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SYNCHRONOUS CULTURES OF ALGAE[1,2]

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Introduction

It has long been known to algologists that some algae belonging to the classes Dinoflagellatae and Chlorophyceae show in their natural habitats a diurnal periodicity of cell division with the division taking place only during the night (38, 72, 168). Laboratory experiments in which cultures of some diatoms (27, 155) and unicellular green algae (1, 35) were subjected to diurnal alternations of light and dark showed that cellular division occurred rhythmically corresponding to the light and dark regime. In these laboratory experiments, however, except for *Scenedesmus* grown under alternating light-dark periods lasting 12 hr each[3] (35), cellular division occurred, contrary to the ecological observations mentioned above, sometime during the light period. Most of these experiments were performed from the viewpoint of the diurnal ecology of algae, and were not of the sophisticated, modern, synchronous culture experiments subsequently developed, i.e., the use of large populations of synchronized cells as an enormously amplified model of a single cell to study various cellular events occurring in the life cycles of algal cells.

It is only a little more than a decade ago that numerous papers reporting on synchronous cultures in the above-mentioned sense, using various microorganisms, appeared in various fields of cell research [cf. the monograph edited by Zeuthen (219)]. This review deals with the synchronous cultures of photoautotrophic algae which have been reported since 1953 when the first relevant study using *Chlorella* was published by a group of Japanese workers (203) [see a review by Hoogenhout (59)]. Since reviews on earlier works made by the Japanese workers using *C. ellipsoidea* have been made elsewhere (41, 200–202, 204), they will be dealt with as briefly as possible except when pertinent in making clear the differences in the methodology used or in the results obtained with other algae. For information on synchronous cultures

[1] The survey of literature pertaining to this review was concluded in July 1965.

[2] Abbreviations: NAD: nicotinamide-adenine dinucleotide; NADP: nicotinamide-adenine dinucleotide phosphate; PCA: perchloric acid; Pi: inorganic phosphate; poly-Pi: inorganic polyphosphate; SDN: sulfur-containing deoxyribopolynucleotide(s); SPN: sulfur-containing peptide-ribonucleotide complexes; TCA: trichloroacetic acid. For the algae which are repeatedly referred to in this review, the following abbreviations, preceding the species names, will be used: *C.* for *Chlorella*, *Chl.* for *Chlamydomonas*, *E.* for *Euglena*, *H.* for *Haematococcus*, *S.* for *Scenedesmus*, and *U.* for *Ulva*.

[3] The light-dark alternation of this kind will be expressed as $12:\overline{12}$.

of some algae under heterotrophic conditions, see the reviews by other authors (69, 135). Reference is also made to other articles on the rhythmicity of growth not accompanied by cell division (e.g., 140) or the phenomena called "phased cell division," in which only a certain small fraction of a cell population performs division in each light-dark cycle (16, 54, 69, 91, 199).

Methodology and Materials

The obvious requisite for a synchronously grown algal population to be used as a model of a single cell is that cells in the cultures must be made to keep as closely as possible their paces of growth and division. When individual cells have the property of dividing into different numbers of daughter cells, the cells must show as similar "division numbers" as possible. Such a requisite has not always been fulfilled because of the algal species used or the method of synchronization applied. The method most widely in use for synchronizing algal cultures is the so-called "programmed" light-dark regime (206), in which random cultures of algae are subjected to regular changes of light and dark periods, whose lengths are selectively chosen by trial and error beforehand. Representative data of synchronous cultures of various algae obtained by this method are given in Table I. In most of the cases listed in the Table, the synchrony was "complete"—a terminology proposed by German workers (100, 144)—which means that the division of all, or practically all, cells takes place during a certain period (usually the dark period) of one light-dark cycle. In the third column are given the division numbers, n and n', the former being the real number of daughter cells liberated from individual mother cells and the latter the average value obtained for the whole population. The bold-face n-values are those observed most frequently in respective experiments. As may be seen, the n- or n'-values are fixed at around two in some species (represented by *E. gracilis*), whereas the value fluctuated markedly in other algae. In some cases, especially in the two strains, 211–8b and 7-11-05, of *Chlorella*, different values were obtained even when the same strain was placed under the same light-dark regime. The wide range of fluctuation of the division number is a manifestation of heterogeneity of cells in the algal population used. Soeder (169) and Soeder & Ried (174) observed in "synchronized" populations of *Chlorella*, strain 211-8b, four groups of cells showing different n-values, 2, 4, 8, and 16, and it was noted that cellular division occurred faster in the cells with the higher n-values. On minor changes of conditions, the same strain synchronized by the programmed light-dark regime showed the phenomena called "group synchrony" and "partial synchrony" (93, 138, 143, 213). These phenomena have been interpreted by Senger (165) as being caused by the existence in cultures of at least two groups of cells with different n'-values. Lorenzen [(93, 99); see also (87)] maintained that the most satisfactory synchrony of their *Chlorella* could be realized when the life cycle was made as short as possible by using adequate temperatures and light intensities. This

TABLE I

REPRESENTATIVE DATA OF SYNCHRONIZED CULTURES OF ALGAE EFFECTED BY THE METHOD OF PROGRAMMED LIGHT-DARK REGIMES

Algae	Culture Conditions[a]	Division No.[d]	References
Euglena gracilis	13.5:$\overline{13.5}$ (25°, ——)	n': 1.9 to 2.1	136, 137
(strain Z) *gracilis*, var.	14:$\overline{10}$ (25°, 3.5 klux)	n': 1.9 to 2.6	32
bacillaris	16:$\overline{8}$ (20°, ——)	$n=2$	19, 20, 22, 135
Chlorella	16:$\overline{12}$ (30°, 9 klux)	n': various values	36, 86, 95, 98,
pyrenoidosa (strain 211–8b)		between 8 and 22	100, 158, 165
		$n=8, 16, 32$	165
	16:$\overline{8}$ (25° to 28°, 8 klux)	$n=2, 4, 8, 16$	174
	14:$\overline{10}$ (30°, 9 to 10 klux)	n':various values between 12 and 17	86, 87, 98, 99
high-temperature	9:$\overline{9}$ (38.5°, ——)	$n=4, 8, 16, 32$	191
strain (7–11–05)	9:$\overline{15}$ (39°, 4 klux)	n': 40 ?	184, 188
		$n=$mostly 16	176
		$n=32, 64,$ or more	183
Scenedesmus			
obliquus	15:$\overline{9}$ (25°, 10 klux)	n':6.5	107
	14:$\overline{10}$ (25°, 10 klux)	$n=45$	120, 121
quadricauda	16:$\overline{8}$ (30°, 8 klux)	n':3	73
		$n=2, 4, 8$	73
Ankistrodesmus			
braunii	16:$\overline{8}$ (20°, 3 klux)	$n=4$?	213
Haematococcus			
pluvialis	24:$\overline{24}$ (25°, 8 klux)	$n=2, 4, 8$	73
Chlamydomonas			
moewusii	12:$\overline{12}$ (25°, 800 f.c.)	$n=8$	11, 12, 13
eugametos	15–18:$\overline{12}$ (25°, 1400 f.c.)	$n=8$[b]	217
Spirogyra			
majuscula	14:$\overline{10}$ (——)	$n=2$	148
Ulva mutabilis[c]	17:$\overline{7}$ (19° to 21°, 8 to 12 klux)	$n=2$	101
Porphyridium			
aerugineum	24:$\overline{24}$ (18°, 4.5 klux)	$n=4$	58

[a] Dashes mean "no descriptions."

[b] But n was 2, 4, or 8 when the light period was shorter than 12 hr.

[c] Sporophytes of a mutant strain "slender."

[d] See text for explanation.

was not true with populations of *C. ellipsoidea* that had been made homogeneous before starting the synchronous culture (see later).

In contrast, the populations of *Euglena* used by several workers seem to have been far more homogeneous. Recently, Petropulos (136) devised an automatic sampling apparatus for synchronizing *Euglena* by the method of programmed light-dark regime. Some species of algae are known to have a capacity for repeating, under continuous illumination, a certain number of apparently synchronous cell cycles subsequent to repeated light-dark regimes [*U. mutabilis* (101); *C. vulgaris* (105); the high-temperature *Chlorella* (5, 25, 57, 70); *Chl. moewusii* (13); *Ankistrodesmus falcatus* (140)]. In such a process of "after-oscillation," however, the synchrony deteriorates sooner or later. Attempts have been made to synchronize *Chlorella* by periodically regulating, under continuous illumination, the provision of CO_2 to the culture (166) or by periodically diluting the culture with new medium (158). These methods, however, gave only unsatisfactory results. In work by some German investigators (96, 138, 166), *Chlorella* was subjected to light-dark alternation under mixotrophic conditions by adding glucose to the medium with the intention of supplementing the photosynthate formed during the light period. Also under mixotrophic conditions, Pogo & Arce (147) obtained some synchrony of growth of *E. gracilis* by using diurnal temperature changes.

Using *C. ellipsoidea* as material, the Japanese workers (see 202, 206) compared various possible methods of synchronization, and obtained the most homogeneous synchrony by a method which they called the "method starting from a homogeneous population" (204, 206). In this method use is made, as the starting material for a culture, a homogeneous population of young and small cells—referred to as D-cells—which are separated, by differential centrifugation, from less homogeneous mixtures of D-cells. The characteristic of this method is that the light-dark alternation is not previously programmed. The alternation is made by observing, under a microscope, the appearance of cells which changes gradually with the progress of cell development. The mature cells—referred to as L_3-cells (see later)—which do not require light for further development, can be recognized by their characteristic appearance. (The Japanese workers routinely call this the "smiling face" of L_3-cells.) The light is turned off when the cells in the culture are all "smiling." The "smiling" cells, when kept in the dark, sooner or later enter into the "laughing" stage (L_4-stage, see later) which is soon followed by the process of cellular division. The switching from dark to light, which is performed after dilution of the culture to the original population density, is also made after all the "laughing" cells have completed their division into daughter cells. The culture of *C. ellipsoidea* synchronized in this way retains a high degree of homogeneity of population in repeated light-dark cycles, showing always a division number of almost exactly 4.0 (under the condition of light saturation).

Use of this culture technique has enabled the Japanese workers to dis-

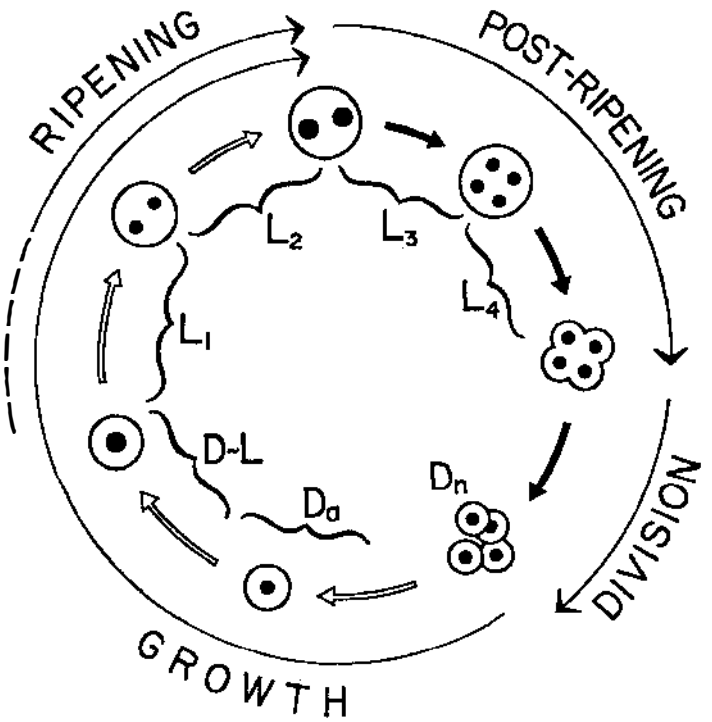

FIG. 1. Schematic representation of the change of cell status (cell size and nuclear pattern) at different stages and "phases" during the life cycle of *C. ellipsoidea* (201, 206). The white arrows indicate the light-dependent processes, whereas the black arrows show the transformations occurring in darkness. For further explanation, see text.

tinguish seven different stages in the cell cycle of *C. ellipsoidea*. For convenience of reference and comparison with results by other workers, the events occurring in the cell cycle of this alga are schematically illustrated in Fig. 1 (201, 206). The characteristics of the seven stages in question are as follows. D_n: the young cell newly produced in the dark from a mother cell; D_a: the photosynthetically most active cell derived from D_n upon illumination; D-L: the transient stage between D and L; L_1: the "unripened L-cell" which is large in size, but not yet ripe enough to undergo cellular division when incubated in the dark; L_2: the "half-ripened L-cell" which can only partially divide when kept in the dark; L_3: the ripened L-cell which can divide completely when incubated in the dark; and L_4: the stage which immediately precedes the liberation of daughter cells. The "growth phase" is the phase requiring illumination, which starts from the D_n-stage and extends to the L_3-stage. The "ripening phase" which requires light seems to start from some-

where around the D-L-stage and ends at the L_3-stage. The "post-ripening phase," which does not require light, begins at the L_3-stage and continues until the stage of L_4-cell which soon undergoes division.

Use of homogeneous populations of D-cells—separated either by differential centrifugation (163, 211) or by differential sedimentation (189, 190)—as starting material for the synchronous culture of *Chlorella* was recently adopted by other workers. Using the technique of fractional centrifugation, respectively, Sorokin (181, 183) obtained two fractions consisting mostly of D- and L-cells from nonsynchronous populations of the high temperature *Chlorella*.

The algae most frequently used as material for synchronous cultures are unicellular forms which proliferate asexually and show no complicated differentiation. Some species, such as *Chlamydomonas*, which have both sexual and asexual means of proliferation, have also been used as material, but the processes investigated were, in most cases, those of vegetative proliferation. Kates & Jones (80), using *Chl. reinhardtii* and *Chl. moewusii*, which have been known to be induced to gametogenesis by removal of the nitrogen source from the medium (159), compared the process of synchronized vegetative development (with a division number of two) with that of gametogenetic development (division number of four). Vegetative growth of some multicellular algae [(101, 148); (see Table I)] has also been studied using the technique of synchronous culture. Lövlie (101) compared two related strains of *U. mutabilis* Föyn, a wild type, and a special mutant derived therefrom. Whereas the wild type could not be grown satisfactorily synchronously because it displayed a rather complicated differentiation pattern, the sporophytes of the mutant showed little differentiation and grew with a fairly good synchrony. On the basis of his study of these two related forms, Lövlie discussed the genetic control of cell division and morphogenesis in this higher form of marine alga. Another organism, which was recently used as material in a synchronous culture, is the nitrogen-fixing blue-green alga, *Chlorogloea fritschii*. With this alga, Fay, Kumar & Fogg (33) obtained—by subjecting the cultures to different light and temperature conditions—populations rich in young and small cells (Stage I) or rich in larger matured cells (Stage IV), and they followed the changes of cell characteristics which occurred during the transformations from Stage I to Stage IV and vice versa.

What are the Causes of Synchronization?

It has been observed with *C. ellipsoidea* that, at the stage around L_3, the light-independent process leading to cellular division is competitively—although only temporarily—suppressed by the process of photosynthetic growth (60, 64). Similar phenomena were also observed by other workers and were called the "photoinhibition" of mitosis (91) or of cellular division (84, 178, 184). Use of such terms should, however, be made with due reserve, since it has been shown by Tamiya et al. (206) that, at least with *C. ellipsoidea*, it is not the process of cellular division per se that is suppressed by

light. That the growth (or protein synthesis) in microorganisms, in general, temporarily slows down or even stops during the process of cellular division has been universally observed [see, e.g. (14, 33)]. Hase, Mihara & Tamiya (47, 48) [see also (131)] showed that the competition mentioned above occurs between the light-independent formation of some substance(s) necessary for division (see later) and protein synthesis which is largely a light-dependent process.

In different species of algae, the "photoinhibition" of cellular division is not equally strong. Seemingly exceptional cases are those of *C. pyrenoidosa*, Pringsheim strain 82 (190), and *Chl. moewusii* (13) whose division has been reported to be uninfluenced by light. Very strong photoinhibition was observed in various species of Euglenineae (91), *C. pyrenoidosa*, strain 211-8b (145), and the high-temperature *Chlorella* (84). In these two strains of *Chlorella*, the longer the light period applied, the more retarded was the occurrence of cellular division in darkness, and a larger number of D-cells was produced (145), which is not so in *C. ellipsoidea* (206). No doubt, the strong "photoinhibition" of cellular division was the main factor causing the synchrony of growth and division in these species [cf. Cook (20)].

With a certain alga, on the other hand, observations indicate that the darkness exerts a suppressing effect upon the process of cellular division. This was reported by Lövlie (101) for the mutant of *U. mutabilis* mentioned earlier in which the division was retarded when the cells were exposed to a temporary dark period during an earlier light phase of the cell cycle. According to Lövlie, this is the reason division of *Ulva* cells could be synchronized by the programmed light-dark regime. Such an effect of darkness, however, has not been observed with other algae thus far investigated.

As far as *C. ellipsoidea* investigated by the Japanese workers is concerned, the photoeffect discussed above cannot be regarded as an important factor in causing the division synchrony. Studies performed by Morimura (116) (see later) on the effect of temperature and light intensity upon the cell cycle of *C. ellipsoidea* have led the Japanese workers to the concept that there must be a certain division-inducing factor(s) which induces cellular division largely independent of the nuclear and cellular substances that have accumulated during the light period. Experimental data show that the accumulation (or action) of this factor which provokes the division is markedly temperature-dependent, but light-independent.

As a counterpart of the division-inducing factor assumed by the Japanese workers, a pace-maker (*Zeitgeber*) of cellular division was postulated by the German authors for *C. pyrenoidosa* (93, 96, 100, 138, 143, 145, 165, 166, 174). The *Zeitgeber* is stated to be the time of beginning of the light period in their programmed light-dark regime. The action of this *Zeitgeber* is claimed to be a temperature-independent process. According to these authors, the life cycle of *Chlorella* involves two phases: (*a*) the phase of "preparation," in which the substances necessary for the formation of daughter cells are accumulated, the process being dependent on various conditions such as the length of the light

period, intensity of light, availability of CO_2, and temperature; and (*b*) the phase of "division" which takes place—independently of the conditions enumerated above—"automatically" at a definite time which is determined by the time of beginning of the light period. When, under unfavorable conditions, the accumulation of substances in the preparative phase is not sufficient for the formation of daughter cells, the cells cannot divide in response to the order given by the *Zeitgeber*. In such cases, the cells divide after being exposed to two or more cycles of light-dark alternation ("group synchrony"). Such a phenomenon has also been observed with some other algae (e.g., 53, 198) whose division is strongly sensitive to light.

Recently, a new statement concerning the *Zeitgeber* was made by the German authors (138, 145) who observed, on subjecting their *Chlorella*, strain 211-8b, and the high-temperature *Chlorella* to different light-dark regimes, that the incipient division occurred at definite times after the beginning of the dark period. It was stated that the beginning of the dark period acted as the *Zeitgeber* in these cases. Whether or not the action of this second *Zeitgeber* is also temperature-independent was not mentioned in their papers. If, in these algal species, the time of division is determined by the pace of artificially programmed light-dark regimes because of the strong photosensitivity of their process of division, it is understandable why it was concluded that the action of the *Zeitgeber(s)* in these algae is temperature-independent.

Morphological and Physiological Events Occurring in Normal Cell Cycles

Cell organelles.—Changes of nuclear pattern in *C. ellipsoidea* during its life cycle have been studied by Tamiya et al. (206) (see Fig. 1). Working with *C. pyrenoidosa*, Lorenzen (94) reported that the nuclei became temporarily unstainable with the Feulgen reagent towards the end of the growing phase. A similar phenomenon was later reported by Bernstein (13) for *Chl. moewusii*. In the same alga, the presence of extranuclear Feulgen-positive particles (chloroplast DNA?) was noticed. This observation has not been confirmed with certainty with other algae. The behavior of various cell organelles during the cell cycle of *Chlorella* was studied with the electron microscope by Murakami, Morimura & Takamiya (123) and Soeder (170, 172). The latter author (172) reported that, in *Chlorella*, 211-8b (which was called *C. fusca*, Shihira & Krauss in his paper), the first step of cytokinesis started along the axis, in which the nucleus, the dictyosomes, and the pyrenoid were marshalled in a row. Of the many pictures taken by Murakami and coworkers with *C. ellipsoidea*, most interesting are those of cells at the stages around L_2 and L_3. Before the occurrence of nuclear division, pairs of mitochondria and Golgi bodies were found which were symmetrically located on both sides of the nucleus. This strongly suggested that the division of both these organelles occurs autonomously (or self-dependently) preceding the nuclear division. The division of the chloroplast [for relevant literature, see (39)] also appears to occur prior to the nuclear and cytoplasmic divisions

(172). Electron microscope pictures indicating the autonomous division of mitochondria in *Micromonas squamata* (104) have been presented by Manton (103). Ris (156) reported on electron microscopic evidence suggesting the presence of DNA in the mitochondria of the same alga. Schmidt (163) and Murakami, Morimura & Takamiya (123) observed that, in *Chlorella* cells, the number of volutin granules increased with the development of cells. The behavior of pyrenoids during the division of chloroplast is not yet quite clear (169, 172, 190). Synchronous cultures of several species of Scenedesmaceae were recently run by Komárek (81). With *S. quadricauda*, for example, the morphology of coenobia, which has been adopted as an important generic characteristic in conventional taxonomy, was found to change markedly with the stages of the algal life cycle.

Photosynthetic activity.—It has been reported for *C. ellipsoidea* (127, 203) that, both under light-saturated and light-limited conditions, the D_n-cells showed relatively weak photosynthetic activity (rate of O_2-production per unit cell dry weight). The highest photosynthetic activity was found later at the D_a-stage, after which the activity decreased progressively until the stage of incipient cell division. Essentially the same phenomena were reported later by many workers using other strains of *Chlorella* [(29, 36, 93, 142, 176, 177, 183, 195); see, however, (95, 190, 194)]. According to Gerhardt (36), the activity of *Chlorella* cells in performing the Hill reaction approximately paralleled the photosynthetic activity at different developmental stages. That certain changes in the makeup of the photosynthetic apparatus during the life cycle of *Chlorella* occur is also indicated by the changes in the morphology of the chloroplast lamellae (123), in the mode of fluorescence of chloroplasts (28, 29) and its modification by the effects of some plasmolytica (30, 31), by changes in the Emerson "enhancement effect" and in the transient phenomena of O_2 evolution upon illumination (171), and perhaps also by the changes in electron spin resonance signals (209).[4] By measuring the light-limited rates of photosynthesis and the chlorophyll contents of *Chlorella* cells, Nihei and co-workers (127) inferred that the relative quantum efficiency of photosynthesis was two to three times higher in D-cells than in L-cells [see also (185)]. With the high temperature strain of *Chlorella*, Sorokin [(177, 180); see also (183)] observed that the photosynthetic activity of young cells gradually increased, while that of matured cells markedly decreased during the measurement. The same author (177) reported that, on measuring the photosynthetic activity of younger cells, the photosynthetic quotient O_2/CO_2 increased temporarily to the enormous value of ten. Earlier Nihei (125) and Nihei et al. (127) observed a temporary increase of the same ratio (up to 3.3) at a certain stage of L-cells of *C. ellipsoidea* (in the presence of phosphate in the medium). On the other hand, *C. pyrenoidosa* studied by

[4] The difference was observed with cells grown under light of varying intensity. Tamiya and co-workers (203) have demonstrated that the D-cells predominate in cultures grown under weak light and the L-cells in cultures incubated under strong light.

Gerhardt (36), showed a constant O_2/CO_2 ratio of about unity throughout the course of the cell cycle.

Respiratory activity.—The Japanese workers (127, 161, 203) have observed that, with *C. ellipsoidea*, the endogenous respiration (per dry weight of cells) was weakest in D_n- and D_a-cells, and highest in the cells at later L-stages [see also (173)]. The exogenous glucose respiration was highest at the D-stage and lowest at the stage around L_2 (161). Ried (151) and Ried, Müller & Soeder (152, 154) obtained essentially the same results with *C. pyrenoidosa*. In their sensitivities towards ultraviolet light (161) and various poisonous substances (152), the exogenous glucose respiration of D-cells resembles the endogenous respiration of L-cells. Sensitivities of endogenous respiration of D-cells toward several poisonous substances suggest that the process is linked to the phosphate metabolism of algal cells (152). Some irregular changes of respiratory activity, which were different from those observed by the authors mentioned above, were reported for the high temperature *Chlorella* (25, 188). The respiratory quotient of endogenous respiration was around unity throughout the life cycle of *C. ellipsoidea* (127), while, in *C. pyrenoidosa*, it increased from 1.2 to 1.3 to about two at later stages of the light period (154, 173). Kates & Jones (79) reported that the (endogenous) respiratory activity of *Chl. reinhardtii* showed, both in its vegetative and gametogenetic developments, a decrease followed by an increase during the growth phase. The vegetative daughter cells displayed twice as high a respiratory activity (per cell) as that of the gametes.

Assimilation (or absorption) of various exogenous substances.—Changes of percentage content of N, P, K, S, Mg, and Fe in synchronized *C. ellipsoidea* have been investigated by some Japanese workers (51, 75). For P, N, and S, similar measurements with results more or less different from those reported by the Japanese workers were made with the high temperature *Chlorella* (70, 163) and *S. obliquus* (120). Using a synchronized culture of a diatom, *Navicula pelliculosa*, Lewin (92) observed that the cells took up Si from the medium at a constant rate throughout the cell cycle.

Effects of light intensity and colored lights.—The effect of the intensity of light (provided either by incandescent or fluorescent lamps) on the cell cycle of *Chlorella* has been studied by several workers (116, 158, 186). Morimura (116) observed that, with *C. ellipsoidea*, the rate of growth of D-cells was higher at greater light intensities, but that, irrespective of the difference in the growth rate, the incipient cell division occurred simultaneously. Similar results (but with some irregular fluctuations) were later observed with the high temperature *Chlorella* in the temperature range of 20° to 40° (187). What was remarkable in Morimura's observations was the difference in the division number which was smaller at lower light intensities. The decrease of division number at lower intensities of available light was also observed for *Chl. moewusii* (13) and *C. pyrenoidosa*, strain 211-8b (158). An exceptional observation was reported by Sorokin & Krauss (187) for the high temperature *Chlorella*, in which the division number decreased (at 25°) or remained

almost constant (at 39°) with the increase in light intensity. With *C. pyrenoidosa*, Ruppel (158) noticed that the ratio of carbohydrate to protein decreased with a decrease in light intensity.

During the past ten years evidence has accrued indicating that light quality affects the distribution of absorbed carbon among various products of photosynthesis [see, e.g. (85, 124)]. A favorable effect of blue light, as compared with red light, upon incorporation of absorbed carbon (^{14}C) into amino acids has been demonstrated with asynchronous populations of *Chlorella* (17, 55). Comparison of the effects of colored light on the synchronous culture of *C. pyrenoidosa* was first performed by Kowallik (82) and Pirson & Kowallik (138, 141). They applied blue, red, and white lights in a regime of 16:$\overline{12}$ (at 30°), the light intensities being adjusted to give the same growth rates of algal cells. It was found that the ratio of carbohydrate to protein in algal cells was higher with red light and lower with blue light as compared with the value obtained with white light. In the red-light experiment, carbohydrate synthesis predominated at the beginning of the light period and was gradually followed by protein synthesis (82). A converse relation was observed in the blue-light experiment. Interesting observations were also made of the modes of formation of RNA and DNA. Whereas RNA synthesis occurred faster in blue light than in red light, DNA was formed in a higher amount in red light than in blue light. During the dark period following exposure to red light, the cells divided earlier with a higher division number ($n' = 11$ to 12) than the cells that had been exposed to blue light ($n' = 7$ to 8). The daughter cells produced in the red-light experiment were smaller than those produced in the blue-light experiment, although they contained the same amount of DNA per cell.

Rather perplexing results, however, were, obtained later by Kowallik (83) who continued cultures on a light-dark regime with colored lights for as long as ten months (about 250 cycles). The division numbers obtained in the red-light and white-light experiments were the same ($n' = 13$ to 14), and this remained unchanged throughout the whole course of the experiment. In the blue-light experiment, however, it was observed that, after six to ten months, the behavior of the cells was completely changed giving rise to smaller D-cells (with larger n'-values) than those of the red- or white-light-adapted cells. This change of character of blue-light-adapted cells could not be rectified by exposing the cells to 100 cycles of light-dark alternation with white light. Moreover, when the blue-light-adapted cell population was transferred to the light-dark regime with red light, more than 30 per cent of the cells were unable to perform cell division.

Effect of temperature.—The effect of temperature on synchronized algal cells was investigated first by Morimura (116) with *C. ellipsoidea* (temperatures tested: 9°, 16°, and 25°; light intensity: 10 klux). It was observed that, although the rates of growth and onset of cellular division were markedly temperature-dependent, the division number remained the same (4.0 to 4.1) at the three temperatures tested. A similar temperature-dependency of

growth and cellular division was later reported for the high temperature *Chlorella*, in which, however, the division number markedly increased with the increase in temperature (187). Kinetic analysis of Morimura's results showed that the processes of "ripening" and "postripening" (see Fig. 1) were—especially at lower temperatures—profoundly temperature-dependent [see also (178, 203)].

In the experiments of the German workers using *C. pyrenoidosa* subjected to programmed light-dark regimes, the temperature effect was investigated only in the narrow range between 20° and 30°. Their observations could not be made at higher or lower temperatures, since there occurred, at those temperatures, the phenomenon of "group" or "partial" synchrony [see (97, 165)].

Biochemical Events Occurring in Normal Cell Cycles

Nitrogen metabolism (amino acids, peptides, amines, and protein).—Changes in content of protein and related substances in synchronized algal cells have been studied by many workers using *Chlorella* (51, 60, 64, 115, 145, 158, 162, 163, 194), *Scenedesmus* (106, 107, 121, 122), and *Chlamydomonas* (79, 217). Using *C. ellipsoidea*, a most extensive investigation was made by Kanazawa (76) on the changes in content of free and bound forms of amino acids during the algal life cycle. The content of bulk protein (per unit dry weight of cells) remained relatively constant throughout the course of the life cycle, showing a decrease to some extent during the actively growing phase. An essentially similar course of change of protein content in *S. obliquus* was observed by Müller (121). According to Kanazawa, the amino acid composition of *Chlorella* protein remained relatively constant during the algal life cycle, and it was found to be almost identical with the composition of structural protein of isolated chloroplasts studied by Weber (215, 216) [see also (108)]. Analytical results, essentially similar to those of Kanazawa, were recently reported by Hare & Schmidt (40) for the high temperature *Chlorella*. [For the amino acid composition of *Chlorella* protein, see also (9).]

Among the free amino acids found in *C. ellipsoidea* (76), the most dominant were glutamic acid (plus glutamine) and alanine followed by proline and serine [see also (208)]. The levels (per unit dry weight of cells) of these amino acids changed in different ways during the algal cell cycle, but, by and large, they increased during the light period and decreased during the dark period. There is ample evidence now that, in green cells, protein synthesis occurring under photosynthetic conditions directly utilizes intermediate or early products of photosynthesis [see, e.g. (8, 9, 124, 218)]. A part of the amino acids formed as early products of photosynthesis is incorporated—either as such or after being transformed into other forms—also into peptides which accumulate abundantly during the light period and decrease abruptly during the dark period (76). The most dominant component in the peptide fraction of *Chlorella* was arginine followed by glutamic acid, glycine and cyst(e)ine. The peptides that accumulate during the light period seem to

have significance as reservoirs of building blocks for the synthesis of protein and other nitrogenous cellular substances in the dark. Recently, Kanazawa, Kanazawa & Morimura (77) isolated seven kinds of arginine-containing peptides from *Chlorella* cells, and determined their constituent amino acids. These peptides are either strongly basic or polar in nature and they are thought to play, besides serving as reservoirs of amino acids and amino groups, some important roles in the formative metabolism of algal cells, conceivably in connection with those of nucleic acids or nucleoproteides. In this connection, the biochemical roles played by some aliphatic amines (spermidine, putrescine, and ethanolamine), which were recently detected in *Chlorella* cells by Kanazawa, Yanagisawa & Tamiya (78), seem also to be of interest.

Ripened cells of *C. pyrenoidosa* (34, 82, 100, 121, 138, 145, 154, 158, 162) and *S. obliquus* (106, 121) have been reported to have a capacity for synthesizing protein in the dark using exogenously provided nitrate [see, however, Wetherell (217)]. Kanazawa (76) showed that, with *C. ellipsoidea*, the capacity for assimilating nitrate in the dark was lacking in D-cells but present in L_3-cells, although the rate of protein synthesis in the dark was only about one-fourth of that occurring during the light period ($D_n \rightarrow L_3$). It was also revealed that the nitrogen utilized in protein synthesis during the dark period was derived in approximately equal quantities from exogenous nitrate and from endogenous amino acids and peptides.

With synchronized *S. obliquus*, Meffert [(106), see also (107)] observed that the changes in quantity of raw protein and hot-TCA-soluble nitrogen were determined by the stages of cellular development, while that of cold-TCA-soluble nitrogen was determined, not by the developmental stages, but by the conditions of light and dark. This statement was confirmed to be partly true by Kanazawa (76). In their experiment using a N-fixing blue-green alga *Chlorogloea fritschii*, Fay, Kumar & Fogg (33) found that the N-fixing capacity was stronger at the stage corresponding to D_a of *Chlorella* than at the stage of cellular division.

Nucleic acids and nucleotides.—Changes in quantity and quality of nucleic acids in *Chlorella* cells during their life cycle have been investigated in detail by Iwamura and co-workers (60, 63–66). Whereas the formation of RNA proceeds almost in parallel with that of protein, the formation of DNA takes place only slowly during the growing phase and occurs abruptly at a considerable rate at the stages of ripening ($L_2 \rightarrow L_4$). Such a sudden increase of DNA occurring at a certain stage of ripening was later reported for *C. pyrenoidosa* (34, 82, 94, 100, 138, 141, 143, 144, 158, 193, 195, 214); the high temperature *Chlorella* (57); and *E. gracilis*, strain Z (32) [but not for another strain of *E. gracilis* (19) and *Chl. moewusii* (12)]. With partially synchronized cultures of *Chl. reinhardtii*, Sueoka (196) performed "transfer experiments" from a ^{15}N
ture" of DNA of that alga. With *C. ellipsoidea*, Iwamura (60) observed that the dark incubation of algal cells at later stages of development caused a

decrease of RNA to some extent [see also (193, 195)]. According to Wanka (214), the accumulation of RNA in *C. pyrenoidosa* temporarily ceases during the stage of active DNA formation. Existence of DNA in chloroplasts in *Chlorella* cells was first reported by Iwamura (61) and later confirmed by Hase, Miyachi & Mihara (49). [For the chloroplast DNA in other, asynchronously grown, algae see (15, 37, 157, 160).] According to Iwamura and co-workers, a part of the chloroplast DNA, which comprises about 10 per cent of the total DNA in *Chlorella* cells (67), shows a lower buoyant density and a higher adenine-thymine content compared with nuclear DNA (61), and has the property of performing partial turnover of phosphorus when the cells are illuminated (62). The phosphorous turnover in chloroplast DNA was found to occur more markedly in deoxyadenosine-monophosphate and thymidylic acid than in the corresponding cytidine- and guanosine-monophosphates, and to be accelerated appreciably by chloramphenicol (62). Recently, Richter & Senger (149, 150) isolated from synchronized cells of *C. pyrenoidosa* a DNA-RNA complex, whose RNA moiety was rapidly labeled when the cells were subjected to short pulses with ^{32}Pi. The complex is thought to be an intermediate in the transfer of information from DNA to RNA.

Various nucleotides and nucleosides contained in the acid-soluble fraction of *C. ellipsoidea*, as they changed in quantities during the algal life cycle, were investigated by Iwamura, Kanazawa & Kanazawa (65, 66) [see also (34, 158)]. The content per cell of total ribonucleotides increased roughly in parallel with the increase of total cell mass. The behavior of individual ribonucleotides varied greatly. The ratio ATP/ADP was higher in D-cells than in L-cells, a contradiction of the statement of Curnutt & Schmidt (26), according to whom the ratio was constant throughout the life cycle of the high temperature *Chlorella*. Concerning the behavior of deoxyribonucleotides in *C. ellipsoidea*, the following observations were made (66). The content of deoxyribonucleotides (in moles per unit dry weight of cells) was low in D-cells, increased only gradually with the growth of cells until they rose abruptly at the stage of active DNA-synthesis, after completion of which the level dropped very rapidly to that originally observed in the D-cells. Whereas DNA synthesis from deoxyribonucleotides is a light-independent process [(60); see also (193, 214)], the formation of deoxyribonucleotides seems to be essentially a light-dependent process (66).

Studies by Hase and co-workers [reviewed in (41, 200, 201, 204)] on the sulfur metabolism in *C. ellipsoidea* have led to the discovery of two interesting forms of S-containing nucleotide complexes which are thought to play important roles in the processes of ripening and division of algal cells. One is a sulfur-containing peptide-ribonucleotide complex(es) designated as SPN, and the other, a sulfur-containing deoxyribopolynucleotide(s) conveniently called the SDN-compound(s). For the chemical nature and behavior of these substances under various environmental conditions, see the reviews mentioned above. It may only be remarked here that SPN is a complex, most

probably acting as a pool or intermediate in the synthetic processes of nucleic acids, protein, etc. (48), while SDN was revealed to be a substance whose accumulation in algal cells is causally related to the occurrence of cellular division. In fact, ^{35}S-feeding experiments to S-starved *Chlorella* cells showed that the cells could perform division only when the quantity of SDN attained a certain level. A similar phenomenon was found later for another S-containing substance, CoA, by Otsuka (131). Under controlled ^{35}S-supply to S-starved cells, it was found that the accumulation of SDN and CoA—and, therefore, the occurrence of cellular division—was competitive with the process of protein synthesis.

A substance similar to or identical with SPN was later found in yeasts (42, 44), the high temperature *Chlorella* (70), *E. gracilis* (21), and *Chl. moewusii* (71). Recently, Virupaksha & Shrift (210) isolated from *C. vulgaris* five kinds of S-containing nucleoside-peptides, in which peptides, containing sulfur in the form of cyst(e)ine, or cyst(e)ine and methionine, are linked to uridine by ester linkages.

A substance apparently identical with SDN was later obtained from *Scenedesmus* (88, 89) and *E. gracilis* (21). Using ^{35}S as a tracer, Cook & Hess (21) observed that the increase of SDN in synchronized *Euglena* cells occurred simultaneously with the onset of prophase, and the final level of the compound attained paralleled the number of daughter cells released later. Vraná & Fencl (212) reported that a certain S-compound(s) contained in the cell extract from *C. pyrenoidosa* had the marked effect of accelerating the division of synchronized cells of the same alga. This substance is stated to be thermolabile and to be accumulated in algal cells in the later stages of the cell cycle. Whether or not this substance is identical with SDN is not clear.

The behaviors of the two coenzymes NAD and NADP in the life cycle of *C. ellipsoidea* were investigated by Oh-Hama, Morimura & Tamiya (129). On the per cell basis, the quantity of NADP, which is believed to be acting in the process of photosynthesis, remained constant during the stages of active photosynthetic growth and increased during the ripening stages. NAD was found to behave conversely, increasing during the growing phase and remaining almost at a constant level during the ripening phase.

Phosphorous metabolism (*polyphosphates*).—Inorganic polyphosphates (poly-Pi) in synchronized *Chlorella* cells were first investigated by Nihei (125, 126) followed by a number of Japanese and American workers. Miyachi and co-workers (112, 113) demonstrated the existence, in the cells of *C. ellipsoidea*, of at least four types of poly-Pi which they named poly-Pi's A, B, C, and D. Poly-Pi "A" (acid-soluble) is contained in volutin granules and is associated with RNA (49). It shows varying condensation numbers, mostly larger than 20 (2). By growing uniformly ^{32}P-labeled D-cells synchronously in a "cold" standard medium, Miyachi & Miyachi (114) followed the change in distribution of ^{32}P in different types of poly-Pi's as well as in some important P-containing cellular substances during the algal cell cycle. From the results obtained, they inferred that, in earlier stages of ripening, poly-Pi "A"

was formed from "C" (see below), and that, at later stages, "A" acted as a P-donor for the syntheses of substances such as DNA and phosphoprotein (113, 115). According to Baker & Schmidt (4), the level (percentage of total cellular phosphorus) of acid-soluble poly-Pi in the high temperature *Chlorella* remained almost constant throughout cell development.

"C" is a group of poly-Pi's which is extractable with a strong KOH-solution and coprecipitable with the potassium perchlorate that is formed upon neutralization of KOH with PCA. It is also thought to act as a P-donor for the syntheses of DNA, phosphoprotein, etc. What is remarkable with "C" is that its formation and mobilization for the syntheses of DNA and phosphoprotein are induced by light (74). "B" is a poly-Pi that is acid-insoluble but soluble in weak alkali. In its role, "B" resembles "D" which is extractable with strong alkali together with "C," but not coprecipitable with potassium perchlorate upon neutralization (112). Under normal photosynthetic conditions, both "B" and "D" function as P-reservoirs and are consumed only under P-deficiency in the formation of RNA and phospholipids (113). A similar role as a P-reservoir was attributed to the acid-insoluble poly-Pi in the high temperature *Chlorella* (6, 57). [See also (5, 7, 56).] According to Miyachi and co-workers (113), "B" acts as the P-donor for RNA synthesis, not in the light, but in darkness. The fate of acid-insoluble poly-Pi during the life cycle of the high temperature *Chlorella* has been investigated by a group of American workers (4, 57, 164). When normally grown algal cells were transferred—immediately prior to the onset of nuclear division—to a P-free medium, the acid-insoluble poly-Pi decreased, while nucleic acid-P, lipid-P, and acid-soluble phosphates increased (7). By supplying phosphate to P-starved cells of *C. pyrenoidosa*, Kuhl (86) investigated the process of accumulation of acid-insoluble poly-Pi in algal cells. The accumulation was considerably enhanced by illumination (to a larger extent in the presence than in the absence of CO_2) and it was suppressed by various inhibitors of photosynthesis but not by 2,4-dinitrophenol. On the other hand, the formation of the acid-insoluble poly-Pi in the dark was inhibited by both dinitrophenol and KCN, but not by the other inhibitors of photosynthesis tested. [For the poly-Pi's in asynchronously grown *S. quadricauda*, see (134).] Using a method different from those used by the Japanese workers, Correll & Tolbert (23, 24) separated, from *C. pyrenoidosa*, six forms of poly-Pi-RNA complex and followed the changes in their quantities and chemical properties during the algal cell cycle. As a whole, the quantity of the complex decreased during the stage prior to nuclear division. The processes of oxidative and photosynthetic phosphorylation in *Ankistrodesmus braunii* were investigated by Simonis (167) using algal cells taken from their synchronous cultures.

Carbohydrates and lipids.—Ruppel (158) reported that, in *C. pyrenoidosa*, the content (per unit dry weight of cells) of total carbohydrate increased during the light phase and decreased—with concomitant increase of protein—during the dark period [see also (100, 138, 145)]. With *C. ellipsoidea* Hase, Morimura & Tamiya (51) and Otsuka (130) observed that, during the

whole course of the algal life cycle, starch formed the major part of the carbohydrates, and sucrose was the main sugar extractable with hot 80 per cent ethanol. For the change of carbohydrate content during the life cycle of *S. obliquus*, see Müller (121). Change in the amount of a membrane substance, chitosan [see (128)], during the life cycle of *C. ellipsoidea*, was investigated by Mihara (111).

Ruppel (158) observed that, in *C. pyrenoidosa*, the percentage of lipid decreased during the light period and increased during the dark period [see, however, (51)]. Detailed analysis of the lipid fraction from *C. ellipsoidea* was performed by Otsuka (130). When compared in terms of moles of fatty acids per unit dry weight of cells, wax was dominant in the lipid fraction throughout the course of the algal cell cycle. Free fatty acids and fat were present in comparable amounts, their ratio being, however, somewhat higher in D_a-cells than in L-cells. It was also noted that the iodine value of the lipid fraction was higher in D_a-cells than in L-cells. Change in the phospholipid content in *Chlorella* cells during the life cycle was studied by Miyachi & Miyachi (114) and by Baker & Schmidt (4). According to the latter authors, the content of lipid-P (percentage of total cellular P) decreased during the earlier stages of cellular development and increased during nuclear division.

A detailed analysis of sterols in synchronized cells of *C. ellipsoidea* was performed by Otsuka (132). The percentage of ergosterol, which is the major component of sterols in *Chlorella* cells (68), was nearly constant throughout the algal life cycle. The level of Δ_5-3β-ol steroid decreased strikingly during the stage from L_2 to L_3, whereas the content of corticoid-type compounds increased during the transformation from D- to L_1-stage and decreased remarkably during the stages from L_1 to L_3.

Pigments.—In their first paper dealing with the synchronous culture of *C. ellipsoidea*, Tamiya et al. (203) reported that the percentage of chlorophyll was relatively low at the D_n-stage, became highest at the D_a-stage, and then decreased gradually with the progress of ripening. Sorokin (179) also reported that the chlorophyll content in the high temperature *Chlorella* decreased with the progress of ripening. For *C. pyrenoidosa*, strain 211-8b, there are different reports made by German workers: Lorenzen (93, 95) maintained that the percentage of chlorophyll remained constant throughout the course of the algal life cycle; Ruppel (158) stated that it decreased at the beginning of the light period and later showed a gradual increase; and, according to Gerhardt (36), the maximum content was observed at a certain stage of the light period. Both German and Japanese workers observed that, during the whole course of the algal life cycle, the ratio of chlorophylls *a* to *b* remained almost constant at around 3:1 (36, 133, 171). For the modes of synthesis of chlorophyll and carotinoid in synchronized *E. gracilis*, see Cook (19). Fay, Kumar & Fogg (33) observed that the amounts of phycocyanin and chlorophyll in *Chlorogloea fritschii* were highest and lowest, respectively, at the stages corresponding to the D_a- and L-stages of *C. ellipsoidea*.

Vitamins.—Using *C. ellipsoidea*, Morimura (117) investigated the

changes in level of 11 of vitamins during the algal life cycle. For the details of his experimental results, reference is made to a review article by Tamiya (202). The fate of ascorbic acid in synchronized *Chlorella* and that of thiamine in *Scenedesmus* and some other microalgae were investigated by Gerhardt (36) and Kafka (73), respectively.

Enzymes.—Using synchronous cultures of the high temperature *Chlorella*, Cole & Schmidt (18) observed that the activity (per cell) of aspartate transcarbamylase increased considerably during the growth phase. The rate of increase fell off at the stage of incipient cell division, which was ascribed to the effect of some heat-stable inhibitor formed at that stage. Kates & Jones (79) followed, in detail, the changes in specific activities of glutamate dehydrogenase, alanine dehydrogenase, and malate dehydrogenase during the vegetative and gametogenetic development of *Chl. reinhardtii*. Observations made by Oh-Hama, Morimura & Tamiya (129) for the coenzymes, NAD and NADP, and by Otsuka (131) for CoA have been described in earlier sections.

Modifications Caused by Various Unphysiological Factors

Ultraviolet light and strong visible light.—Sasa (161) observed that the inhibitory action of ultraviolet light upon various cell activities of *C. ellipsoidea* increased in this order: endogenous respiration of D-cells < exogenous glucose respiration of D-cells and endogenous respiration of L-cells < photosynthesis < overall cell proliferation. The same order of sensitivities was observed by Lee (90) on irradiating asynchronous cultures of *C. ellipsoidea* with the γ-ray from cobalt-60. In relation to the inhibition of overall cell proliferation, ultraviolet irradiation applied at the L_2-stage was considerably more potent than that given at the D-stage (161). By applying sublethal doses of ultraviolet light to synchronized *E. gracilis*, Petropulos (137) observed that chlorophyll formation was inhibited more strongly (but with a lower "target number") by the irradiation applied just before cell division than by that given during cellular division.

Chlorella cells at the stage around L_2 or L_3 are also sensitive to strong visible light. The strains 211-8b and 7-11-05 used by the German and American authors are apparently far more sensitive than *C. ellipsoidea* used by the Japanese workers. With their strain, Pirson (138) and Pirson & Ruppel (146) observed that when—towards the end of the light period—the cultures were diluted (e.g., 30-fold) with a new culture medium, some cells became unable to divide and eventually bleached (the so-called "dilution shock"). This is a drastic action of light which might be related to, but is evidently qualitatively different from, the photoeffect such as observed in normal synchronous cultures of *C. ellipsoidea*. The "dilution shock" of the German workers was observed with white or blue light, but not with red light (146). The strong photosensitivity of the strain used by the German workers is also manifest in the phenomenon termed by them as the "cold shock" (98, 144). When the cells at some stage of ripening (seemingly corresponding to L_2 or L_3) were

exposed, under aerobic conditions, to a temperature of 4° for 2 hr in the light (4 to 9 klux), cell division was halted and there occurred a considerable loss of chlorophyll and photosynthetic activity. Such an injurious effect of cold was not observed when the "shock" was given in darkness. That the harmful effect of strong visible light is much stronger at low temperature than at high temperature is a well-known fact (186, 203, 207).

Hypertonicity of media.—Ried (151) and Ried & Soeder (153) investigated the effect of high tonicity of the culture medium (27 times that of normal) upon synchronized *Chlorella* cells. Using the same alga, Döhler (28, 29) studied the effect of some plasmolytica upon the fluorescence of chlorophyll at different stages of the cell cycle.

Omission of essential nutrient elements from the medium.—The effect of deprivation of each major element from the normal nutrient medium was first studied by Hase, Morimura & Tamiya (51) with *C. ellipsoidea*. Except in the case of S-deficiency, the cells performed one round, but not more, of the cell cycle, showing more or less low growth rates and division numbers compared with the control. In the case of S-deficiency, the D-cells apparently grew normally up to the L_1-stage, but, at this stage, the development of the cells was completely arrested and no division occurred. For the many interesting observations made by Hase and co-workers (43, 45–48, 50, 52) on feeding the S-starved cells with ^{35}S-sulfate under various environmental conditions, see the reviews by Hase (41) and Tamiya (200, 201). Otsuka (130) found with *C. ellipsoidea* that in the S-deficiency [as also known to be true for the case of N-deficiency (192)], carbohydrates were converted into fatty acids and lipids [see also (102)], whose composition was different from that of normally grown cells, i.e., lacking the sulfolipid discovered by Benson, Daniel & Wiser (10) in normal photoautotrophic cells in general. The supply of the S-source alone to the S-starved cells was effective in restoring the lower level of carbohydrates, but not in decreasing the abnormally elevated level of lipids. For normalizing both the quantity and quality of lipids of S-starved cells, the supply of an N-source together with an S-source was required.

The effects of Mg- and N-deprivation were studied in detail by Galling (34) using synchronized *C. pyrenoidosa* [see also (3, 93, 139)]. The process which was most strongly suppressed by Mg-deficiency was RNA synthesis. Less suppressed were the syntheses of protein, chlorophyll, and DNA, and most insensitive was the synthesis of carbohydrates. A marked effect of Mg-deficiency was the accumulation in the cells of PCA-soluble N-compounds, in which were found various kinds of mononucleotides, some free bases (hypoxanthine, uracil, and orotic acid), and amino acids. The omission of N-sources from the medium naturally affected the syntheses of all kinds of nitrogenous substances and caused an accumulation of lipids (34). Synthesis of DNA was found to be more resistant to N-deficiency than the syntheses of protein and RNA. The recovery processes of Mg- and N-starved cells when provided with the respective deficient elements were different in that re-

covery from Mg-deficiency occurred only in the light, while recovery from N-deficiency took place independent of light.

The effect of P-deficiency on the life cycle of *C. pyrenoidosa* was studied by Schmidt (163). The growth of cells proceeded apparently normally up to the stage of the onset of nuclear division when growth practically halted with only a few cells undergoing nuclear or cellular division.

Effects of antimetabolites and other substances.—Using *C. ellipsoidea* as material, a group of Japanese workers (118, 205) performed extensive studies on the effects of various antimetabolites and other substances upon the algal life cycle. For the many observations made with more than 50 different substances, see the reviews by Tamiya (200–202). The effect of thiamine upon the synchronous cultures of *C. pyrenoidosa*, *S. quadricauda*, and *H. pluvialis* was investigated by Kafka (73), and Metzner & Senger (110) studied the effects of diphenylamine and streptomycin upon the mixotrophically synchronized cultures of *C. pyrenoidosa*. Senger (166) and Soeder, Ried & Strotmann (175) observed that, with *C. pyrenoidosa*, CO_2 supplied in excess inhibited the liberation of daughter cells from mother cells. A suppressing effect of CO_2 upon cell division of the high temperature *Chlorella* was also reported by Sorokin (182) who stated that the inhibitory action of excess CO_2 was counteracted by bicarbonate added to the medium [see, however, the criticism by Mer & Causton (109)]. Recently, Süss et al. (197), using synchronized *C. pyrenoidosa*, made the interesting observation that the algal cells could act as a host for the tobacco mosaic virus (TMV). On treating the culture with free TMV-RNA, the algal cells maintained their synchrony, and after two rounds of the cell cycle, showed new synthesis of TMV-RNA as demonstrated by the assay of infectivity of cell-extracts on *Nicotiana tabacum* var. Xanthi.

Concluding Remarks

Although various aspects of cellular events occurring in some primitive algae have hitherto been made clear by using the technique of synchronous culture, they are, no doubt, only an extremely small facet of all the events taking place in the life cycles of these algae. Especially scanty is our knowledge about the behavior of various cell organelles and enzymes and their control mechanisms which must be subject to changes in many different ways during the algal cell cycle. Studies with isolated enzymes and cell organelles require large quantities of cells which are difficult to obtain with the culture arrangements usually used for synchronization. To meet such a requirement, Morimura, Yanagi & Tamiya (119) recently constructed a large-scale culture chamber (40 liters in capacity) for synchronization of *Chlorella*.

The algae, either unicellular or multicellular, which perform sexual proliferation, have proved to be far more difficult to synchronize than those having only asexual means of proliferation. An auspicious beginning of synchronizing these algae has already been made by Kates & Jones (79) with *Chlamy-*

domonas and by Lövlie (101) with a mutant strain of *Ulva*. It is expected and hoped that the elaboration of the synchronizing techniques applied to these algae will open new avenues of approach to the elucidation of various biological phenomena of higher orders, such as genetic control of development, morphogenesis, differentiation, etc.

By way of conclusion, the writer wants to re-emphasize the importance of the effort of making the "synchronous" cultures as homogeneous as possible insofar as one intends to use a large cell population as a magnified replica of a single cell. "Completeness" of synchrony is by no means a decisive test for the homogeneity of the population, a fact which, unfortunately, seems to have not been duly realized by some workers. In working with algae whose populations are likely to become heterogeneous on being subjected to some "synchronizing" techniques, awareness of and efforts to improve, the heterogeneity are deemed to be highly relevant. If this point is ignored, it would be, as Schmidt (163) has stated, "probably impossible to obtain a quantitative and reproducible picture of the detailed and subtle biochemical and physical events" occurring in the life cycles of algal cells.

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