SYNCHRONIZATION OF CELL DIVISION

ALLAN CAMPBELL

Department of Bacteriology, University of Michigan, Ann Arbor, Michigan

A culture of unicellular organisms growing at maximal rate under constant conditions contains cells at all stages of the division cycle. The relative frequencies of cells of different ages—*i.e.*, the *age structure* of the population—is calculable if one knows the distribution of individual doubling times. The calculation assumes only that the age structure remains constant from one moment to the next. This review is concerned with reports of situations in which the age structure deviates from the calculable one, and varies with time in a somewhat periodic manner.

DEFINITIONS

Balanced growth. The concept of unbalanced growth has been used by Cohen and Barner (7), to describe situations in which the normal ratio of synthesis of nuclear to cytoplasmic components is disturbed. For our purpose, it will be convenient to say that growth is *balanced* over a time interval if, during that interval, every extensive property of the growing system increases by the same factor.

Consider a population which has been growing under constant conditions for a very long time. This is best achieved in a continuous culture device (29), but is probably approximated by a batch culture which has been in the logarithmic phase for several generations. It is logically necessary that, so long as the constant conditions prevail, growth will be balanced over any time interval, either finite or differential.

Now consider one cell within the population. As time proceeds, it will give rise to a clone of descendants. Over any small interval of time, the growth of the cell will, in general, not be balanced. It might, for example, synthesize all of its nucleic acid at one stage of the division cycle, and its protein uniformly throughout the cycle. If one assumes that the doubling time is precise [*clock mode* of growth (14)], the growth of the clone will be balanced only over intervals which are integral multiples of the doubling time. The growth of the population which is the aggregate of all these clones is balanced over arbitrary time intervals, because events in different clones are not in phase with each other.

When conditions are not constant, as when the stationary phase is approached, growth need not be balanced, but there is no logical reason it could not be. Neither need it be balanced if some one extensive property is increasing exponentially. The concepts of logarithmic growth and balanced growth are thus independent.

Synchronization. We will not attempt so precise a definition of *synchronization* or *degree of synchronization*. The limits of their utility will become clearer as the data are discussed. Roughly, *synchronization* denotes any process in which the events in different cell lines are put into phase with each other. A *completely* synchronized culture would be one in which, at any given moment, every cell was doing exactly the same thing.

Real systems lie somewhere between this ideal and the completely random situation of a culture in balanced logarithmic growth. It is desirable to have a quantitative expression for the extent to which this ideal is approached. The term *degree of synchronization* has sometimes been used for this purpose.

The usual procedure is to pick some stage of the division cycle which ordinarily lasts for only a small fraction of the doubling time and to measure the percentage of cells which are in that phase. Thus, yeast might be said to be "60 per cent synchronized" if 60 per cent of the cells are budding at once (2); and *Tetrahymena* could be considered 85 per cent synchronized because 85 per cent of the cells are dividing at one time (46).

Unfortunately, the term is ambiguous. If we chose to consider, instead of the number of cells undergoing division, the number at, for instance, anaphase, we would record a smaller degree of synchronization.

This ambiguity does not alleviate the need for some such expression, however. The fact that 85 per cent of the cells are dividing at one time places them in a group which are rather close to being completely in phase with one another. The

other 15 per cent might be dividing at random, for all we know.

The greatest hazard lies in the fact that an excess of cells at a particular stage may indicate either synchronization or a prolongation of that stage. For example, it is well-known that colchicine will arrest the growth of mammalian cells at metaphase. One would not call such a colchicine-treated population *synchronized* unless one could find conditions under which, upon removal of the colchicine, the arrested cells recovered uniformly and underwent subsequent division cycles in phase with each other.

METHODS OF SYNCHRONIZATION

Methods of synchronization can be classified into 2 categories:

(A) In *prior treatments*, a culture is treated in some manner and then allowed to grow unrestrictedly. The synchronized growth is observed over a period during which the external growth conditions remain constant. A prototype of the prior treatment is the development of sea urchin eggs after fertilization. If conditions are so arranged that the eggs are all fertilized within a period which is short compared to the doubling time, they will then all divide at close to the same moment, and may remain in phase for several cell generations (28).

(B) In *periodic treatments*, the culture is subjected to periodic changes in external conditions. The experiment is so arranged that the period of these changes equals the doubling time of the cell. The treatment is continued throughout the time that synchronized growth is observed.

A possible prototype of the periodic treatment is one which does not concern cell division at all, but rather a replication process comprising several divisions. The malaria parasite is known to enter the human blood cell and to multiply there in such a manner that one can observe a sequence of identifiable stages in the development of the infected cell. Finally the cell is destroyed, and the new parasites are liberated into the bloodstream.

One might expect that, in a patient who had had the disease for some time, events in different blood and tissue cells would be completely out of phase. Actually, they are out of phase in recent infections, but as time proceeds come into phase. Periodic changes in body temperature and other symptoms are correlated with similar periodic changes in the stages of the infected blood cells, which all liberate their parasites at about the same time (5).

There is some evidence that this phasing is due to the life cycle of the parasites' coming into phase with the normal diurnal cycle of the patient. The alternative would be a feedback mechanism in which the effects on the physiology of the host of particular stages of the parasite's life cycle affect the growth of the parasite at other stages of its cycle.

In principle at least, the cell division process might be induced to come into phase with an externally imposed periodic process in a similar manner. Cycles of chilling and warming (23, 37, 39), starvation and replenishment (6), and illumination and darkness (43) have been used with some success; but a real demonstration that the periodicity induces the synchronization has yet to be made.

EXPERIMENTAL SYSTEMS

Those microorganisms for which synchronization has been reported with at least a moderate amount of supporting data are listed in table 1. Early reports with only suggestive data have not been included. They are mentioned by DeLamater (9).

In the table, the term "growth temperature" is used for any temperature at which the organisms can grow indefinitely. "High" and "low" temperatures are outside the growth range of the organisms.

Those systems which have been studied most thoroughly will now be described in more detail.

(I) (4, 22–24). The generation time of *Salmonella typhimurium* is 45 to 50 min at 25 C and 18 to 20 min at 37 C. If a growing population at 25 C is placed at 37 C, there is a rapid increase in deoxyribonucleic acid (DNA) followed about 30 min later by a burst of cell division in which the viable count doubles. At 25 C 91 per cent of the cells appear binucleate in thionine-stained preparations. The percentage of quadrinucleate cells increases to a value of 50 per cent 25 min after the temperature shift; then the cells divide, and the fraction of binucleate cells increases to 91 per cent at 60 min.

Salmonella cultures can also be subjected to a periodic regime of 8 min at 37 C followed by 30 min at 25 C. After several such cycles, total DNA, turbidity, and viable count almost double during

TABLE 1

Microbial systems in which synchronization of cell division has been reported

Type of Treatment	Organism	Method of Treatment	Reference
Periodic	*Nitzschia palea*	Changing illumination	(43)
Periodic	*Salmonella typhimurium*	Shifts of growth temperature	(23)
Periodic	*Saccharomyces chevalieri*	Starvation for glucose	(6)
Periodic	*Tetrahymena pyriformis*	Shifts to high temperature	(46)
Prior	*Bacillus cereus*	Spore germination	(12)
Prior	*Bacillus megaterium*	Shifts from low temperature to growth temperature	(18)
Prior	*Diplococcus pneumoniae*	Single shift of growth temperature	(16)
Prior	*Escherichia coli*	Filtration or centrifugation	(26)
Prior	*Escherichia coli*	Multiple temperature shifts between growth temperatures	(37)
Prior	*Escherichia coli*	Starvation for thymine	(1)
Prior	*Salmonella typhimurium*	Single shift of growth temperature	(23)
Prior	*Saccharomyces cerevisiae*	Starvation for nitrogen	(2)
Prior	*Saccharomyces cerevisiae*	"Dissimilation"	(31)
Prior	*Saccharomyces chevalieri*	Starvation for glucose	(6)
Prior	*Tetrahymena pyriformis*	Aging	(3)
Prior	*Tetrahymena pyriformis*	Multiple temperature shifts between high and growth temperatures	(39)
Prior	*Chlorella ellipsoidea*	Starvation followed by exposure to high light intensities	(42)
Prior	*Nitzschia palea*	Shift of light intensity	(43)

each 37 C interval and remain fairly constant at 25 C. The culture does not remain well synchronized if, after several such cycles, it is allowed to grow unrestrictedly at 37 C.

(II) (38–40, 46). At 32 to 34 C ("sublethal temperatures") *Tetrahymena pyriformis* will increase in mass, but will not divide. Short exposures to this temperature are not lethal. Periodic treatments, in which time is allowed for 1 division to occur at 24 C between successive exposures to sublethal temperatures result in some synchronization.

However, the best synchronization was obtained with a prior treatment. The culture undergoes a series of alternating exposures to 33.9 and 28.5 C, the period at 28.5 C being sufficiently short so that the cell division mechanism is unable to recover from one temperature shock before the next one is applied. After several such shocks, the cells are placed at 28.5 C and allowed to grow unrestrictedly. At this point, they are 2 to 4 times their normal size. The next 2 or 3 divisions occur more rapidly than the ordinary maximal growth rate, and are fairly synchronous.

(III) (1, 7). The growth properties of a thymine requiring mutant of *Escherichia coli* have been intensively studied by Barner and Cohen. If thymine is the limiting nutrient in a growth medium, the cells, upon exhausting it, will cease to divide or to synthesize DNA, but will continue to make ribonucleic acid (RNA) and protein. If such unbalanced growth goes on for more than 30 min, death results. If, after 30 min of starvation, thymine is added back to the culture, the cells divide all at once. The second doubling follows almost immediately. During several subsequent generations, the viable count appears to double in periodic steps.

(IV) (11, 18). The generation time of *Bacillus megaterium* at 34 C is 34 min. If a growing culture is placed for 30 min at 12 or 15 C, the nuclear material contracts into a compact mass. If it is then returned to 34 C, relatively little cell division occurs during the first 40 min, and the nuclear material resumes more or less its former appearance. The viable count then doubles in about 15 min, during the latter stages of which contraction of the nuclear material is visible in some cells. Subsequent divisions are less well synchronized. Good synchronization occurs only if the period of exposure to 15 C is between 30 and 70 min.

(V) (6, 41). If either *Escherichia coli* or a respiratory deficient strain of *Saccharomyces chevalieri* is starved for glucose, the replenishment of the glucose leads to some synchronization. Measurements have been made on the periodic increase of several cell components of *Escherichia coli* after starvation. With *Saccharomyces*, besides simple starvation, a periodic process has been used. The culture is periodically diluted by a factor of 2 with fresh growth medium at a time interval (3 hr) twice as long as the normal doubling time. The numbers of nonbudding cells, budding cells, and cell pairs pass through successive maxima during the course of the cycle.

MECHANISM OF SYNCHRONIZATION

Periodic treatments. One can construct many simple models which predict that a periodic treatment should cause synchronization. They are all based on the notion that 2 periodic processes occurring within the same system should seek their own phase relationship.

A specific model is illustrated in figure 1. Suppose that, in the ordinary division cycle, the cell progresses through a sequence of stages A, B, etc. Now suppose that, under a different set of conditions, the cycle consists of the same sequence, but that the relative time spent between, say A and B rather than B and C, is different in the 2 cases. If we suddenly shift the growth conditions from those pictured in figure 1a to those of figure 1b, we expect the growth during the next cycle to be somewhat synchronized, because the majority of cells will be between A and B, which occupies a small portion of the cycle in 1b.
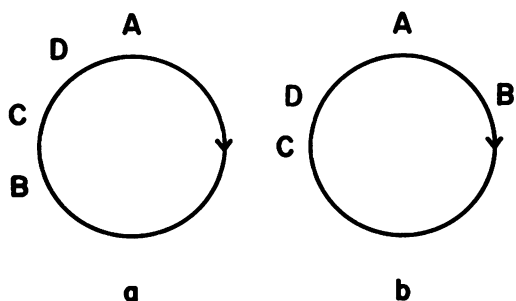

Figure 1. Possible model for synchronizations involving shifts of external conditions. It is assumed that under two different sets of conditions (a and b), the cell passes through the same sequence of stages during the normal doubling cycle, but that the relative amount of time spent at particular stages is different in the two cases.

Suppose we perform a series of shifts between the 2 conditions of such duration that 1 period of the regime corresponds to 1 doubling of the cells. The model as described predicts an approach to complete synchronization as time proceeds.

If synchronization were to occur in this manner, another prediction would be possible. A population which had been growing logarithmically under the conditions of 1a should, when placed suddenly under those of 1b, have a growth curve depending on the relative lengths of the different sections of the growth cycle under the 2 conditions. If the same experiment were done in the reverse direction, the growth curve would depend on the same relationship and should be calculable from the first one. In the case of *Salmonella*, such reverse shifts have been made (4) and the results are negative. Shifts from 25 to 37 C cause synchronization, whereas those from 37 to 25 C do not.

It is hardly surprising that this model, which was chosen on the basis of mathematical rather than physical simplicity, should fail its first serious test. All this means is that the state of the cell, for the purpose of such growth studies, cannot be completely specified by attaching a value to any one independent variable. The experimental result does bear further examination, however, since most alternative schemes for synchronization would lead one to predict an effect qualitatively in the same direction as that required by the model.

Prior treatments. The ideal prior treatment might be one in which the cells were brought completely into phase with no effect on their general physiology. Whether or not this ideal is approachable depends on the normal variation of division times among the cells.

With protozoa, this variation can be very small. Prescott (34) has collected several hundred dividing amoebae and cultured them together. After 24 hr, all the cells divide over a span of 1.4 hr, and 70 to 90 per cent over a 40 min period. This corresponds to a standard deviation of about 0.022 generations. The curves of Zeuthen on *Tetrahymena* cultures started from single cells likewise indicate a low figure. Exposure of the amoebae to strong visible light causes unequal division, which increases the variability of the division time, and gives negative sister-sister correlations (35).

The situation with bacteria has been sum-

marized by Powell (32, 33). For *Escherichia coli,*
Streptococcus faecalis, Aerobacter aerogenes, and
Proteus vulgaris, the standard deviation is about
0.3 generations, and for *Bacillus* sp. it can be as
high as 0.5. This agrees with the less extensive
observations of Kelly and Rahn (20). With such
high standard deviations, the distribution curves
are noticeably skewed and are best fitted by
functions other than the normal curve. Large
positive sister-sister correlations were observed
by Powell, but mother-daughter correlations were
not significant. He believes, however, that con-
siderable negative mother-daughter correlations
might be obscured for technical reasons.

Powell has presented a detailed analysis of the
relationship of the distribution of doubling times
to that of the stationary age distribution of a
growing population. An adequate treatment of
the general, nonstationary case has not yet been
made. We may view any prior treatment for syn-
chronization as being a matter of placing a pop-
ulation with an arbitrary initial age distribution
(in particular, one in which all cells have close to
to the same age) under a defined set of conditions.
These conditions then determine the probability
that a cell of a given age will divide in subsequent
time intervals. The age distribution must ulti-
mately approach the stationary one characteristic
of the imposed conditions. Its transient forms will
depend on the distribution of doubling times.

Figure 2 shows theoretical growth curves on
the following assumptions: (a) The cells have
divided completely in phase at time 0. (b) The
doubling times follow a normal distribution. (c)
There are no correlations within clones.

Negative mother-daughter correlations would
result in better synchronization than is shown by
the curves. In the absence of such correlations,
any prior treatment resulting in sharper curves
than are shown here must be assumed both to
have put the cells into phase and to have reduced
their variability in subsequent cycles.

The only organism for which we have some
idea of the standard deviation, σ, of the individ-
ual doubling times under conditions similar to
those in synchronized cultures is *Tetrahymena.*
Even allowing for the fact that synchronization
at the first division is imperfect, growth becomes
unsynchronized more rapidly than one would
predict simply on the basis of normal variation at
subsequent divisions.

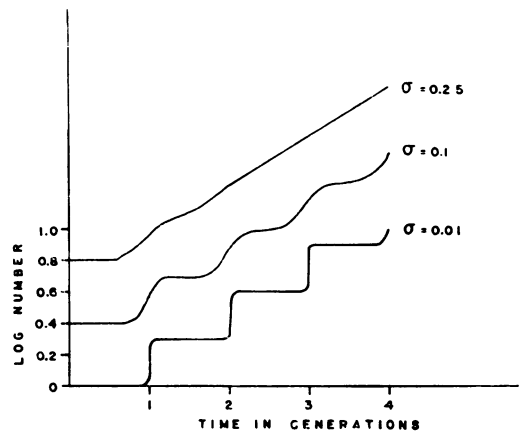A possible explanation is the fact that the

Figure 2. Theoretical synchronization curves.
Assumptions: All cells divide at time 0. Individual
doubling times normally distributed with standard
deviation σ. No correlations within clones.

synchronized divisions are occurring during a
period when the cells are recovering from stress.
Growth is unbalanced, the generation time is low,
and the population is heterogeneous with respect
to size. It is conceivable that severe randomizing
effects come into play as the recovery period ends.

Prescott's and Zeuthen's data on small num-
bers of cells may also be influenced by some un-
conscious selection. It may be that any large
population will contain some individuals which
are aberrant (either genetically or physiologically)
in appearance and/or generation time. In a small
number of clones such as Zeuthen examined,
these might have happened to be absent; or, in
both instances, such animals may simply not
have been chosen for the experiment. If no such
aberrancies occurred, one could predict from
Prescott's data that a culture started from a
single amoeba would remain detectably in syn-
chrony for over a hundred generations!

Data on mechanism of synchronization. With
Tetrahymena (41), exposure to sublethal tempera-
tures causes the inactivation of some unknown
process necessary to cell division, and a fixed
time at optimal temperature is required to repair
this damage. The damage cannot be a complete
block of DNA synthesis, since much DNA is
formed during the alternating temperature
period.

Falconi and Szybalski (11) have measured the
increase of nucleic acid, protein, turbidity, and
other growth properties in *Bacillus megaterium*

as a function of time at 15 C. Such cultures show good synchronization if returned to 34 C at around 50 min, and poorer synchronization after longer or shorter periods of chilling. The various growth properties increase at different rates; so that synchronization can be said to occur only when the cells are at "a particular stage of unbalanced growth."

Interference with normal DNA synthesis may be a critical feature of some synchronizations. This is suggested by (a) the ability to synchronize by thymine starvation, and (b) the abrupt rise in DNA content of *Salmonella* cultures following a shift from 25 to 37 C (22). In both cases, changes in DNA are the earliest detectable consequences of the treatment.

Besides filtration and spore germination, which require little comment, the main group of synchronizations not obviously related to the above types are those resulting from (a) approach to stationary phase in complex media, (b) starvations for glucose, (c) incubation of algae in darkness or in dim light. These may have a common mechanism of energy exhaustion. A possible clue to their mechanism is found in the reported changes of respiratory and photosynthetic quotients during the doubling cycles of *Escherichia coli* and *Chlorella ellipsoidea*, respectively (see next section). Exhaustion of substrate could make impossible the creation of a sufficiently reduced state of protoplasm to allow division to occur.

APPLICATIONS OF SYNCHRONIZATION

General considerations. The most usual purpose of measurements on synchronized cultures is to infer the order of events during the normal doubling cycle of a microorganism. Some of the limitations of this approach will be discussed first.

(A) In *periodic* systems, all changes in populational properties are ultimately compelled to assume the period imposed by the investigator. Thus, correlations of such properties with the time of cell division or with each other may be fortuitous. For example, the reviewer has attempted to study the reversion phenomenon in long term adapting yeasts using synchronized populations (Campbell, J. Bacteriol., in press). A sharp change in the percentage of galactose positive colonies is almost exactly coincident with a similar sharp rise in viable count. However,

further investigation showed that the change in populational composition is mostly, if not entirely, due to the process (glucose starvation) employed to achieve synchronization.

(B) With periodic treatments, the growth must ultimately be balanced over every increase of a factor of 2. With *prior* treatments, it need not be balanced at all. One example is *Tetrahymena*, where the average cell volume decreases 2 to 4 times during the first few hours of synchronous growth (39). Another is pneumococcus, where 2 periodic processes (cell division and transformability) become phased, but with different periods (15). Since the average normal doubling cycle of freely growing cells must be balanced, the chemical changes occurring within one cycle of such synchronous growth cannot be the same as those of the normal cycle.

It is also clear that changes during the first division or two after prior treatment may have no relevance to the normal cycle. Unfortunately, synchronization is generally best during this period. Barner and Cohen (1) have summarized the situation: "In all the reported methods for controlling cell division by means of temperature or chemicals, it must be recalled that the cell is subjected to stresses resulting in a situation of unbalance approaching pathology to a greater or lesser extent. Synchronization is initiated by a phase of recovery from the imposed stress and unbalance. The relation of such recovery phenomena to phenomena occurring in normal division and interphase can not yet be considered to be unequivocally clear."

Chemical studies. In some higher plants, DNA synthesis occurs exclusively during one stage of the division cycle (17). The analogous experiments with single bacterial cells would be technically more difficult. The results of work with synchronized cultures are summarized in the following paragraphs. They do not yet provide a definitive picture of the normal doubling cycle, largely because of the limitations just discussed. They do, however, provide the investigator of growth processes with a number of standardized systems in which the increases of particular cell components have been partially separated in time. These will undoubtedly find many uses.

(I) Lark and Maaløe (22) have measured DNA, RNA, turbidity, and cell counts as a function of time for both single and periodic temperature shifts with *Salmonella typhimurium*. Soon after

a single shift the DNA content rises abruptly. Following these increases, the curves continue fairly smoothly, with small wiggles suggesting some synchronization of a second increase. Throughout the period, RNA and turbidity increase smoothly and exponentially. In cultures which have been through several cycles of alternate growth at 25 and 37 C, DNA, turbidity, and colony count all virtually double during the short interval at 37 C. RNA increases both at 25 and at 37 C, somewhat more rapidly at 37 C.

(II) Barner and Cohen (1) have measured the DNA content of cultures of Escherichia coli recovering from thymine starvation under 2 conditions: (a) Thymine is added after 30 min of starvation. The amount of DNA increases in an odd stepwise curve. The period of the curve is about equal to the doubling time of the cells, but the DNA increases by a factor of about 3 during the first division cycle and about 1.5 during the second. The increase of DNA precedes cell division by about 15 min. (The normal doubling time is 53 min, but during the synchronized divisions it appears to be less.) (b) Thymine starvation and replenishment are carried out in synthetic medium supplemented with amino acids. Thymine is added after 20 min of starvation. Viable count follows a stepwise curve, but DNA begins immediately to increase smoothly and exponentially.

During the entire period of study, the DNA content per cell is abnormally high. Since the original population starved was growing exponentially, one might expect the amount of DNA per cell to be larger than in the original culture immediately preceding cell division, and smaller immediately after. In point of fact, it is larger throughout the time studied (2 to 3 divisions).

During starvation and replenishment, turbidity increases as in a growing, nonstarved culture. RNA follows a different curve, but shows no stepwise increases.

It seems probable that, as with Tetrahymena, the cells are larger than normal at the onset of synchronous division, and decrease in size during these divisions, which occur with a shorter period than the normal doubling time.

(III) Scott et al. (41) have studied RNA, DNA, and total cell count in B. megaterium recovering from chilling and in E. coli recovering from glucose starvation. DeLamater (9) has presented additional data from the B. megaterium system. The most striking feature is that in some experiments the DNA content rises (frequently doubles) very abruptly preceding cell division.

(IV) If Bacillus cereus spores are germinated and the young vegetative cells grown in heart infusion broth at densities high enough to retard normal growth, Giemsa staining shows a rather uniform change from uninucleate to binucleate condition occurring 90 to 120 min after germination. No stepwise increases are observed in DNA or RNA (12).

(V) Maruyama (25) has fractionated Escherichia coli cultures through filter paper piles (26). The cells fall into 2 groups located around 2 different peaks on such a pile. The behavior of the 2 fractions on subsequent growth in synthetic medium corresponds to that of 2 fractions obtained by centrifuging at specific gravity 1.245 and separating those cells at the top ("small cells") from those at the bottom ("large cells"). Both groups show stepwise increases in viable count. Biochemical studies were done on a culture from large cells, where the generation time is 50 min.

During a 40-min period when viable count is constant, there are increases first of RNA, then of protein, and last of DNA. Division then ensues, at which time DNA remains constant and RNA begins to increase. In the 20 min following division, all 3 components increase. The rate of oxygen uptake follows a smooth curve, but the respiratory quotient shows 2 peaks; one is immediately before cell division, when it attains the value of 1.6. It then drops below 1 during division. This should result in making the protoplasm more reduced immediately before division, which is the same effect observed with Chlorella (see VII).

The method has the advantage that the cells are subjected to no deliberate stress in the process of synchronization. Data on cycles beyond the first will be required before one is positive no effects of the fractionation procedure are modifying the normal course of synthesis.

(VI) Ogur et al. (31) have measured the amount of DNA in commercial yeast cake after placing it in growth medium. There is a period of about 2.4 hr during which the total number of cells plus buds observable by direct count doubles. The DNA content of the culture doubles during the same period and follows essentially the same curve. There is an indication that the DNA increase slightly precedes division.

(VII) *Chlorella ellipsoidea* divides by multiple fission. On reexposure to bright light after several days in dim light, the cell number remains constant for about 40 hr; then almost all cells divide between 40 and 60 hr, giving about 5 progeny per cell (19). Preceding division, DNA content rises sharply, and cell mass temporarily ceases to increase. There are marked changes both in photosynthetic activity and photosynthetic quotient. The latter results in measurable changes in the average oxidation state of the protoplasm during the cycle.

(VIII) Even in plasmodia which are quite large (25 to 50 mg dry weight), all of the nuclei of *Physarum polycephalum* divide in very close synchrony (8, 13). This must mean that the state of the common cytoplasm determines when the nuclei will divide; although one cannot tell what nucleo-cytoplasmic interactions may occur during the cycle.

The synchronization in this case is internal to the organism. The individual nuclei could be considered analogous to cells placed in a periodically changing environment, where feedback from their own activities might or might not be critical. On this analogy, the limitations of periodic treatments could be said to apply here, with the disadvantage that we do not know what the nature of the treatment is. However, these limitations matter only if one is concerned with synchronization first and with nuclear division second.

Incorporation studies with labeled orotic acid have shown that, during the 16 to 20 hr division cycle, RNA synthesis occurs throughout interphase, whereas all detectable DNA synthesis takes place during an interval 20 to 70 min after division (30).

Cytological studies. The results of cytological work on synchronized bacterial cultures have been critically reviewed and evaluated by Robinow (36). He has concluded that the observations of Lark *et al.* (23) and Hunter-Szybalska *et al.* (18) cannot be unequivocally interpreted, because of aggregation of nuclear material due to salt effects. This viewpoint stems from the finding of Whitfield and Murray (44) that the clumping of nuclear material when cells are chilled is very sensitive to the salinity of the medium.

Size distribution studies. Scherbaum (38) has analyzed the distribution of cell sizes in *Tetrahymena* populations subjected to and recovering from temperature shock. Apparently, during the series of shocks the cells which are initially small grow proportionally less than those which are initially large. The result is that the synchronized populations are actually more heterogeneous than ordinary logarithmic phase cultures.

Effect of division phase on other properties. Hotchkiss (15) has found that pneumococcal populations returned to normal temperature after chilling show periodic changes both in division rate and transformability. The viable count doubles (observed factor 1.9) during each period of the "division rate" curve, indicating synchronization. The transformability curve becomes out of phase with the division rate curve in later cycles, which makes it difficult to locate a particular "transformation sensitive" stage in the normal division cycle (14).

Romig *et al.* (37), using *Escherichia coli* synchronized by temperature cycling, report that the lethal effects of radiation are greatest immediately following cell division, but that the proportion of mutants among the survivors is highest immediately before division. Bruce and Maaløe (4) have uncovered some complications involved in interpreting such results. Ducoff (10) has shown that the division delay induced by sublethal X-ray doses is about the same for synchronized as for unsynchronized *Tetrahymena* populations.

The response of synchronized cells to bacteriophage infection has been investigated by two groups. Lark and Maaløe (22) have shown that the frequency of lysogenization of *Salmonella typhimurium* by a phage (A) doubles abruptly about 6 min after a temperature shift from 25 to 37 C and then slowly returns to its original value. This doubling occurs simultaneously with cytological changes which are interpreted as a doubling of the nuclear material (24).

Barner and Cohen have infected, with T2, *Escherichia coli* cells recovering from thymine starvation, at various times after thymine replenishment. They observed no significant change in adsorption, latent period, or burst size between such cultures. An input multiplicity of 0.25 was used, and about 15 per cent of the phage adsorbed. In another series of experiments, cultures were infected (presumably at high multiplicity) and DNA synthesis was followed. The rate of DNA synthesis by the infected cells is greater the later the infection is performed, and roughly

parallels the amount of bacterial DNA present at the time of infection.

Single cell studies. The above experiments must be compared with the direct measurements of small groups of cells which divide synchronously either because of (a) immediate common descent from a single cell or (b) deliberate selection and pooling of cells at a certain division stage.

In the case of protozoa, it appears that for both *Tetrahymena* and *Amoeba* the cell mass increases linearly rather than exponentially during most of the interphase (34, 45). When the mass has doubled, it remains constant for a period prior to division, and in the middle of division begins to increase at twice the original rate. Measurements of respiratory rate, total protein, and optical density all give similar curves.

All in all, the picture is very similar to that in synchronized cultures of *Chlorella* and of *Tetrahymena*. The latter case is complicated by the decrease in average cell volume during the synchronized divisions, so that the respiratory rate does not double when the cell number does.

CONCLUSIONS

From the above information one can construct the first dim outlines of what Mazia (27) calls "the life history of the cell." Most investigators agree that DNA is synthesized discontinuously rather than continuously, generally sometime during interphase. Before division there is a period which, in some cells at least, is characterized by a cessation of mass increase and by the protoplasm becoming more reduced. The mechanism of many known synchronizations can be explained (not necessarily correctly) in terms of these facts.

The advantage of constructing some working picture from the crude data presently available is that a model of the cell cycle which is even partially correct should lead to the rational development of better procedures for synchronization. The field should then enter an autocatalytic phase.

REFERENCES

1. Barner, H. D., and Cohen, S. S. 1956 Synchronization of division of a thymineless, mutant of *Escherichia coli*. J. Bacteriol., **72**, 115–123.
2. Beam, C. A., Mortimer, R. K., Wolfe, R. G., and Tobias, C. A. 1954 The relation of radio-resistance to budding in *Saccharomyces cerevisiae*. Arch. Biochem. Biophys., **49**, 110–122.
3. Browning, I., Brittain, Madeleine S., and Bergendahl, Janet E. 1952 Synchronous and rhythmic reproduction of protozoa following inoculation. Texas Repts. Biol. and Med., **10**, 794–802.
4. Bruce, V., and Maaløe, O. 1956 The ultraviolet inactivation of synchronously dividing *Salmonella typhimurium*. Post-irradiation effects on viability. Biochim. et Biophys. Acta, **21**, 227–233.
5. Brumpt, E. 1949 The human parasites of the genus *Plasmodium*. In *Malariology*, pp. 65–121. Edited by M. F. Boyd. W. B. Saunders Co., Philadelphia.
6. Campbell, A. 1956 Repeated synchronization of cell division in a long term adapting yeast. (Abstract.) Bacteriol. Proc., pp. 40–41.
7. Cohen, S. S., and Barner, H. D. 1954 Studies on unbalanced growth in *Escherichia coli*. Proc. Natl. Acad. Sci. U. S., **40**, 885–893.
8. Daniel, J. W., and Rusch, H. D. 1956 Growth of a plasmodial slime mold in pure culture on a soluble medium. (Abstract.) Federation Proc., **15**, 513–514.
9. DeLamater, E. D. 1956 Bacterial chromosomes and their mechanism of division. In *Bacterial anatomy*, pp. 215–260. Edited by E. T. C. Spooner and B. A. D. Stocker, 6th Symposium Soc. Gen. Microbiol. Cambridge University Press, Cambridge, England.
10. Ducoff, H. S. 1956 Radiation-induced fission block in synchronized cultures of *Tetrahymena pyriformis* W. Exptl. Cell Research, **11**, 218–220.
11. Falcone, G., and Szybalski, W. 1956 Biochemical studies on the induction of synchronized cell division. Exptl. Cell Research, **11**, 486–489.
12. Fitz-James, P. C. 1955 The phosphorus fractions of *Bacillus cereus* and *Bacillus megatherium*. Can. J. Microbiol., **1**, 525–548.
13. Güttes, E. Unpublished data. Referred to by J. Daniel and H. Rusch in a paper presented at a meeting of the American Society for Experimental Pathology (see reference 8).
14. Hoffman, J. 1953 *The size and growth of tissue cells.* Charles C. Thomas, Springfield, Ill.
15. Hotchkiss, R. D. 1955 Bacterial transformation. J. Cellular and Comp. Physiol., Suppl. 2, **45**, 1–22.
16. Hotchkiss, R. D. 1954 Cyclical behavior in pneumococcal growth and transformability

occasioned by environmental changes. Proc. Natl. Acad. Sci. U. S., **40**, 49–55.

17. HOWARD, ALMA, AND PELC, S. R. 1951 Nuclear incorporation of P³² as demonstrated by autoradiographs. Exptl. Cell Research, **2**, 178–187.

18. HUNTER-SZYBALSKA, M., SZYBALSKI, W., AND DELAMATER, E. 1956 Temperature synchronization of nuclear and cellular division of *Bacillus megatherium*. J. Bacteriol., **71**, 17–24.

19. IWAMURA, T. 1955 Change of nucleic acid content in *Chlorella* cells during the course of their life cycle. J. Biochem. (Tokyo), **42**, 575–589.

20. KELLY, C. D., AND RAHN, O. 1932 The growth rate of individual bacterial cells. J. Bacteriol., **23**, 147–153.

21. LAMANNA, C., AND MALETTE, M. F. 1953 *Basic bacteriology*, pp. 233–235. The Williams & Wilkins Co., Baltimore.

22. LARK, K. G., AND MAALØE, O. 1956 Nucleic acid synthesis and the division cycle of *Salmonella typhimurium*. Biochim. et Biophys. Acta, **21**, 448–457.

23. LARK, K. G., AND MAALØE, O. 1954 The induction of cellular and nuclear division in *Salmonella typhimurium* by means of temperature shifts. Biochim. et Biophys. Acta, **15**, 345–356.

24. LARK, K. G., MAALØE, O., AND ROSTOCK, O. 1955 Cytological studies of nuclear division in *Salmonella typhimurium*. J. Gen. Microbiol., **13**, 318–326.

25. MARUYAMA, Y. 1956 Biochemical aspects of cell growth of *Escherichia coli* as studied by the method of synchronous culture. J. Bacteriol., **72**, 821–826.

26. MARUYAMA, Y., AND YANAGITA, T. 1956 Physical methods for obtaining synchronous culture of *Escherichia coli*. J. Bacteriol., **71**, 542–546.

27. MAZIA, D. 1956 The life history of the cell. Am. Scientist, **44**, 1–32.

28. MAZIA, D., AND DAN, K. 1952 The isolation and biochemical characterization of the mitotic apparatus of dividing cells. Proc. Natl. Acad. Sci. U. S., **38**, 826–838.

29. NOVICK, A. 1955 Growth of bacteria. Ann. Rev. Microbiol., **8**, 97–110.

30. NYGAARD, O., GÜTTES, SOPHIA, AND RUSCH, H. P. 1957 Synthesis of DNA in relation to nuclear division in a plasmodial slime mold. (Abstract.) Federation Proc., **16**, 368.

31. OGUR, M., MINCKLER, S., AND McCLARY, D. O. 1953 Desoxyribonucleic acid and the budding cycle in the yeasts. J. Bacteriol., **66**, 642–645.

32. POWELL, E. D. 1955 Some features of the

generation times of individual bacteria. Biometrika, **42**, 16–44.

33. POWELL, E. D. 1956 Growth rate and generation time of bacteria, with special reference to continuous culture. J. Gen. Microbiol., **15**, 492–511.

34. PRESCOTT, D. M. 1955 Relations between growth and cell division, I. Exptl. Cell Research, **9**, 328–337.

35. PRESCOTT, D. M. 1956 Relations between growth and cell division, II. Exptl. Cell Research, **11**, 86–94.

36. ROBINOW, C. F. 1956 The chromatin bodies of bacteria. Bacteriol. Revs., **20**, 207–242.

37. ROMIG, W. R., SOCOLOFSKY, M. D., AND WYSS, O. 1956 Effects of radiation on synchronized cultures of *Escherichia coli*. (Abstract.) Bacteriol. Proc., p. 52.

38. SCHERBAUM, O. 1956 Cell growth in normal and synchronously dividing mass cultures of *Tetrahymena pyriformis*. Exptl. Cell Research, **11**, 464–476.

39. SCHERBAUM, O., AND ZEUTHEN, E. 1954 Induction of synchronous cell division in mass culture of *Tetrahymena pyriformis*. Exptl. Cell Research, **6**, 221–227.

40. SCHERBAUM, O., AND ZEUTHEN, E. 1955 Temperature-induced synchronous divisions in the ciliate protozoon, *Tetrahymena pyriformis*, growing in synthetic and proteose-peptone media. Exptl. Cell Research, Suppl. **3**, 312–325.

41. SCOTT, D. B. M., DELAMATER, E. D., MINSAVAGE, E. J., AND CHU, EILEEN C. 1956 Sequences of metabolic events during growth of synchronized bacteria. Science, **123**, 1036–1037.

42. TAMIYA, H., IWAMURA, T., SHIBATA, K., HASE, E., AND NIHEI, T. 1953 Correlation between photosynthesis and light-independent metabolism in the growth of *Chlorella*. Biochim. et Biophys. Acta, **12**, 23–40.

43. VON DENFFER, D. 1948 Die planktische Massenkultur pennater Grunddiatomeen. Arch. Mikrobiol., **14**, 159–202.

44. WHITFIELD, J. F., AND MURRAY, R. G. E. 1956 The effects of the ionic environment on the chromatin structures of bacteria. Can. J. Microbiol., **2**, 245–260.

45. ZEUTHEN, E. 1953 Growth as related to the cell-cycle in single cell cultures of *Tetrahymena pyriformis*. J. Embryol. and Exptl. Morphol., **1**, 239–249.

46. ZEUTHEN, E., AND SCHERBAUM, O. 1954 Synchronous divisions in mass cultures of the ciliate protozoon *Tetrahymena pyriformis* as induced by temperature changes. Proc. Symposium Colston Research Soc., **7**, 141–156.