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Diet in relation to depression and recovery in the ciliate *Didinium nasutum* [1].

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(With 6 figures in the text.)

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Introduction.

Investigations on pure-line cultures of protozoa have demonstrated repeatedly that under certain conditions the experimental lines become more and more depressed as the length of the period of culture increases. All such depressions were formerly attributed

[1]) It is a pleasure to acknowledge in connection with this investigation my indebtedness to Prof. MAX HARTMANN for his kindness in placing the facilities of his Department at my disposal and for his constant interest in the work.

to some sort of senescence inherent in the organisms themselves (Maupas, 1888), but it has since been shown that in many cases the depression is partly or wholly the result of unfavorable cultural conditions. Various investigators, working on relatively unrelated forms, have found that certain protozoa can live without depression for great numbers of generations — perhaps indefinitely — under favorable conditions, though under slightly unfavorable conditions the same protozoa exhibit marked depression, which becomes more pronounced as generations pass and which often results in the death of the experimental animals. Thus it is clear that the effects of the depression are cumulative; in general, each generation is more depressed than the preceding. Examples of depression under unfavorable conditions and extended survival under adequate conditions are afforded by the investigations of Enriques (1905) on *Glaucoma* and *Stylonychia*, Metalnikov (1922) on *Paramecium caudatum*, Woodruff and Moore (1924) on *Spathidium*, Bělař (1924) on *Actinophrys*, Woodruff (1929) on *Paramecium aurelia*, and Beers (1926, 1928) on *Didinium*. (Reference may be made to Jennings, 1929, for a detailed review of this type of work in protozoan genetics.) It is of interest to note that similar results have been obtained by Hartmann (1924) in studies on *Eudorina*, one of the plant-like protista, and by Lynch and Smith (1931) on *Proales sordida*, a rotifer which reproduces exclusively by parthenogenesis and which, like protozoa, is therefore well suited for studies on pure-line inheritance.

Certain investigations have dealt with the inheritance of such induced depressions after the return of the animals to favorable cultural conditions, though the studies have not been sufficiently extensive to warrant any general conclusions. Middleton (1918) induced depression in pure lines of *Stylonychia pustulata* by culture at high temperatures and found that the depression persisted for approximately 20 days after the lines were returned to the normal temperature. Jollos (1921), working on *Paramecium aurelia*, induced depression in the fission rate by subjecting the animals to calcium nitrate and found that the depression was inherited for four months after the animals were restored to normal conditions of culture. Lynch and Smith, however, in their studies on *Proales*, found no inheritance of the induced depression (induced in this case by insufficient food and affecting longevity and fecundity); the animals recovered in the first generation when culture under adequate conditions was resumed.

With regard to the question of depression in *Didinium nasutum*, I have shown in a previous study (1928) that pure lines of this ciliate when cultured on a diet restricted to starved paramecia exhibit a cumulative depression which, if sufficiently prolonged, ends in the death — rarely in the encystment — of the lines. That such a depression is actually the result of dietary inadequacies was shown by culturing control lines of the same clone on well-fed paramecia. Such lines showed no depression. This type of depression in *Didinium* manifests itself most clearly in a marked decrease in the fission rate, in the production of structurally abnormal individuals, and in the death of the lines after a period of culture varying from 20 to 40 days (25 to 50 generations). Other symptoms of depression include an increase in the death rate, a decrease in the encystment rate, seemingly with loss of the ability to encyst, difficulties in food ingestion, and a conspicuous and characteristic vacuolation of the cytoplasm.

The present study is a continuation of the preceding. It deals primarily with the problem of recovery in lines of *Didinium* which are depressed by a diet of starved paramecia, though special mention will be made of certain structural abnormalities which accompany depression. In other words, it attempts to answer these questions: What is the capacity of *Didinium* to recover from such a condition of depression when adequate cultural conditions — meaning a diet of well-fed paramecia — are restored? Will the symptoms of depression persist or will they disappear at once after the resumption of the adequate diet? Can the genetic constitution of the animals be more or less permanently altered by culture under bad conditions?

Material and methods.

This study concerns three clones of *Didinium*, designated as A, B, and C. Since the experimental procedure was the same for all three clones, it will suffice to restrict our discussion of methods to only one of the clones, A.

Two sets of pure lines, designated as group 1 and group 2, were established with individuals of clone A. It is obvious that the didinia of both groups, having been derived asexually from a single specimen, were genetically identical. The depressed group (group 1) consisted of ten sub-lines, this large number being necessary in consequence of the high mortality in the group; however, in the last stages of depression the number of progeny produced daily in this

group was not sufficient to maintain even ten lines and the number was of necessity reduced to six or four. The control group (group 2) consisted of a smaller number of sub-lines — usually four. Depression was induced in group 1 by culture on a diet of starved paramecia. The lines of group 2 received, on the contrary, only well-fed paramecia as food.

The didinia were grown on depression slides in 0.01 per cent modified KNOP solution, to which the paramecia were added. In the preparation of the modified KNOP solution three stock solutions were made up, as usual: 10 per cent Ca $(NO_3)_2$, 5 per cent KNO_3, and 5 per cent $MgSO_4 \cdot 7H_2O$. A 1 per cent solution was then prepared by adding the following amounts of the stock solutions to 150 cc. distilled water: 10 cc. Ca $(NO_3)_2$, 7.5 cc. KNO_3, and 7.5 cc. $MgSO_4$. Then, a 0.01 per cent solution was made up by adding 1 cc. of the 1 per cent solution to 99 cc. distilled water. Finally, 5 cc. of $NaOH—KH_2PO_4$ buffer mixture having a hydrogen ion concentration of p_H 6.8 was added to each 100 cc. of the 0.01 per cent solution, this p_H being eminently suitable for the culture of both *Paramecium* and *Didinium*. After the addition of the buffer mixture the final solution is not actually a 0.01 per cent solution from the standpoint of total salt content, but for convenience it will be referred to as 0.01 per cent modified KNOP solution. (For the preparation of ordinary KNOP solution, see BĚLAŘ, 1928.) The distilled water used in making up all solutions was obtained from a Jena-glass still; the buffer mixture was prepared according to the directions given by CLARK, 1928.

The food of group 1 was prepared as follows: Thousands of well-fed specimens of *Paramecium caudatum*, which were grown in hay infusion, were concentrated by centrifuging into approximately 1 cc. of infusion. This amount of infusion plus paramecia was poured into 200 cc. of 0.01 per cent modified KNOP solution contained in a culture dish. To be sure, a considerable number of bacteria were transferred to the KNOP solution along with the paramecia. Since KNOP solution is an extremely poor medium for the growth of bacteria, the supply was soon exhausted by the paramecia. After a week or ten days in KNOP solution, the paramecia were distinctly emaciated; they were unusually small and sluggish and contained no conspicuous food inclusions. Fifty of these starved paramecia had an average length of only 182 micra, whereas fifty well-fed specimens of the same clone (such as were supplied the didinia of group 2) had an average length of 228 micra. Preliminary

to being fed to the didinia of group 1, the starved paramecia were concentrated to some extent by centrifuging. They were further concentrated and, at the same time, washed by filtration. In this process the paramecia and KNOP solution were poured into an ordinary paper filter, the meshes of which were small enough to retain the paramecia. By passing additional amounts of fluid through the filter, the paramecia were thoroughly washed and brought into fresh KNOP solution. They were then pipetted out of the filter and were ready to be used as food for group 1.

In the preparation of the food of group 2, paramecia were taken from flourishing hay-infusion cultures and were partly concentrated by centrifuging. Further concentration and washing were effected by filtration. In the filtration process the hay infusion was gradually, and, to all practical purposes, completely replaced by KNOP solution, so that both types of paramecia — starved and well-fed — were contained in essentially the same medium before being fed to the didinia. All paramecia used in the entire study belonged to the same clone.

It is understood that no limitation was placed on the quantity of food received by the lines of either group; paramecia were available as food in excess at all times. Regarding all ordinary chemical and physical factors, such as temperature, light, and the methods of washing and handling the slides and pipettes, the two groups were treated entirely alike. Transfers of certain of the progeny were made daily to fresh environments. Frequent inspections of both groups prevented the occurrence of completed conjugation.

Permanent mounts were made of certain abnormal specimens of group 1. These were stained *in toto* on the slide in BECHER'S anthracene blue. A stock solution was prepared by dissolving 0.1 gm. of anthracene blue in 100 cc. of 5 per cent aqueous aluminum sulfate. The animals were stained overnight in a solution consisting of one part stock solution and 19 parts water.

History of the depressed lines and control lines of clone A.

The cultural histories of groups 1 and 2 of clone A are summarized in Table 1. A record of the fission rate of the two groups is presented graphically in Fig. 1. Table 1 gives daily averages of the fission rate of the lines of both groups and daily averages of the encystment rate and death rate in group 1. In Fig. 1 these

Table 1.

Cultural histories of three groups of lines of *Didinium nasutum*. Clone A. Group 1 was fed on starved paramecia; groups 2 and 1 R, on well-fed paramecia. Group 1 R was established on the twenty-third day with surplus animals of group 1. Note decline in group 1; absence of decline in group 2 (control group); recovery in group 1 R. Encystment and death rates are given in percentages.

Days of culture	Group 1				Group 2		Group 1R		
	Total number of fissions to date	Average number of fissions per day	Encystment rate	Death rate	Total number of fissions to date	Average number of fissions per day	Average number of fissions per day	Encystment rate	Death rate
1	3.0	3.0	5·5	0.0	3.1	3.1			
2	5.1	2.1	8.4	2.5	6.4	3.3			
3	7.4	2.3	5.5	0.0	9.1	2.7			
4	9.2	1.8	6 6	2.5	12.1	3.0			
5	11.1	1.9	6.6	3.4	15.3	3.2			
6	12.6	1.5	10.5	2.5	18.1	2.8			
7	14.6	2.0	10.0	0.0	21.5	3.4			
8	15.5	0.9	12.5	5.5	24.5	3.0			
9	17.4	1.9	5.0	3.4	27.2	2.7			
10	19.0	1.6	3.0	2.5	29.7	2.5			
11	20.4	1.4	3.3	8.3	33.1	3.4			
12	22.3	1.9	0.0	5.5	36.4	3.3			
13	23.9	1 6	6.4	4.5	39.4	3.0			
14	25.4	1.5	0.0	3.2	42.5	3.1			
15	27.2	1.8	0.0	8.3	45.2	2.7			
16	28.8	1.6	3.6	7.5	48.2	3.0			
17	30.5	1.7	0.0	7.3	50.9	2.7			
18	31.8	1.3	4.5	6.5	54.1	3.2			
19	32.7	0.9	0.0	12.2	57.6	3.5			
20	33.2	0.5	3.5	15.4	60.6	3.0			
21	34.3	1.1	0.0	6.2	63.1	2.5			
22	34.7	0.4	3.3	15.8	65.8	2.7			
23	35.2	0.5	0.0	16.6	69.1	3.3			
24	35.6	0.4	0.0	27.8	71.9	2.8	2.0	0.0	0.0
25	36 4	0.8	0.0	35.5	74.8	2.9	3.0	0.0	0.0
26	36.6	0.2	0.0	66.7	77.8	3.0	3.2	0.0	0.0
27	36.6	0.0	0.0	100.0	80.8	3.0	3.0	0.0	0.0
28					83.0	2.2	2.8	0.0	0.0
29					86.5	3.5	3.0	0.0	0.0
30					90.0	3.5	2.8	0.0	0.0
31					92.7	2.7	3.4	0.0	0.0
32					95.6	2.9	2.9	2.5	0.0
33					98.9	3.3	3.0	0.0	0.0
34					102.2	3.3	3.4	0.0	0.0
35					105.1	2.9	2.6	0.0	0.0
36					108.1	3.0	3.0	0.0	0.0
37					110.8	2.7	2.8	0.0	0.0

daily averages of the fission rate are averaged further for two-day periods. The encystment rate and death rate were calculated,

following the method of CALKINS (1915), from the number of encystments or deaths in the group and from the total number of animals present at the time of each daily transfer. This method does not give strictly accurate results for the death rate, for a specimen may die, disintegrate, and disappear completely in the interval between successive transfers. However, the depressed didinia of group 1 usually underwent a slow death, moribund specimens gradually becoming spherical and more and more swollen, sluggish and transparent. In view of this fact, the method is sufficiently accurate for the purposes at hand. The encystment rate and death

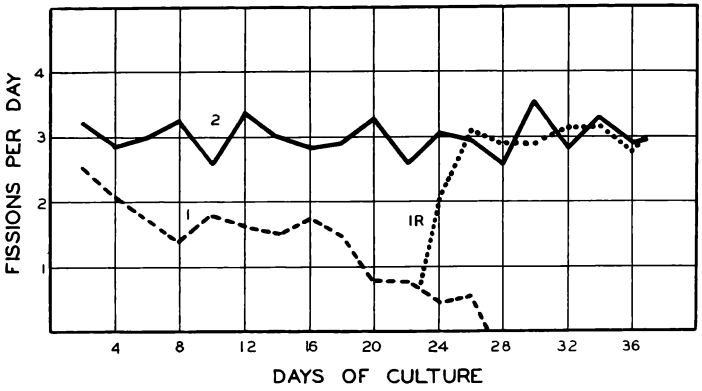

Fig. 1. Graph of division rates in three groups of lines of *Didinium nasutum*. 2, ————, control group, cultured on well-fed paramecia; 1, – – – – –, depression group, cultured on starved paramecia; 1R, ... , recovery group, established with surplus animals of group 1 on the 23rd day and cultured on well-fed paramecia. Clone A. The division rates are averaged for successive two-day periods.

rate of group 2 are not included in Table 1, for they were so low as to be quite insignificant. Only one cyst was produced in group 2 and only two individuals died. We may pass over for the present the lines under the heading "group 1R" of the table and return to these later when the matter of restoration of the animals to an adequate diet is taken up. The lines of group 1R were established with depressed animals of group 1 shortly before the death of the latter.

Table 1 and Fig. 1 call special attention to the following features of the cultural histories: The fission rate of group 2 (well-fed lines) showed no decline as generations passed; it was at all times higher than that of group 1 and it was as high at the end of the experiment, when the lines were discontinued, as at the beginning. The fission

rate of group 1 showed, on the contrary, a consistent decrease as generations passed; it fell to zero on the twenty-seventh day in consequence of the death of all remaining lines of the group. Only 36.6 generations per line were produced in group 1 in the 27 days, whereas 80.8 were produced in group 2.

The encystment rate of group 1 was relatively high during the first third of the period of culture; throughout the remainder of the period it was consistently low (Table 1). Evidently the animals encysted in the early part of the cultural period as a result of the inadequate nature of the food. Prolonged subjection to this same inadequate diet appears to bring about the partial or total loss of the ability to encyst. No doubt this is due to the inability of the animals to store up sufficient food materials of the proper kind to permit the production of cysts. In contrast to the high encystment rate, the death rate of group 1 was low in the first third of the cultural period (Table 1). After this time it showed a marked increase. It became consistently higher, until it reached 100 per cent on the twenty-seventh day and thus terminated the cultural history of the lines.

Restoration of the depressed lines of clone A to an adequate diet.

In order to ascertain whether the depression in group 1 would persist after the resumption of an adequate diet, surplus didinia of the lines of this group were transferred to clean slides and fresh Knop solution and were supplied with well-fed paramecia. In establishing these recovery lines didinia were chosen that were structurally normal to all appearances, for structurally abnormal individuals die almost without exception, regardless of their diet. Many of the didinia used were, however, smaller and less active than normal individuals, and occasionally one of them would burst upon attempting to ingest a large, well-fed paramecium.

We may follow in detail, as typical of the outcome of the transfer to an adequate diet, the history of five lines established with five surplus individuals of group 1 on the twenty-third day of culture. The history is summarized in Table 1 under the heading group 1 R, and the fission rate is represented graphically in Fig. 1.

During the first day of culture on a diet of well-fed paramecia the fission rate of group 1 R showed a striking increase. Two generations were produced within the first 24 hours, whereas only 0.4 generation was produced in parent group 1 (Table 1). During the

second day the lines of group 1 R produced an average of three generations per line; after only one day of culture on well-fed paramecia their fission rate attained the level of that of control group 2. Group 1 R was cultured in parallel with group 2 for a period of 14 days, and during this period both groups exhibited practically the same rate of fission (Table 1 and Fig. 1). A total of 40.9 generations per line were produced in group 1 R during the 14 days and 41.7 generations per line in group 2 in the same time. In the meantime the lines of group 1 had already succumbed to the injurious effects of their inadequate diet, as has been noted. No structural abnormalities appeared in group 1 R, no deaths occurred, and only one individual encysted. Since the fission rate of group 1 R equalled that of the control group throughout the second day of culture on well-fed paramecia, and since the animals of group 1 R showed no indications of depression or decline during the remainder of the 14-day period, it is evident that recovery was complete after a maximum of 24 hours of culture on well-fed paramecia.

Results similar to the foregoing were obtained in all cases in which depressed animals of group 1 were restored to a diet of well-fed paramecia. For example, six individuals of group 1 were restored to a diet of well-fed paramecia on the 21st day, at a time when group 1 was producing less than one generation per day (Table 1). One of the six died without dividing, but the five remaining individuals produced an average of 2.2 generations per line in the first 24 hours of culture on well-fed paramecia. On the second day the five recovery lines produced an average of 3.1 generations per line; on this day (the 23rd of Table 1) group 2 produced 3.3 generations. This recovery group was cultured in parallel with group 2 for 16 days. In this time it produced an average of 46.8 generations per line, whereas group 2 produced 47.7 generations per line (22nd to 37th days of Table 1). No abnormal individuals developed in the recovery group, no encystments occurred, and only three deaths were observed. These results show that recovery was complete after 24 hours of culture on well-fed paramecia.

A third and final case of recovery in clone A concerned four didinia of group 1 which were restored to the diet of well-fed paramecia on the 25th day. All of them lived, and on the first day they produced an average of 1.5 generations per line; on this day (the 26th of Table 1) parent group 1 produced only 0.2 generation, and control group 2 produced 3 generations. On the second day the fission rate of the recovery group was actually appreciably

higher than that of group 2; the recovery group produced 3.5 generations, and group 2 only 3 generations. However, this fact, in view of the future history of the recovery lines, was of no significance. The recovery group was cultured in parallel with group 2 for 12 days, in which time the recovery group produced 36.3 generations and group 2 produced 36. Abnormalities were quite absent in the recovery group, and the encystment rate and death rate were insignificant. Again, the results show an immediate recovery in the depressed animals when they were transferred to the adequate diet of well-fed paramecia.

Results obtained with clones B and C.

The results obtained with these clones were entirely in accord with those just described for clone A. Group 1 of clone B (fed on starved paramecia) exhibited a typical cycle of decline which ended in the death of all of the lines on the 33rd day of culture with the production of a total average of 46.4 generations per line. As usual, the encystment rate decreased and the death rate increased with the passage of generations. Group 2 of clone B (well-fed) showed no decline as generations passed. A total of 98.7 generations per line were produced in group 2 in the 33-day period in which groups 1 and 2 lived synchronously. The effect of restoration to a diet of well-fed paramecia was tested in two groups of depressed animals. The first group consisted of six sub-lines which were derived from surplus didinia of group 1 on the 26th day of culture; the second recovery group consisted of four sub-lines which were established on the 30th day. The results, which were the same in both cases, may be summarized briefly as follows: When depressed animals of group 1 were transferred in the last stages of decline to a diet of well-fed paramecia, their recovery was complete within approximately 24 hours (two generations); i.e., on the second day they began to divide as fast as the control group; the number of encystments and deaths fell, with rare exceptions, to zero; and all other symptoms of depression disappeared.

Group 1 of clone C survived only 22 days on the inadequate diet. However, the fission rate of this clone was higher than that of either of the preceding clones, so that 41.5 generations were produced in group 1 in the 22 days and 77 in group 2 in the same time. The encystment rate in group 1 showed the usual decrease, though it did not fall to zero at the end of the cultural period.

Two cysts, to all appearances normal ones, were produced on the last day of the period. The death rate showed the usual increase, until on the 22nd day all remaining specimens, with the exception of the two cysts, died. The effect of transfer to an adequate diet was tested in three groups of depressed animals. The first recovery group was established with five depressed didinia of group 1 on the 15th day, the second group with four specimens on the 18th day, and the third with three specimens on the 20th day. All recovery groups had a similar history — the depressed animals upon restoration to an adequate diet of well-fed paramecia recovered within about 24 hours or after the passage of about two generations, whereupon the fission rate assumed the level of that of the control group.

Discussion.

From the foregoing results, it is evident that the type of depression induced in *Didinium* by a diet of starved paramecia does not persist, but disappears almost immediately — within 24 hours or two generations — when normal and adequate cultural conditions are restored. The fact that the fission rate is slightly lower in the first 24 hours than later can scarcely be regarded as indicative of the inheritance of the depression; this period is merely one of intense assimilative activity and recovery. Evidently, this type of depression in *Didinium* is unlike the depression induced by MIDDLETON (1918) in *Stylonychia* by culture at high temperatures and by JOLLOS (1921) in *Paramecium* by means of various chemicals. However, the dissimilarities in the nature of the unfavorable cultural factors used to induce depression in the three studies (temperature, chemicals, and dietary insufficiency) are in themselves sufficient to account for any differences in the experimental results. It is of interest to note in this connection that the present results are strikingly in accord with those of LYNCH and SMITH (1931), who, as stated previously, induced depression in a rotifer by means of a dietary factor (insufficient food) and found that the animals recovered in the first generation when transferred to a favorable medium.

The precise nature of the unfavorable dietary factor which operates to induce depression remains to be ascertained. It is obvious that the protoplasm of well-fed paramecia is much richer in reserve food substances than that of starved paramecia. This fact, together with the very rapid recovery of the didinia when given well-fed paramecia, indicates that the decline is the result

of dietary deficiency. The didinia when grown on starved paramecia have ample food from the standpoint of quantity, but it is not entirely of the right kind. Nevertheless, the fact that well-fed paramecia contain immense numbers of undigested bacteria in food vacuoles must not be overlooked, for these bacteria are also ingested when *Didinium* feeds. The nutritional importance of the bacteria thus secondarily ingested by *Didinium* remains unevaluated. Finally, it is possible that substances mildly toxic to *Didinium* accumulate in the cell-bodies of the paramecia in consequence of their starvation and resultant altered metabolism and that these substances are of importance in inducing depression in *Didinium*.

Abnormalities accompanying depression.

I have mentioned previously (1928) the production of abnormal specimens in considerable numbers when *Didinium* is cultured on starved paramecia, and numbers of abnormalities appeared in the lines of group 1 of all three clones of the present study. Certain of these abnormalities are of unusual interest. Typical ones are shown in Figs. 3, 4, 5, and 6. A normal full-grown specimen is shown for comparison in Fig. 2. The normal specimen shows the single sausage-shaped macronucleus, the contractile vacuole, the two latitudinal bands of fused cilia or membranelles which characterize the genus, the clear cytoplasmic region around the mouth, and the trichites of the seizing organ. The micronuclei, being small and difficult to distinguish in whole mounts, are not indicated in the figures.

The conspicuous abnormalities which appeared in the depressed lines were usually associated with incomplete fission, the daughter cells of a normal transverse fission remaining fused together. As was noted by Thon (1905), an approaching fission manifests itself first in a doubling of the number of ciliary girdles. A normal specimen preparing to divide exhibits four bands of cilia, even before the nucleus begins to divide, and long before the cytoplasmic constriction appears. Regarding the present abnormalities, the number of ciliary bands serves therefore as an indication of the number of individuals which are potentially present in a single monster. But since the number of ciliary bands does not usually agree with the number of mouth openings or with the number of macronuclei, the ciliation is not a reliable criterion by which monsters may be analyzed into component individuals. Fig. 3, for example, shows

an abnormal *Didinium* that has six ciliary bands, indicative of three incompletely separated individuals, though only two mouth openings and two macronuclei are present. Both mouths appear to be normal and functional. Only one contractile vacuole is present. A similar abnormal specimen is shown in Fig. 4. Six ciliary bands are present, though two of them merge into one for half the distance around the circumference. Two macronuclei are present, though each has approximately twice the length of a normal macronucleus. The terminal mouth is perfectly formed and appears to be functional. The existence of a second mouth and second anterior end, both imperfect, is indicated at the side of the specimen. Again, only one contractile vacuole is present. The individual shown in Fig. 5 has eight bands of cilia and three macronuclei, one of which is more than twice as long as normal. Two of the nuclei show terminal lobulations. The apical mouth and seizing organ appear to be normal. An imperfect mouth and seizing organ have formed at the side, and two of the ciliary girdles are so disposed as to encircle in a more or less normal manner this imperfect laterally placed anterior end. This monster, like the others, has a single contractile vacuole. It was the largest of all abnormal specimens that developed in the depressed lines. It had a length of 265 micra, whereas the specimens shown in Figs. 3 and 4 and the normal individual in Fig. 2 measured only 180 micra in length.

In addition to the contractile vacuole, other cytoplasmic vacuoles of considerable size are indicated in Figs. 3, 4, and 5. These vacuoles contained fluid. They are absent in normal didinia. Their presence may be taken as further evidence of depression due to dietary inadequacies, for Vieweger (1925) has noted that similar vacuoles accompany inanition in *Colpidium colpoda*.

Not only were abnormal individuals of gigantic proportions produced in the depressed lines, but miniature specimens measuring only 100 micra or less in length occurred as well. One of these is shown in Fig. 6. This individual has a small irregularly shaped macronucleus and has only one ciliary girdle. Such individuals are usually derived by imperfect fission from one of the giant specimens. They rarely feed, and they die almost invariably without dividing.

The giant individuals are of special interest when considered from the standpoint of an axial gradient in the rate of metabolic reactions in *Didinium*. Child (1914) directed attention to this type of axial gradient in ciliate infusoria by showing that the anterior end of *Stentor*, *Stylonychia*, *Paramecium* and other ciliates is more

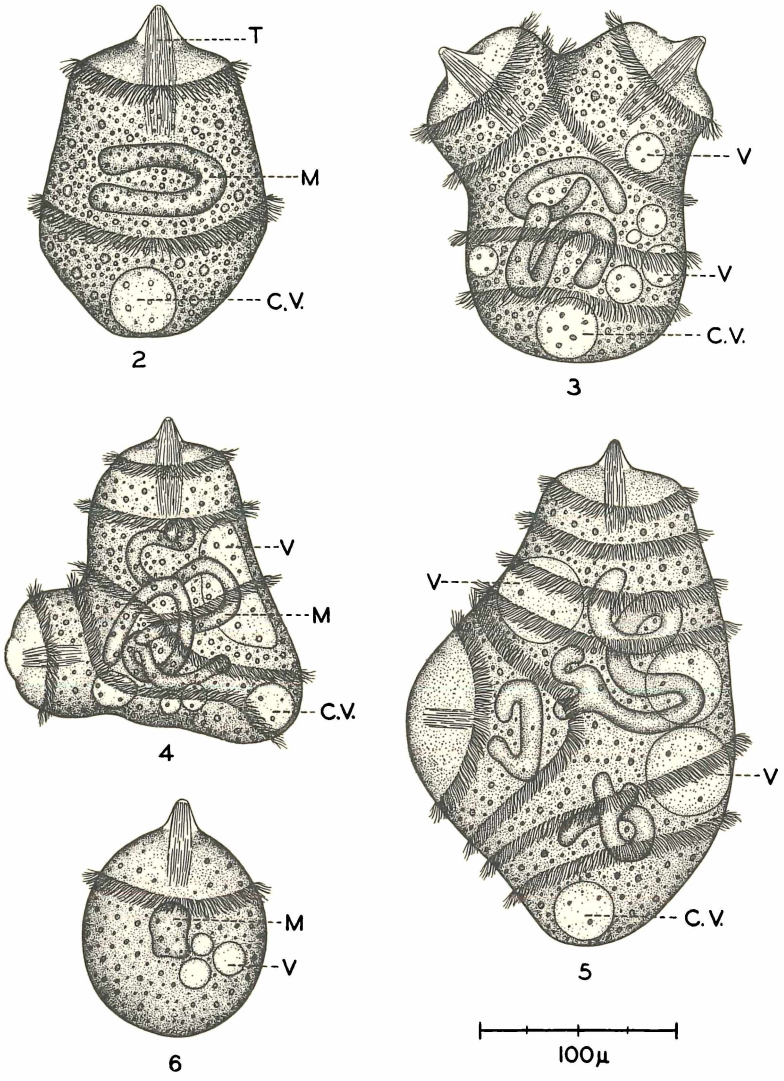

Fig. 2. Normal full-grown specimen of *Didinium nasutum*. — Figs. 3—5. Abnormal specimens of *Didinium nasutum* which developed in pure lines that were cultured on starved paramecia. Such monsters resulted from incomplete fission. — Fig. 6. Small abnormal specimen of *Didinium nasutum* having only one ciliary band and an imperfect macronucleus. From a line cultured on starved paramecia. C V contractile vacuole; M macronucleus; T trichites of the seizing organ; V cytoplasmic vacuoles that were characteristic of the abnormal specimens.

susceptible to KCN than the posterior end. His observations on *Paramecium* were extended by CHILD and DEVINEY (1926), who used, in addition to KCN, other chemicals and ultraviolet radiation. The results showed in general the presence of an axial gradient in susceptibility, and this susceptibility gradient was considered to indicate the presence of an axial gradient in metabolic processes along the longitudinal axis.

The giant individuals just described also speak for the existence of an axial gradient, which expresses itself in this case in the degree of differentiation of the incompletely separated animals along their respective longitudinal axes. Let us consider the manner in which the abnormal specimen shown in Fig. 3 was actually observed to be derived from a normal *Didinium* which was isolated at one of the daily routine transfers. This normal specimen when first observed had four ciliary bands and a single macronucleus. Soon the nucleus divided, but the cytoplasmic division which completes a normal fission was suppressed. The potential anterior daughter cell retained the seizing organ of the parent cell, as usual; but the seizing organ of the posterior daughter cell, being prevented from developing normally by the failure of the cytoplasm to divide, began to form at one side and later shifted anteriorly, thus giving rise to the condition of anterior duplication shown in the figure. Likewise, the ciliary girdles rearranged themselves, so that each anterior end acquired the normal ciliary complement of two bands. Simultaneously, two additional bands developed posteriorly. But with the differentiation of the anterior ends, the regulatory processes ceased. The anterior ends fuse just posterior to the trichites of the seizing organs. No evidence of duplication appears at the posterior end of the monster, as is shown by the single contractile vacuole. Normal didinia were never observed to develop from such giant individuals, though one or more small specimens may be constricted off, as has been mentioned.

A similar gradated condition of antero-posterior differentiation, but less advanced than the preceding, is shown in Fig. 4. Here, the anterior end of the posterior daughter cell of an abortive fission was still in the process of development at the side when the specimen was killed. Only a limited amount of ciliary rearrangement has taken place. The anterior girdle of the lateral anterior end is complete, the posterior incomplete. Posteriorly, the two individuals fuse into one, as is emphasized by the presence of a single contractile vacuole.

A less advanced stage in the differentiation of a laterally placed anterior end is shown in Fig. 5. This individual evidently represents an incomplete separation of four didinia, as is indicated by the eight ciliary bands and three macronuclei, one of which is more than twice as long as normal. An anterior end is seen to be differentiating at the left side of the abnormal individual. A limited number of trichites are visible in this end; the cytoplasmic elevation in which the trichites lie is largely devoid of food inclusions, as is the normal circum-oral region; and two ciliary bands have assumed a position with reference to the developing seizing organ which approaches the normal. Like the other giant individuals, this specimen has only one contractile vacuole.

From the foregoing, it would seem that when a normal cytoplasmic division is inhibited in a dividing *Didinium*, the potential posterior daughter cell may differentiate its anterior end at the side, but the two individuals remain unseparated posteriorly. The developmental potentialities are not sufficiently pronounced posteriorly to effect a complete differentiation of a posterior end in each individual or a complete separation of the two individuals. Hence, the evidence adduced from such abnormalities indicates the presence of an axial gradient in the capacity for differentiation in *Didinium*. This gradient, which may be considered in the light of a developmental gradient, is, like the susceptibility gradient, indicative of a physiological gradient along the longitudinal axis.

Summary.

This study deals with the production of depressions in pure lines of *Didinium nasutum* and with the recovery of the lines from such depressions.

Depression was induced in three clones by culturing the didinia on starved paramecia. The depression of the lines expressed itself primarily in a marked decrease in the fission rate, in the production of structural abnormalities, and in an increase in the death rate. Furthermore, the depression was cumulative; it became more pronounced as generations passed, so that all lines of the three clones ultimately died out or encysted after a period of culture which varied from 22 to 33 days (36 to 46 generations). The encystment of the lines in the last stages of decline was an exceptional occurrence, for they seemed to lose their ability to encyst as generations passed. Control lines of the same clones were cultured on

well-fed paramecia. These lines showed no depression. In general, they divided twice as fast as the depressed lines.

The depressed animals when restored in the last stages of depression to a diet of well-fed paramecia showed a strikingly rapid recovery. After 24 hours of culture on well-fed paramecia, in which time about two generations were produced, they were dividing as fast as the control groups, and all indications of depression had disappeared. Such recovery lines were cultured in parallel with the control lines for a period of two weeks (about 40 generations), in order to ascertain whether their recovery was permanent. In all cases the recovery lines divided at the same rate as the control lines and showed no symptoms of decline. Hence, the depression was not inherited after the restoration of the depressed animals to an adequate diet of well-fed paramecia.

The depression is attributed to dietary deficiency and not merely to insufficient food, for starved paramecia were present in excess at all times.

Certain of the abnormalities that occurred in the depressed lines are of special interest in that they concern the rearrangement of cytoplasmic material following an incomplete fission. In the production of such an abnormality the ciliary bands and the nucleus divided as usual, but the cytoplasm failed to divide. The posterior daughter cell then differentiated its anterior end at the side of the parent individual. This anterior end shifted forward, so that the two daughter individuals were separated anteriorly, but united posteriorly. This condition of antero-posterior differentiation is indicative of an axial gradient in the developmental potentialities along the longitudinal axis.

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