The discriminations involved in this test were quite difficult, according to the reports of the human subjects, and as shown by the fact that all subjects including the chimpanzee made a number of errors.

Product moment correlations were computed between the ranking of each subject and (i) the weight of the stimuli (ranked within each trial-array), and (ii) the ranks assigned by the other subjects. The chimpanzee's order of food selection correlated almost as highly with the actual size (weight) of the stimuli as did the humans' rank ordering ($r = .87$ for Alpha; .93 and .96 for the humans). Furthermore, Alpha's scores had high agreement with the humans'. Her scores agreed with the human scores as well ($r = .89$ and .86) as these agreed with each other ($r = .90$). Alpha's correlations with the judgments of humans were as high as the correlation between her own preferences and the weight of the food.

Clearly, then, chimpanzee selection of larger sizes of food-pieces in a direct choice situation is mediated by visual size perception. The mechanisms of selection appear to be similar to human perception of the same stimulus objects (*5*).

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8 January 1960

Synchronous Division in *Chlamydomonas moewusii*

Abstract. Mass cultures of *Chlamydomonas moewusii* have been synchronized by means of light-dark shifts. Division of at least 91 percent of the population was made to occur in 1/24 of the life cycle of the cells. The advantages of working with synchronized cultures of obligate autotrophs are discussed.

A great deal of attention has been paid to the subject of synchronous division of cell populations in recent years (*1, 2*). The principal reason for this interest has been to provide the investigator with a large amount of cellular material with most, if not all, of that material existing in the same morphological and physiological condition. In addition, an often overlooked reason for producing such cultures is that the endeavor to induce synchrony often reveals fundamental information about cellular mechanisms (*3*). This paper is concerned primarily with the methods used to induce synchronized division in *Chlamydomonas* and with a brief discussion of their importance.

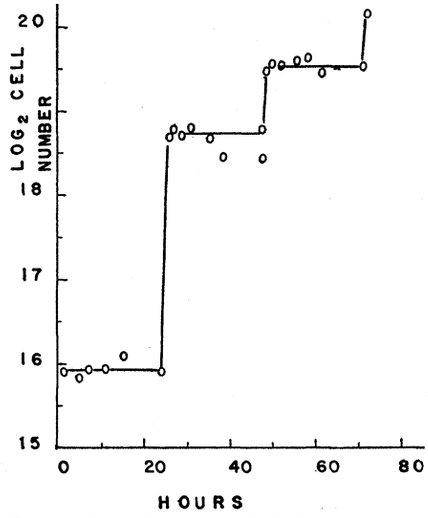

Fig. 1. Growth curve of *Chlamydomonas moewusii* showing synchronous division as a function of a light-dark cycle.

The minus strain of the heterothallic, obligately photosynthetic protist, *Chlamydomonas moewusii* (Burkholder and Provasoli strain) was used. The cells were cultured in 2 liters of liquid medium (*4*) buffered at *p*H 6.8 in 4-liter Pyrex bottles. The cultures were maintained at 25°C and were aerated continuously with a mixture of 95 percent air and 5 percent carbon dioxide. The cells were stirred gently at 10-minute intervals for 30 seconds with a magnetic stirrer. Illumination was provided by six 15-watt, cool white, fluorescent lamps. The light intensity was approximately 800 ft-ca incident on one surface of the growth vessel.

As a result of previous experiments concerned with the investigation of the behavior of the cells in constant light (*3, 5*), a regimen of a 12-hour light, 12-hour dark cycle was chosen in order to synchronize the growth and division of the culture. All the experiments reported here were performed with cultures inoculated with cells previously grown on the same light-dark cycle. However, other experiments have shown that this treatment of the stock culture is not a requirement for the induction of synchrony. The results of this choice are plotted in Fig. 1. All population curves were prepared by counting fixed cells directly in a hemocytometer. Below 250,000 cells/ml, the error of this counting method did not exceed 15 percent; the average error was 12 percent. Above 250,000 cells/ml, the error did not exceed 8 percent. When the population was determined in cultures containing fewer than 50,000 cells/ml, samples were concentrated by centrifugation before counts were made. No attempt was made to correlate hemocytometer counts with viable counts. However, the cytological observations, presented below, concerning the abundance of eight-celled mother cells would preclude the presence of large numbers of nonviable cells.

The curve indicates an eightfold increase in the population above the initial number of inoculated cells during the first cycle. The smaller increases (less than complete doublings) in cell number during the second and third cycles are due to the limiting conditions for population growth imposed upon the medium by the large number of cells produced during the first cycle. Optimum growth, as determined by eightfold increases of about 90 percent of the population during a single cycle, ceases after the population has reached a density of about 400,000 to 480,000 cells/ml, which represents an inoculum of about 50,000 to 60,000 cells/ml. Measurements made on cultures begun with very small inocula (10, 25, and 100 cells/ml) indicated eightfold increases occurred during each cycle until the limiting number was reached. Twenty experiments performed both with cultures begun with small and large inocula produced the same results reported here.

True synchronization of growth and division is believed to have occurred because of the following evidence. (i) Examination of the curve reveals no increase in cell number between 0 and 23 hours. The cells were exposed to light during the first 12 hours and to darkness the second 12 hours. The entire increase in population then, as revealed by the curve, occurred during the 23rd and 24th hour (that is, the 12th hour of darkness). In 2 experiments out of 12 in which the inoculum was large (about 50,000 cells/ml), an increase in cell number occurred until the 25th rather than the 24th hour. Thus, "cytokinesis" usually occupied only 1/24 of the entire life cycle. (ii) Examination of the cells throughout the growth cycle revealed a steady shift in cell types from the true flagellated, ellipsoid, vegetative cell, through the nonflagellated spherical cell, to the multinucleated sphere ("mother cell") which gave rise to the eight independent, flagellated, vegetative cells.

Analysis and evaluation of the data reveal both the highest percentage and the highest degree of synchrony (that is, the shortest amount of time during which division occurs) yet obtained for

any species of cell. In the first case, taking into account the percentage error of the method of counting, most probably no less than 91 percent of the population was found to divide synchronously. This figure is based upon a maximal eightfold predicted increase in cell number which was then verified by cytological examination. In the second case, the actual duration occupied by the production of new cells was only 1 hour out of 24 hours.

In similiar attempts to produce synchronously dividing cultures of *Chlorella pyrenoidosa* (*6*) and *C. eugametos* (*7*) by light-dark cycles, fractional centrifugation was found to be necessary to separate the newly released daughter cells from those mother cells which had not yet completed division. On the cycles used, all of the cells could not be kept in phase by varying the light-dark periods alone. Either these two species are more refractive to synchronization than *Chlamydomonas moewusii,* or the period of light to which the cells were exposed was too long. It appears to be important to provide no more light than is necessary for the cells to initiate mitosis, the latter being a light-dependent reaction in these species. Cytokinesis and release of the daughter cells are light-independent reactions. Provision of light beyond the minimum amount of time required for mitosis tends to initiate a second life cycle for a portion of the population, and thus encourages asynchrony.

Recently, Scherbaum reported a new parameter, the synchronization index (SI) for comparing the degree of synchrony in different systems (*8*). The originally derived equation was formulated for cells which divide once to form two cells. The system reported here for *Chlamydomonas* divides three times to produce eight cells. The equation has been modified, so that in

$$SI = n' X (1 - t/gt)$$

n' represents the percentage of cells undergoing synchronous division, t represents the time during which the new cells are being produced, and gt represents the calculated time required for a single cell to produce eight daughter cells during asynchronous exponential growth. In order to determine gt, the "true" generation time was measured first. The latter is an expression of the time taken for a population to double during the exponentional growth phase. It was determined by growing cultures in constant light under asynchronous conditions (*8*). The value was found to be 4 hours. Since a single cell actually gives rise to eight cells during exponential growth and not two, the "true" generation time is not an indication of what is occurring and thus cannot be used in the equation. Theoretically, three times the "true" generation time, or 12 hours, would be necessary for the production of eight cells in constant light. This value of 12 hours was corroborated experimentally. It presents the same type of information as that provided by the term *generation time*; that is, it reveals the amount of time necessary for an *average* cell to produce progeny under "constant" conditions. The synchronization index was 0.83. The range of synchronization indices as computed by Scherbaum (*9*) for all of those species for which data were available was between 0.26 and 0.69.

In conclusion, it has been shown that a single light-dark shift is sufficient to produce a highly synchronized culture of *C. moewusii,* perhaps the highest of any species to date. It would appear that this species, in spite of its "atypical" division pattern, would be an ideal one to study the mechanisms underlying artificially induced growth and division.

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21 January 1960

Subfractionation of Human Serum Enzymes

Abstract. Evidence is given for the subfractionation by starch gel electrophoresis of human serum cholinesterase, aromatic esterase, and acid phosphatase. These subfractions and unfractionated leucine aminopeptidase are localized with respect to the protein zones of the Smithies starch gel pattern.

The remarkable heterogeneity of serum proteins, including enzymes, is being increasingly evidenced today. Proteins, including many that have hitherto been considered homogeneous, are being separated into distinct subfractions by newer and more powerful methods, of which starch gel electrophoresis, introduced by Smithies in 1955, is a leading example. The present work has employed this method to contribute evidence for the subfractionation of several serum enzymes that have been long studied for their biochemical and clinical importance.

The procedure is essentially that given in Smithies' first detailed paper (*1*). Soluble starch (15 gm) (*2*), suspended in 0.03*M*, *p*H 9.00 ± 0.03, borate buffer (100 ml), is heated with swirling to sudden formation of a homogeneous, viscous solution, which is quickly evacuated to remove air bubbles, and poured into plastic trays (2.0 by 25 by 0.65 cm) to cool overnight (final gel, *p*H 8.5 ± 0.05). A small paper rectangle, saturated with the serum sample, is inserted into a vertical slit in the gel and sealed over with a glass cover slip. A coat of waterproofing plastic is applied over the entire exposed gel surface from a spray can. A paper wick (six thicknesses of blotting paper) connects each end of the gel with its corresponding electrode chamber, filled with the same buffer. Electrophoretic conditions are room temperature, constant current 2 ma/cm of gel width, initial gradient 6 volt/cm for a 6-hour run, correspondingly less voltage and current for an overnight 18-hour run. Each gel is cut horizontally into two slices approximately 3 mm thick, each of which may be tested differently. Subsequent cross comparisons are facilitated by a toothed pattern cut through one side of the gel before slicing [after Grouchy (*3*) and Moretti *et al.* (*4*)].

Histochemical methods employed are: (i) protein (blue-black zones), aniline blue black (*1*); (ii) cholinesterase (purple zones), beta-carbonaphthoxycholine iodide (or its 2-bromo-6-naphthyl derivative), *p*H 7.4 Veronal buffer, calcium ions, naphthanil diazo blue B (tetrazotized *o*-dianisidine) as coupling agent (*5*); (iii) aromatic esterase, alpha-naphthyl acetate (purple-gray zones) or beta-naphthyl acetate (red-purple zones), *p*H 7.0 phosphate buffer, diazo blue B as coupling agent (*6*); (iv) acid phosphatase (purple-red zones), naphthyl AS phosphate, *p*H 5.4 acetate buffer, manganese ions, diazotized *o*-aminoazotoluene as coupling agent (*7*); (v) leucine aminopeptidase (orange-red zone), *l*-leucyl-beta-naphthylamide • HCl, *p*H 7.1 tris(hydroxymethyl)aminomethane buffer, diazotized *o*-aminoazotoluene as coupling agent (*8*). Where applicable, the substrate solution is made $10^{-4}M$ in eserine (cholinesterase inhibitor). In most cases incubation for 20 minutes at room temperature is adequate to develop zones. (Control incubations ruled out the possibility of interference by native serum phenols.) After the excess