	2.44	2.58	2.43	2.75					
Average survival time of starved sisters of D.	C												1.13	1.17	1.00	
Average survival time of starved.	D	2.40	2.24	1.88	1.80	1.84	1.79	1.74	1.77	1.56	1.78	1.59	1.11	1.14	1.05	
Difference.	E	.02	.04	.70	.72	.67	.76	.70	.81	.87	.97		.02	.03	.02	

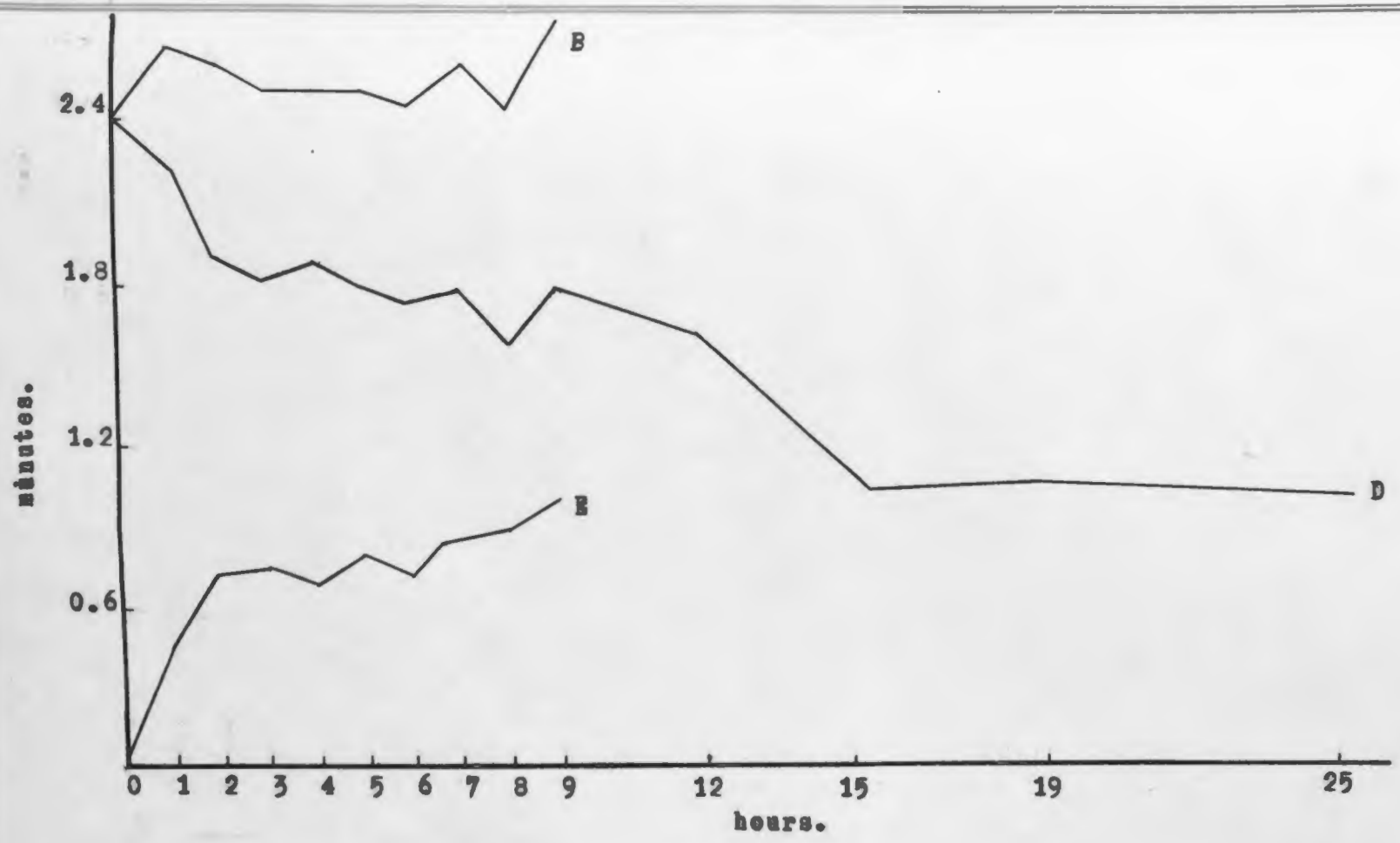

Figure III.

Survival time of starved and yeast fed Paramecia in KNC. See table VI.

The abscissa represents time in hours between division and death of Paramecia.
The ordinate represents survival time in minutes.

Curve B represents the survival time of yeast fed Paramecia.
Curve D represents the average survival time of the starved sisters of B.
Curve E represents the difference between the average survival times of the fed and starved sisters. That is, it is the difference between the curves B and D.

exactly the same (viz., .02 minutes), even though the actual survival time of sisters immediately after division and after starving for 25 hours is very different. This close correspondence is probably due to the fact that in the case of Paramecia division is practically equal; that is, there is as much food in one daughter cell as in the other. While in the case of Didinia we often have unequal division, and accordingly the difference in survival time is greatest at the age of one-half and one hour.

Attention may be called to the fact that here the Paramecia which were left to age in tap water and food (that is, yeast) were more resistant than those left in tap water alone, and that therefore the tap water as such had no deleterious effect, as might have been suspected in the case of Paramecia grown in hay infusion. Cf. experiment II above.

DISCUSSION.

It is evident then from the preceding six experiments that young Paramecia and starved Paramecia are more susceptible to KNC than old or fed Paramecia. In the case of Didinia, although as far as average differences are concerned the reactions of the starved and fed are like that of Paramecia, when the survival times of the fed animals are considered alone, it is seen that there is a decided rhythm, the resistance increasing up to the fourth hour and then decreasing to the sixth hour, at which time a large percentage has divided. In view of this latter fact it is of interest to note the similar results of Lyon and others in regard to the rhythmic susceptibility of sea urchin eggs. The results of Lyon's experiments (Lyon, '02) on the susceptibility of sea urchin eggs to KNC solutions show roughly, although clearly,

that the susceptibility to KNC reaches a maximum and a minimum at stated intervals. About ten to fifteen minutes after fertilization the egg is especially susceptible to KNC. Again, after the first cleavage and after the second come susceptible periods with a rise in resistance in between. He was working on the supposition that KNC inhibits oxidations, and so it would be possible to test this further by depriving the eggs of oxygen in some other way. This he did by substituting hydrogen for air in the water in which the eggs were to develop. There was a decided similarity between this experiment and the previous one. There was a gradual loss of resistance during the first ten to fifteen minutes after fertilization. Then the resistance increased. This experiment was carried no further than thirty minutes, and so it is uncertain as to whether there was a second and a third susceptible period after the first and second cleavages. He also investigated the effect of heat and cold on developing sea urchin eggs (Lyon, '04) and found that immediately after the entrance of the sperm the egg is more susceptible to heat than a few minutes later. The resistance decreases, reaching a low point just before division, then increases, reaching a maximum right after division, decreasing again to a low limit just before the second division. Similar experiments were performed with cold but they were not carried beyond the first cleavage, so here no rhythm was demonstrated, but he considers the existence of such a rhythm probable. Lyon ('04) also studied the carbon dioxide production of the developing sea urchin egg. His experiments here were not strictly quantitative in character, but he found a slight increase in CO_2 production in the first ten to fifteen minute interval following fertilization. Decreased CO_2 production followed. The interval

during which the eggs were actively dividing into the first two blastomeres was one of active CO_2 production. This was followed by a period of lessened production with a second rise at the time of the second cleavage. Spaulding ('04) also found decided rhythms in the resistance of the developing sea urchin egg to both ether and hydrochloric acid. Mathews ('06) made a study of living eggs of Arbacia and Asterias and examined sections of eggs preserved at definite intervals after fertilization. Comparing Lyon's work on Arbacia and the condition of the egg at the various intervals, as shown by the sections, he concludes that the period of greatest susceptibility is immediately before and during segmentation, and that just after segmentation there is a period of great resistance. He also endeavored to repeat Lyon's work on Arbacia, using Asterias eggs. The results were unsatisfactory so far as sharp and decisive periods of susceptibility were concerned, but they showed clearly that the eggs in certain stages were more susceptible to KNC than in others.

Moore ('15), in his work on artificial parthenogenesis, found that the greatest number of eggs, which had been previously treated with butyric acid, developed when treated with the hypertonic sea water at 40, 60, 90-100 and 115-125 minutes after the fatty acid treatment. In the case of normally fertilized eggs where hypertonic sea water would not have the above "curative" effect, but could be nothing but injurious, he found that with an exposure of 40 minutes a maximun susceptibility occurred just after fertilization and immediately before and during each cytoplasmic division; while maximum resistance is 35 to 45 minutes after fertilization and just after each division. Lillie finds an esmotic swelling taking place in Arbacia eggs when placed in

dilute sea water. The rate of this swelling depends upon the degree of dilution of the water and the condition of the eggs. The fertilized eggs have a higher resistance to the osmotic swelling, and consequent cytolysis, than the unfertilized; but shortly before the appearance of the cleavage furrow the resistance rapidly declines to a minimum at the time of the appearance of the furrow. Immediately after the completion of the furrow the former resistance returns. A similar change is found at the second and third cleavages, and probably occurs at all cell divisions.

In looking for a cause or causes of the variability in resistance of Didinium and Paramecium to KNC we are reminded at once of the fact that Lyon found the periods of susceptibility to KNC of the sea urchin eggs to correspond to those for hydrogen, which might lead to the conclusion that oxidations are interfered with. Warburg, and Loeb and Wasteneys have found that the oxygen consumption of the sea urchen egg is actually decreased by the presence of KNC. Child ('15) finds that stimulated flat worms are more susceptible to KNC than the unstimulated, and also that the worms which he found to have a low resistance to KNC had a high rate of CO_2 production, as determined by Tashiro's biometer. He concludes from this fact that there is a relation between the resistance to KNC and "the rate of metabolic reactions, or certain of them, probably the oxidations." He also finds that there is a marked difference between old and young Planaria, and between starved and fed Planaria of the same age. The young worms and the starved worms show a greater degree of susceptibility than the older worms and the well fed worms, respectively. The results given above for Paramecium and Didinium are the same as those found by Child on Planaria. Now, considering the parallel-

ism between resistance to KNC and CO_2 production and also the resistance to KNC of stimulated and unstimulated worms, Child concludes that the young worms have a greater metabolic rate than the old ones, the starved greater than the fed; and since my results on Paramecia with respect to survival time in KNC seem to be identical with these on Planaria, I was inclined to accept this conclusion and apply it to these protozoa. But recent work in this laboratory on the actual oxygen consumption of Paramecia indicates that in the case of this animal at least KNC has no effect on oxidations, even up to concentrations which kill in a few hours. Lund, '18. And so we must search for the explanation of these differences in some other direction.

If the permeability of the plasma membrane of the protozoan, like that of the surface of the sea urchin egg, changes at different times in its history and with different nutritive conditions, we might use that in explaining its various reactions to KNC. Lillie ('16) has shown that in the case of the egg, permeability is greatest at division and less between divisions; and, as stated above, according to Lyon and others susceptibility to KNC is greatest at division and less during the time between divisions. Didinia show a similar behavior, the resistance increasing up to the fourth hour, but then decreasing again to the fifth hour when 37% of them have divided. The results for Paramecia, however, do not give this sort of a curve; but the resistance increases to a marked extent in hay infusion, and to a smaller extent when fed yeast, up to the very time of the beginning of the formation of the constriction. My data on the reactions beyond this point (that is, the time when the cell is dividing) are

insufficient to warrant conclusions. However, my impression from the few Paramecia which did happen to divide while in the KNC is that the resistance does not decrease at that time.

If, on account of this parallelism with the results on the egg, it is conceded that the susceptibility of Didinia is due to an increase in permeability, the conclusion might be justified that the high susceptibility of the starved animals is due to the same thing. If this is the case, it is obvious that there is an inconsistency in relating permeability and rate of oxidations; for the egg at division has an increase in permeability and also, if we may depend upon the limited data available on this point, an increase in oxidation rate, while a starved Paramecium or Didinium has, according to this hypothesis, an increase in permeability and, according to the facts, a decrease in rate of oxidation. The latter has been found in some recent work in this laboratory, which is as yet unpublished.

It is interesting to note, in regard to the lack of resistance to KNC of the starving cells, that this is in accord with the common experience that tissues suffering from lack of normal blood supply offer less resistance to various toxins and infections than tissues which are well supplied with blood; i.e., well nourished.

It is evident that the experiments given above on Paramecium and Didinium, and the results on eggs of Echinoderms by others, and those on lower invertebrates, offer a promising avenue of approach to an understanding of what some of the fundamental conditions in the cell are which determine the degree of resistance of cells to toxic substances.

SUMMARY.

1. An attempt was made to discover what factor or factors are responsible for the observed differences among individuals of a pure line of Paramecia and Didinia living in the same culture medium; e.g., hay infusion. What are the differences in a protozoan cell which cause difference in response to apparently identical external conditions at different times?

2. Survival time (resistance) of Paramecia and Didinia in solutions of KNC was selected and used as an index because of the generally supposed relation of the toxic action of KNC to the rate of oxidation in cells.

3. All the data on survival time of Paramecium and Didinium were obtained by observations on individual cells and not by estimating the average survival time of large numbers of cells as is usual in such experiments. In this way the variation in resistance of the cell at different times during the period between cell divisions was followed accurately and is summarized in curves.

4. The cessation of movement of Paramecium is a less variable "end point" than cytolysis for determining the death point in KNC solutions.

5. Paramecia can be cultivated in pure line mass cultures in tap water when fed compressed yeast. In this way the chemical composition of the medium and food can be kept more constant than has been possible previously in work on Paramecium.

6. Resistance of Paramecium to KNC when allowed to feed on bacteria shows a marked increase, and when fed on yeast the resistance increases to a smaller degree from the time of division up to the following division. The resistance of Didinia to KNC

when fed with Paramecia increased from the time of completion of division until a maximum was reached some time previous to the second division, and then gradually fell before the second division. This rhythm is directly comparable to that found by Lyon and others on Echinoderm eggs.

7. When Paramecium and Didinium are prevented from obtaining food the resistance to KNC gradually decreases below its value at the completion of division.

8. Starvation of sister cells of Didinia results in a decrease in difference of survival time in KNC. This original difference in sisters is apparently due to the fact that the food content is not always distributed equally to the daughter cells at division. In Paramecia the distribution of food is practically equal, and here the average difference in survival time between sisters is the same immediately after division as it is after a period of twenty-four hours. This small observed difference is however within the limits of experimental error.

9. The difference in resistance of fed and starved Paramecia and Didinia and the rhythmic change in resistance in the case of Didinia between cell divisions which is closely similar to that found in Echinoderm eggs can at present be best explained by assuming that it is due to change in permeability of the cell. Penetration of KNC into the cell and hence its toxic action is dependent upon the degree of permeability of the cell at different times. On this assumption rhythm in susceptibility depends primarily upon rhythm in permeability. The possible relation between rate of intracellular oxidation or nutrition and cell permeability is in the absence of sufficient data an open question.

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