NUTRITIONAL CONTROL OF SEXUALITY IN CHLAMYDOMONAS REINHARDI

By RUTH SAGER* and S. GRANICK

(From the Laboratories of The Rockefeller Institute for Medical Research)

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In initiating an investigation of inheritance in the unicellular green alga, *Chlamydomonas reinhardi*,[1] difficulties were encountered in obtaining regularly, and at will, a high proportion of sexually active cells required for making crosses. The unpredictability of mating behavior made it evident that for adequate control of the material, factors governing sexuality would have to be investigated.

There have been many reports in the literature, beginning with Klebs (1896), that the presence of light and depletion of nutrients may induce sexuality in a number of microorganisms. In this paper we present evidence that in *C. reinhardi*, depletion of one component of the medium, the nitrogen source, leads to the differentiation of vegetative cells into gametes; and that following the addition of a nitrogen source to gametic cells, dedifferentiation occurs to the vegetative state. Light appears to act indirectly, by way of photosynthesis, and is not obligatory for zygote formation of dark-grown nitrogen-depleted cells.

Some applications of these findings to the technical problems involved in crossing various mutant strains are presented in this paper, and some implications for the general problem of gametic differentiation will be discussed.

Materials and Methods

1. The Organism.—C. reinhardi is a heterothallic isogamous alga: there are two mating types which look alike and are referred to as *plus* and *minus*. A diagrammatic sketch of the organism as it appears in liquid culture is shown in Fig. 1. It is motile, swimming by means of two anterior flagella. When cells are grown on an agar surface, flagella are absent. When healthy cells from a solid medium are placed in liquid, *e.g.* distilled water in light or dark, the flagella are extruded within a few minutes and the cells become motile.

The mating process has been described in a number of species of *Chlamydomonas* (*e.g.* Goroschankin, 1890; Klebs, 1896; Pascher, 1927; Moewus, 1933; Gerloff, 1940;

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Skuja, 1949; Lewin, 1952a; Smith, 1951). Although species differences in details of mating behavior have been reported, the process appears to be uniform with respect to the main events: cluster formation, pairing between cells of opposite mating type at their flagellar ends, fusion, and incorporation of the contents of the two parental cells into the zygote.

In order to study the mating process in *C. reinhardi*, it was necessary to distinguish between the normally indistinguishable cells of *plus* and *minus* mating type. This was accomplished by growing one mating type on a nitrogen-deficient medium in the light,

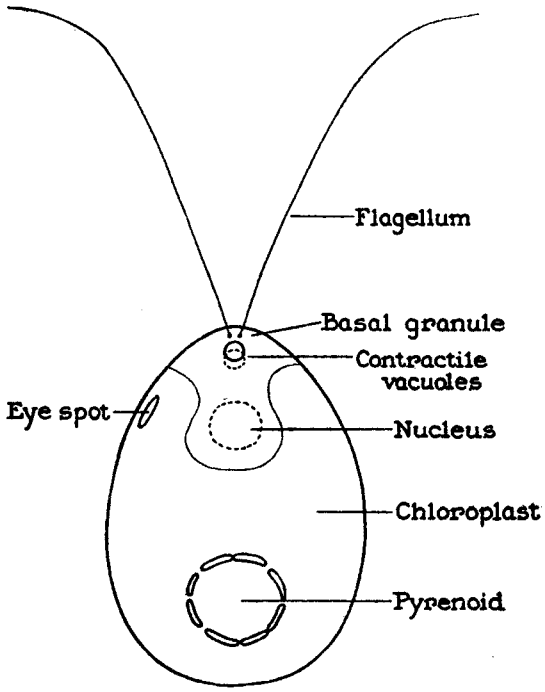

FIG. 1. Diagrammatic sketch of *C. reinhardi* as it appears in liquid culture.

until the cells became distended with large starch grains. The culture of the other mating type, grown on standard medium, consisted of smaller cells of deeper green color and devoid of starch grains. The striking difference in appearance of the cells following this treatment can be seen in Fig. 2. This procedure was used only for observations of mating; in all experiments to be reported in this paper, cells of the two mating types were grown under the same conditions. Other procedures to distinguish between mating types in related organisms, have been used by Lerche (1937) and Lewin (1952 *a*).

When non-flagellated cells of the two mating types, harvested from agar, are mixed in liquid, no mating behavior is observed until the cells have become flagellated and motile. The occurrence of mating between motile cells depends upon nutritional conditions to be described below. Cells with low sexual activity may carry out early steps

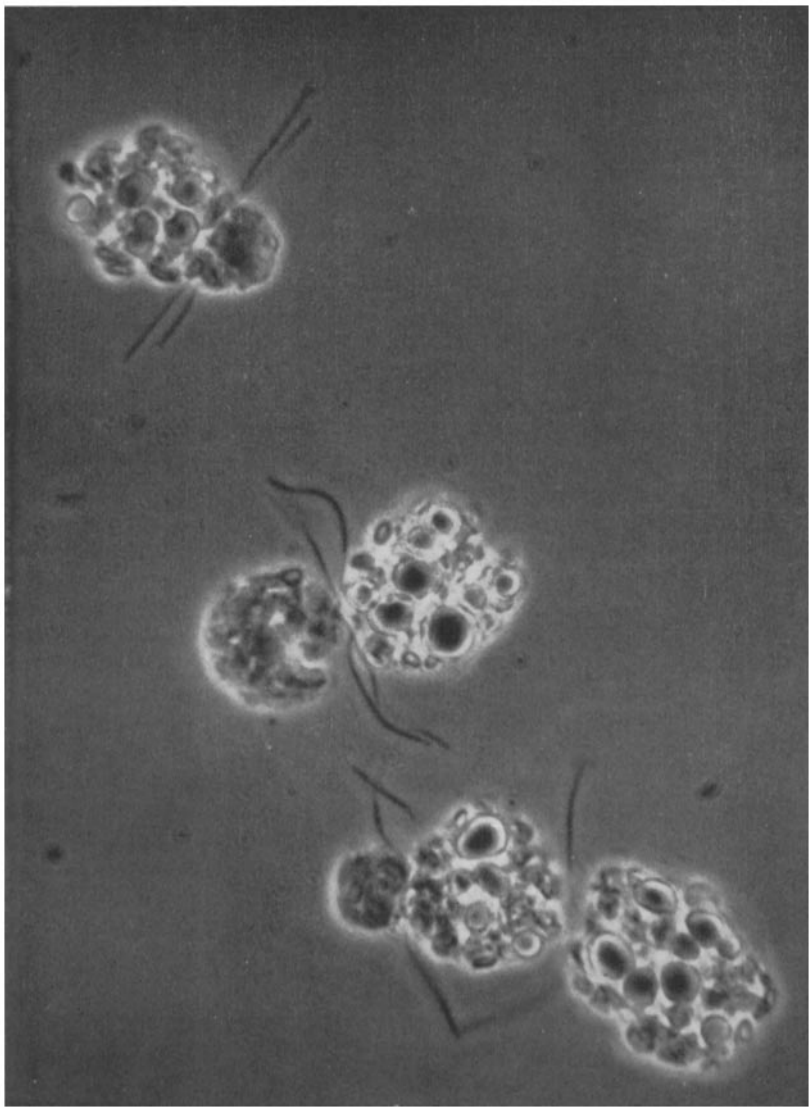

FIG. 2. Early stages in zygote formation as seen with phase contrast microscopy. Pairing has occurred and cells are fusing from the anterior ends. Cells of one mating type, grown on low nitrogen medium, have accumulated large starch grains and are clearly distinguishable from the smaller cells of the other mating type grown on high nitrogen (medium I).

in the mating process, such as cluster formation and pairing, and then separate before fusing to form zygotes; or fusion between pairs may begin, and lead not to zygote formation, but to lysis.

When fully sexual cells, or gametes, or the two mating types are mixed together, the mating process is initiated immediately, culminating within 5 to 30 minutes in zygote formation. The first step is aggregation. Cells of the two mating types come together in clusters, with their flagella actively beating and oriented towards the center of the cluster. Subsequently, the clusters break up and the cells swim off in pairs, which consist of one cell of each mating type. Pairing is at first only flagellar; the pairs swim actively, and only the flagella are in contact. At this stage, the pairing is still reversible; for example, a third cell may swim in and disrupt a pair. Gradually, the anterior ends of the cells come closer together, oriented by the flagella. Then a thin protoplasmic thread may be seen connecting the two anterior ends, at a stage when the cells are still a few microns apart. The anterior ends soon fuse, as shown in Fig. 2, and this may be considered the first irreversible step in the mating process. Subsequently, fusion proceeds rapidly, leading to the formation of the zygote which appears to contain all the cell contents of both parents.

In this paper no attempt has been made to distinguish among the many steps involved in mating. Using zygote formation as the criterion of gametic activity, or sexuality, methods have been investigated for controlling the extent of zygote formation. As will be discussed below, gametic differentiation appears to be triggered by some reaction brought on by nitrogen depletion, which determines whether or not the whole series of steps leading to zygote formation will be initiated.

2. Methods.—Cells of the two mating types were grown separately on agar at 25°C. The media used, medium I for cells grown in the light, and medium II for dark-grown cells, have been described previously (Sager and Granick, 1953) and are listed below.[2] The light intensity employed for growth and mating was 700 to 800 f.-c.; the light source consisted of G.E. fluorescent tubes ("standard cool white" and "daylight"). The two strains used were *21gr*, derived as a single cell isolated from the *plus* mating type stock, and *4Y*, similarly derived from the *minus* mating type stock. These strains are being used extensively in genetic investigations (Sager).

Agar-grown cells were harvested and suspended in sterile distilled water in the dark until motile, generally one-half hour. Subsequently, the suspensions were kept in light or dark as indicated for each experiment, and the cells of the two mating types adjusted to equal density. The extent of gametic activity of the culture under different experimental conditions was determined by counting the proportion of zygotes formed.

In experiments designed to study zygote formation in the dark, cells were grown

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[2] Medium I (pH 6.8) contains, in grams per liter: K_2HPO_4, 0.1 gm.; KH_2PO_4, 0.1 gm.; NH_4NO_3, 0.3 gm.; $MgSO_4 \cdot 7\ H_2O$, 0.3 gm.; $CaCl_2$, 0.04 gm.; $FeCl_3 \cdot 6\ H_2O$, 0.01 gm.; sodium citrate. $2\ H_2O$, 0.5 gm.; trace metal solution, 10 ml.

Medium II (pH 6.2) contains, in grams per liter: sodium acetate. $3\ H_2O$, 2 gm.; $NaH_2PO_4 \cdot H_2O$, 3.67 gm.; K_2HPO_4, 1.15 gm.; NH_4NO_3, 0.3 gm.; $MgSO_4 \cdot 7\ H_2O$, 0.3 gm.; $CaCl_2$, 0.04 gm.; $FeCl_3 \cdot 6\ H_2O$, 0.01 gm.; sodium citrate. $2\ H_2O$, 0.5 gm.; trace metal solution, 10 ml.

Trace metal stock solution contains, in milligrams per liter: H_3BO_3, 100 mg.; $ZnSO_4 \cdot 7\ H_2O$, 100 mg.; $MnSO_4 \cdot 4\ H_2O$, 40 mg.; $CoCl_2 \cdot 6\ H_2O$, 20 mg.; $Na_2MoO_4 \cdot 2\ H_2O$, 20 mg.; $CuSO_4$, 4 mg.

and harvested in a dark room, and microscopic observations of zygote formation were made on cells killed with osmium tetroxide vapor in the dark. In these experiments, the last traces of light were not excluded.

RESULTS

1. The Light Requirement for Gametogenesis.—When light-grown cells from medium I are harvested into distilled water in the dark, and the *plus* and *minus* cells mixed in the dark, no zygotes are formed, even after 24 hours. Only when such a mixture is exposed to the light for several hours does zygote formation occur. These observations indicate that cells which have been

TABLE I

The Light Requirement for Gametogenesis

Experimental conditions		Results (observed after 3 hrs. in dark)
Plus and *minus* cells suspended separately	*Plus* and *minus* cells mixed	
Dark ½ hr.	Light 5 min.	No clusters or pairs
" " "	" 1 hr.	" " " "
" " "	" 2 hrs.	Few clusters
" " "	" 3 "	Clusters + few pairs
" " "	" 3½ "	*Ca.* 50 per cent zygotes
" " "	" 4 "	> 90 " " "
Dark ½ hr.; then light:		
1 hr.	Dark	No clusters or pairs
2 hrs.	"	Few "
3 "	"	Clusters + few pairs
3½ "	"	*Ca.* 50 per cent zygotes
4 "	"	> 90 " " "

grown in the light, have an additional light requirement for zygote formation.

An indication of the nature of this light requirement came from the following experiment. Cell suspensions were exposed to light for various times before and after mixing the *plus* and *minus* cells, and the mixtures were then placed for 3 hours in the dark to allow time for zygote formation. The duration of the light requirement for zygote formation was then determined by counting the proportion of zygotes formed. The results, summarized in Table I, show that an exposure of 4 hours was required, and that light could be supplied before the mating types were mixed, as well as afterwards. Evidently, cells will mate in the dark if their light requirement has already been met before the mating types are mixed.

It appears then, that the light requirement for zygote formation is in fact, a light requirement for the conversion of vegetative to gametic cells; *i.e.*, for

gametogenesis. The relation between the light requirement for gametogenesis and the nutritional state of the cells was then investigated.

2. Effect of Age of the Culture and Nutrient Depletion on Gametogenesis.—A decrease was observed in the light requirement for gametogenesis with ageing of cultures grown on medium I.[2] As shown in Fig. 3 (upper curve) 2 day old cultures grown on medium I have a light requirement of 4 to 5 hours, week old cultures need only about 2 hours of light, and 2 week old cultures about 1½ hours.

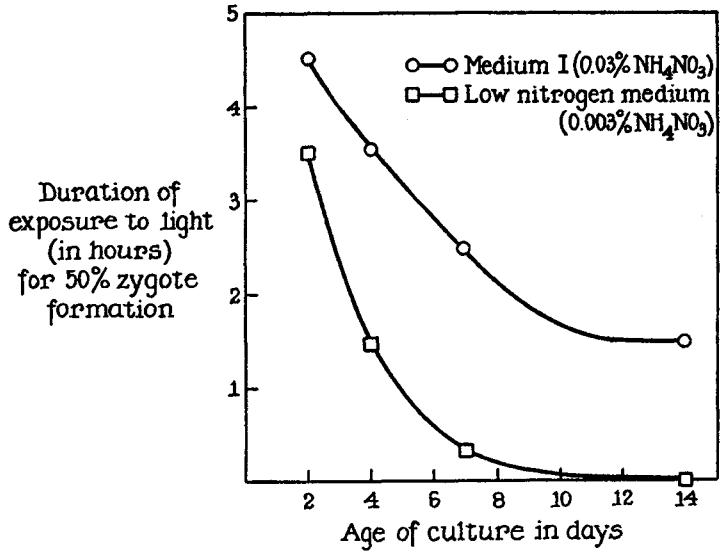

FIG. 3. Effect of age of culture and nitrogen depletion on light requirement for zygote formation. The light requirement decreases with age of cultures grown on either medium I (upper curve) or low nitrogen medium (lower curve). Only in the latter case is the light requirement lost completely as the culture matures.

Since ageing of a culture generally involves depletion of the medium, these results seemed consistent with reports of the beneficial effects of starvation upon induction of sexuality (*cf.* Discussion). It then became of interest to determine whether depletion of any single component of the medium might accelerate gametogenesis.

To test this possibility, cells were grown on a series of media, based on medium I but each deficient in one component. After 1 week, cells from each of these media were tested for gametic activity. Only the cells from the nitrogen-deficient medium had a shorter light requirement for zygote formation than did the control cells grown on medium I. Also, only the low nitrogen cells showed deficiency symptoms: a yellowish color and distention with

starch grains, similar to the nitrogen-deficient cells of Fig. 2. After a second subculture on media deficient in components other than the nitrogen source, cells did show deficiency symptoms; yet they formed very few zygotes.

3. Effect of Growth on a Low Nitrogen Medium upon Gametogenesis.—Cells grown on a low nitrogen medium (medium I with 0.003 per cent NH_4NO_3, $\frac{1}{10}$ the concentration normally used), were tested for gametic activity at intervals during growth of the cultures. The results, summarized in Fig. 3 (lower curve), show that cells grown on a nitrogen-deficient medium have a shorter light requirement for gametic activity during growth of the culture than do cells grown on medium I; and most important, that cells from fully grown nitrogen-deficient cultures have no light requirement for gametogenesis at all.

During growth in the light, the component of the medium most readily depleted appears to be the nitrogen source. This may explain the reported induction of sexuality in various algae by mild starvation; for example, exposure to light for 24 hours in distilled water would lead to depletion of nitrogenous reserves, but not of inorganic components of the cell.

It was then attempted to obtain zygote formation in the dark with dark-grown cultures. Cells grown on medium II in the dark would mate in the light, but they had a long light requirement. There was no zygote formation at all when such cells were mixed in the dark. However, when cells were grown in the dark on a low nitrogen medium (medium II with $\frac{1}{10}$ the NH_4NO_3), and tested for zygote formation in the dark, a small proportion of zygotes was found.

A number of attempts were made to increase the yield of zygotes from dark-grown, nitrogen-deficient cells. The best results were obtained with the most densely grown cultures, washed and resuspended not in distilled water but in buffered acetate at the time of mixing the mating types. The importance of dense and vigorous growth is attributed to the need for sufficient cells to deplete the available nitrogen, and for cells with enough energy reserves to carry through the mating process. The function of acetate in enhancing the yield of zygotes may be that of satisfying this energy requirement. As has been previously reported (Sager and Granick, 1953), *C. reinhardi* can use acetate as sole carbon source for growth in the dark, though it uses the acetate inefficiently.

In some experiments yields as high as 25 per cent zygote formation have been observed after 24 hours. Thus, it has been possible to induce zygote formation in the absence of light with dark-grown, nitrogen-depleted cells.

4. Application of Nitrogen Depletion.—The finding that gamete formation is greatly enhanced by nitrogen depletion has had a number of applications. First, it has made possible the control of mating behavior of strains to be crossed. For genetic analysis, it is important that a high proportion of zygotes

be formed in order to ensure that a random sample of parental cells is represented. For ease of manipulation, the formation of zygotes immediately after mixing of parental clones is also important. By the use of nitrogen-depleted cells in crosses, it has been possible to obtain at will over 90 per cent zygote formation within a short time after mixing the mating type clones.

The ability to obtain zygotes from dark-grown cultures, mixed in the dark, has made it possible to intercross various pigment mutants which are killed

TABLE II

Effect of Suspending Medium upon Zygote Formation

Suspending medium	Zygote formation (>50 per cent after 24 hrs. in light)
Sterile distilled water	+
Medium I	−
" II	−
" I minus nitrogen source	+
" II " " "	+
Components of the medium	
per cent	
0.03 $MgSO_4$	+
0.02 KH_2PO_4	+
0.004 $CaCl_2$	+
0.001 $FeCl_3$	+
0.05 Na citrate + trace metal mixture	+
0.03 NH_4NO_3 (in M/30 phosphate, pH 6.8)	−
M/30 phosphate buffer, pH 6.8	+
Other nitrogen sources	
per cent	
0.06 NH_4Cl (in M/30 phosphate, pH 6.8)	−
0.06 $NaNO_3$ " " " " " "	−
0.1 urea " " " " " "	−
0.1 glutamine " " " " " "	−
0.1 glutamate " " " " " "	±
0.1 glycine " " " " " "	+

in the light due to the presence of porphyrins; *i.e.*, intermediates blocked in chlorophyll synthesis, which act as photodynamic dyes.

The finding that gametic activity requires nitrogen depletion without a concomitant loss of available energy has made it possible to design media for production of gametes from various mutant strains which grow poorly. It has also led to the understanding of previous difficulties in eliciting zygote formation encountered by ourselves and others (Provasoli, Hutner, and Pintner, 1951). Since mating behavior varies so strikingly with nitrogen availability, differences in conditions of growth would be expected to alter the observed sexuality.

5. Reversibility of Gametogenesis.—In the experiments so far reported, it has been the practice to suspend the cells to be tested for zygote formation in distilled water. If a growth medium is used for suspension of cells to be crossed, no zygotes are formed. Both media I and II inhibit zygote formation, as shown in Table II.

To determine whether or not a specific component of the medium was responsible for this inhibition, cells were suspended in each of the components separately, and, as shown in Table II, only the NH_4NO_3 had this effect. Other compounds which will act as sole nitrogen sources for growth, NH_4Cl, $NaNO_3$, urea, and glutamine, all inhibited zygote formation. Glutamic acid, which

TABLE III

Reversibility of Gametogenesis

Treatment of cells	Effect on zygote formation (observed ½ hr. after mixing *plus* and *minus* cells in light)
	per cent
1. Mixed in distilled water	>90
2. Buffered NH_4NO_3 added to separate distilled water suspensions, then:	
plus and *minus* cells mixed after 5 min.	>90
" " " " " " 2 hrs.	50
" " " " " " 3 "	1–5
" " " " " " 4 "	<1
3. *Plus* and *minus* cells suspended separately in buffered NH_4NO_3 for 4 hrs., then washed and resuspended in distilled water, then:	
plus and *minus* cells mixed after 5 hrs. in light	>50
" " " " " " 24 " " dark	<1
" " " " " " 24 " " " + acetate	<1

can be used slightly for growth, showed some inhibitory action, and glycine, which is not utilized, had no effect on zygote formation at all. The concentration required to inhibit zygote formation is of the same order of magnitude as that required for growth.

The rate at which nitrogen compounds inhibit zygote formation was determined, using distilled water suspensions of actively gametic *plus* and *minus* cells. Buffered NH_4NO_3 was added to a portion of each suspension and aliquots of the two suspensions were mixed at intervals. As shown in Table III, the ability to form zygotes was lost within 3 to 4 hours.

The inhibition of zygote formation by nitrogen compounds is reversible. When cells which had been inhibited by NH_4NO_3 were washed, resuspended in distilled water in the light, and the mating types mixed, zygote formation again ensued after several hours. Light was required for this reversal; sus-

pensions kept in the dark with or without added acetate formed no zygotes at all.

6. Carbon and Nitrogen Contents of Vegetative and Gametic Cells.—An attempt was made to correlate the gametic state with an absolute nitrogen concentration per cell or a carbon-nitrogen ratio. Cells grown at different nitrogen levels and harvested at definite intervals with respect to gametic activity, were analyzed for total nitrogen and carbon content.

The results, summarized in Table IV, show that cells with the same light requirement for gametic activity, grown on different levels of available nitrogen, vary widely in nitrogen, carbon, and C/N ratio, correlating not

TABLE IV

Carbon and Nitrogen Content of Vegetative Cells and of Gametes

NH_4NO_3 content of growth medium	Age of culture	Light requirement for mating	C/cell (dry weight basis) mg. $\times 10^{-5}$	N/cell (dry weight basis) mg. $\times 10^{-6}$	C/N
per cent	*hrs.*	*hrs.*			
0.001	58	2	5.57	4.38	12.7
0.005	58	3	4.38	4.57	9.4
0.01	58	3	4.30	7.56	5.7
0.001	87	1	6.85	4.67	14.7
0.005	87	1	7.66	7.51	10.2
0.01	87	1	3.89	6.07	6.4
0.001	125	½	6.60	3.72	17.7
0.005	125	½	4.13	4.20	9.9
0.05	87	24	3.44	7.37	4.68
0.05	87 plus 24 hrs. in distilled water in light	1	2.91	6.25	4.66

with sexuality, but merely with the nitrogen concentration of the growth medium. It would seem then that the induction of sexuality is controlled not by the level of total nitrogen, but by the concentration of some specific nitrogen fraction or compound.

DISCUSSION

The key role of nitrogen depletion in gametogenesis has been demonstrated by two complementary findings: (1) the elimination of the light requirement for gamete formation by growth of cells on a low nitrogen medium, and (2) the reversible differentiation and dedifferentiation of cells between the gametic and vegetative states by the availability of a nitrogen source. Nitrogen depletion favors gametic differentiation; addition of a

nitrogen source favors the reverse. No other component of the medium has these effects.

What is the role of light in this process? Cells which are not sufficiently nitrogen-depleted may require many hours of exposure to light, while suspended in a medium devoid of a nitrogen source, before they differentiate into gametes. Similarly, gametes which have been dedifferentiated by exposure to a nitrogen source, require several hours in the light to reverse the process.

This reciprocal relation between light and nitrogen has led us to propose that light acts indirectly, by providing carbohydrates to tie up the excess nitrogen; in addition, photosynthetic carbohydrates may provide energy for the mating process. On this hypothesis, one would expect that the action spectrum of the light requirement for zygote formation would coincide with the absorption spectrum of chlorophyll. The reports of Smith (1951) that blue, green, orange, and red light are all effective in inducing copulation of dark-grown cells of *C. reinhardi*, and of Lewin (1952 *b*) that the action spectrum for copulation in *C. moewusi* resembles the absorption spectrum of chlorophyll, support this view.

In addition to the photosynthetic role of high intensity light, is there a possibility that low intensity illumination may also influence some specific step in mating? Moewus (1940) has reported that in *C. eugametos* sexuality is determined by particular carotenoid pigments, and that a short exposure to low intensity light is required for a photochemical step which controls copulation. The detailed observations by Smith (1951) and Wendlandt (1951) of mating behavior in *C. reinhardi* do not differ markedly from those reported here, for cultures grown on high nitrogen (medium I). Since such cultures exhibit a light requirement for zygote formation, Smith was led to suggest the existence in *C. reinhardi* of light-induced sexual substances which control the mating reaction. In the experiments reported here, no light was required for zygote formation when nitrogen-depleted cells were used. Consequently we have concluded that for motility and sexuality, no light-induced systems are required. In these experiments, the last traces of light were not excluded during transfer of cells, but no accelerating effects of short exposures to low intensity light were observed.

What evidence is there of the generality of a mechanism of nitrogen depletion in the induction of sexuality in other organisms? Klebs (1896) found that *C. media* requires both light and depletion of the medium for gametic differentiation. He did not study changes in the light requirement as a function of nutrient depletion. Although he observed the inhibition of gametogenesis by nitrate, he considered it merely illustrative of the gametogenetic effect of depletion of any component of the medium.

Since then many workers (Schreiber, 1925; Strehlow, 1929; Lerche, 1937; Maher, 1947; Bold, 1949; Smith, 1951; Lewin, 1952 *b*) have confirmed the

beneficial effects on gamete formation in various algae, of light and of nutrient depletion by ageing of cultures or by suspension of cells in distilled water in the light. Whether or not nitrogen depletion plays the same key role in gametogenesis in other algae as it does in *C. reinhardi* is not known but that it may seems a reasonable inference. However, the particular inability of some species to utilize and to store reduced carbon sources may determine the extent to which it is possible to dispense with the light requirement (Lewin, 1950).

It is particularly interesting to find that mild starvation is a common technique for inducing sexuality in the protozoa (*e.g.* Sonneborn, 1939). Elliott and Hayes (1953) in their studies of conjugating strains of *Tetrahymena*, for example, report that conjugation has never been observed in the presence of nutrients and that mating occurs only between starved cells. Conjugation can be initiated by washing the cells and mixing the two mating types in distilled water. The period between mixing and the appearance of the first conjugation pairs can be decreased by successive washing of the cells. These observations are so similar to ours that it would be very interesting to determine which component of the medium inhibits copulation in these organisms.

The inhibitory effect of a nitrogen source upon gametogenesis may be related to the well known inhibitory effect of nitrogenous fertilizers upon flowering in higher plants (Miller, 1931), in which there appears to be a connection between nitrogen availability and the control of flower bud differentiation. This effect has been difficult to analyze because of the inaccessibility of bud primordia to nutritional control. With the demonstration by Liverman (1952) that addition of carbohydrates can substitute for the high intensity light requirement of short day plants, it should be possible to test for an inhibitory effect of nitrogen compounds in this process.

In *Chlamydomonas*, zygote formation is not only the mechanism for sexual recombination; the zygote is also the only stage in the life cycle which is long surviving in a non-dividing state and resistant to an adverse environment. In this connection zygote formation may be compared with sporulation in bacteria. Hardwick and Foster (1952) have shown that in various aerobic spore formers, starvation by suspending cells in distilled water can induce sporulation, and that the process can be suppressed by glucose and the supression relieved by a nitrogen source.

If one considers that spore formation in bacteria and zygote formation in *Chlamydomonas* are devices for preservation of the organism under adverse nutritional conditions, then the two phenomena appear closely related from an evolutionary standpoint. To a bacterium nutritional difficulties would presumably be detected first as a shortage of carbohydrate, since this is the component required in largest amounts. To a photosynthetic organism, how-

ever, it is limitation of the nitrogen source to which it is most sensitive, since in nature, light is not limiting. Thus, the same sort of evolutionary development leading to selection of low carbohydrate as a triggering device for sporulation in bacteria may be involved in the development of nitrogen deficiency as a necessary prelude to zygote formation. The evolutionary significance of the dual role of the zygote as spore and as sexual stage is another question, but it is of some interest to note that the use of the fertilized egg as overwintering form is a widespread device throughout the plant and animal kingdoms.

SUMMARY

1. Cells of *Chlamydomonas reinhardi* grown in the light or dark on standard medium require an additional exposure to light in the absence of a nitrogen source, in order to become sexually active. As the culture ages, the light requirement decreases.

2. This light requirement is a function of nitrogen depletion, as shown by the observation that cells from cultures grown to maturity on a low nitrogen medium in the light or in the dark, have no additional light requirement for zygote formation. The withholding of no other component of the medium has this effect.

3. In cells requiring light for zygote formation, the light can be supplied before the mating types are mixed, indicating that light is required, not for mating *per se*, but for the conversion of vegetative cells to gametes.

4. Gametes can be dedifferentiated to the vegetative state by any nitrogen compound which the cells can use for growth; and by further exposure to light in the absence of a nitrogen source, these vegetative cells can again become gametic.

5. Cells grown at different nitrogen levels become gametic at widely different cell concentrations of nitrogen and carbon and C/N ratios.

6. It is postulated that the role of light in gametic differentiation is indirect, providing by photosynthesis, energy for the mating process and carbohydrates to tie up excess nitrogenous reserves; and that the concentration of some particular nitrogen fraction or compound determines whether or not gametic differentiation is initiated.

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