Meetings

Cell Synchrony

Approximately 10 years ago the technique of synchronization of cell division was introduced and developed on a variety of cell types, including protozoa, bacteria, and algae. In order to discuss the current status of cell division synchrony, a conference was held at the Biology Division of the Oak Ridge National Laboratory, Oak Ridge, Tennessee, 9–10 October 1964. More than 30 scientists from the United States and abroad met with over 200 Oak Ridge scientists. The program was divided into three sessions: (i) Methods and physiology in cell synchrony (chairman, Otto H. Scherbaum, University of California at Los Angeles), (ii) Biochemical control of synchrony (chairman, A. M. Elliott, University of Michigan), and (iii) Structural and developmental aspects of synchrony (chairman, N. E. Williams, State University of Iowa).

Charles E. Helmstetter (Roswell Park Memorial Institute) described procedures for obtaining synchronous bacterial cultures by selection of cells at division. This method involves selection of cells at division by continuously flowing medium through a population of cells which are bound to the surface of a membrane filter. Only newborn cells are eluted from the surface. Selection of young cells occurs whether the medium flows normally or tangentially to the binding surface. There appears to be no correlation between selection and the size of the cells, but rather with the age distribution of the culture. The best selection was found in small, nonflagellated, nonfilament-forming bacteria.

Light-dark synchronizations of photosynthetic organisms *Chlorella* were discussed by H. Lorenzen (University of Goettingen). He emphasized the general importance of maintaining the shortest possible life cycle for creating the most effective synchronous cycles of growth. The physiological activities and chemical composition of the cells at corresponding times of each succeeding life cycle were described. It was emphasized that in this way complete synchronous cultures can be established and maintained for several weeks.

J. R. Cook (University of Maine) discussed the synchronized division in *Euglena gracilis* subjected to a light-dark cycle (14 hours light, 10 hours dark, at 21.5°C). He compared the synthetic rates of growth between synchronized and exponentially growing *Euglena*, using mathematical formulations derived from the growth equation. It was indicated that the synchronizing procedure has no effect, other than synchronization, on the division behavior of the individual cells.

Temperature-induced synchrony in the ciliate *Tetrahymena pyriformis* was reported by G. M. Padilla, I. L. Cameron, and O. L. Miller, Jr. (Oak Ridge National Laboratory). Repetitive cell division synchrony is induced by a 12-hour temperature cycle (2.5 hours at 27°C and 9.5 hours at 12°C). More than 90 percent of the cells regularly divide in the last hour of the warm period. It was shown that cultures can be kept synchronized indefinitely. Moreover, over 80 percent of the cells are in stomatogenesis just prior to the burst of division. This aspect of the system was developed by G. L. Whitson (ORNL). Pulse-labeling experiments with H^3-thymidine revealed lack of synchrony in macronuclear DNA synthesis, while the micronucleus actively incorporates H^3-thymidine in a synchronized manner during cytokinesis. Techniques for the isolation of polyribosomes by zonal centrifugation from synchronized cells were also described.

A method for obtaining nuclear synchrony in the plasmodial myxomycete *Physarum* was described by J. W. Daniel (University of Wisconsin). In submerged and agitated cultures, the plasmodia are individually but not collectively synchronous. When these plasmodia are poured onto the surface of filter paper, the suspension of microplasmodia fuse into a single, large surface plasmodium. Once synchrony of nuclear division is established it is maintained throughout the organism's growth until the plasmodium overgrows its nutritional source.

Methods for the mathematical analysis of decay of synchronization in ergodic cell cultures have been studied by J. Engelberg and H. R. Hirsch (University of Kentucky). They showed that, by using statistical parameters which describe the cell doubling time distribution function, it is possible to determine the future history of a culture with special reference to the degree and loss of synchronization.

J. J. Blum and V. Kahn (Duke University) described the effect of actinomycin D on temperature-synchronized cultures of *Astasia longa*. They used a repetitive temperature cycle of 17.5 hours at 13.5°C and 6.5 hours at 28.5°C based on the method of synchronization developed by Padilla and James (1960). The cells were treated with actinomycin D for various time intervals during this synchronized cycle. It was found that with increasing time after the beginning of the cold period, the inhibitory effect of actinomycin D on cell division diminishes. If the inhibitor (5 μg/ml) is added at the beginning of the cold period, there is considerable inhibition of the subsequent division while, if the drug is added at a time corresponding to early prophase, no inhibition in doubling is observed at concentrations of up to 15 μg/ml. The effect of actinomycin D on RNA synthesis, the incorporation of labeled uracil into RNA, and the timing of synthesis of acid phosphatase were also described. They showed that while actinomycin treatment generally inhibits cell division and acid phosphatase synthesis to approximately the same extent, a differential sensitivity can be demonstrated by exposing the cells to actinomycin D late in the warm period.

O. H. Scherbaum described the carbohydrate metabolism and control of cytokinesis in *Tetrahymena*. He discussed the effect of restricted respiration and compared it with the effect of the heat treatment that induces synchrony on glycogen synthesis in cells growing abnormally large but not able to start cytokinesis. The accumulation of two acid-soluble phosphory-

lated deoxysugars in heat-treated cells, as it relates to the cytokinetic block, was discussed. Physical-chemical data on these sugars were summarized.

I. L. Cameron (Oak Ridge National Laboratory) discussed specific nutritional synchronization of *Tetrahymena* after replacement of pyrimidines in cultures deprived of these metabolites for 72 hours. A sequence of macromolecular events occurs after this replacement. First, RNA synthesis begins and then protein synthesis is initiated after 1 hour. The cells enter DNA replication after 2½ hours and begin to divide after 6 hours. Data were presented to show that early biochemical events are synchronized but that such synchrony is quickly lost as the cells complete their first cell division. Cameron concluded with a discussion of the increase in polysome material; such increase precedes the beginning of protein synthesis after pyrimidine replacement.

L. Rasmussen (Carlsberg Foundation, Denmark) commented on the delay in cell division in *Paramecium aurelia* after temperature changes and exposure to various chemical agents. He confirmed and extended the studies originated by E. Zeuthen, who demonstrated that there are transition points occurring in heat-synchronized *Tetrahymena pyriformis* when these cells are exposed to heat, fluorophenylalanine, or inhibitors of oxidative phosphorylation. It appears that the addition of such compounds beyond a transition point does not produce any delay in the subsequent synchronous burst of division. Studies of this nature were extended to exponentially growing cultures of the ciliate *Paramecium aurelia* where transition points were also found. The ensuing discussion included a description of this effect in other cells such as the green alga, Ulva, HeLa cells, Limna eggs, and others. G. L. Whitson (Oak Ridge National Laboratory) pointed out, however, that in similar experiments he failed to find transition points in all instances by different treatments, using the same strain of *Paramecium*. Some basic similarities, however, were mentioned.

In a series of studies on the respiration rates of synchronized *Astasia longa*, T. W. James (University of California at Los Angeles) developed a culture flask which can be used for the simultaneous synchronization of these flagellates with a repetitive temperature cycle (17.5 hours at 14.5°C, 6.5 hours at 28.5°C) and the measurement of oxygen consumption. It was found that the degree of synchrony bears a direct relationship to the increase in respiration rate during the cold period. It was also discovered that the respiratory rate of synchronized cells exceeds those of exponentially dividing cells. The results were interpreted as a confirmation of the work of Zeuthen on marine eggs and the work on the synchronized *Tetrahymena*, where there appears to be a decrease in the respiration rate just prior to cytokinesis. It was suggested that the decline in rate that occurs close to the onset of mitosis may be related to the activity of DNA in mitochondria.

J. J. Eiler and R. K. Mehta (University of California Medical Center) discussed the effect of phenylurethane on the ATP content of synchronously dividing *Tetrahymena pyriformis*. It was found that this compound inhibits respiration of the organism and protein synthesis during the 8-hour period of heat treatment, thus resulting in 70-percent depression of the synchronous division. In addition to depression in respiration during protein synthesis, the drug appears to depress the increase of ATP associated with synchronous growth. It was suggested that the effects of phenylurethane are not due to uncoupling but rather to the inhibitory effect of ATP on macromolecular synthesis. However, blockage in the utilization of ATP by an energetically significant reaction cannot be excluded.

A report on the synchronization of mammalian HeLa cells was made by G. C. Mueller (University of Wisconsin). In the presence of FUDR most of the cells stall at a point just prior to the initiation or beginning of DNA synthesis. Mueller noted that DNA is synthesized in an orderly process involving early and late replicating fractions of DNA. Incorporation of bromodeoxyuridine into DNA during the early replicating period results in the loss of cloning ability. However, introduction of bromodeoxyuridine into the late replicating DNA has little effect on cell survival. Experiments with actinomycin and puromycin indicated that replication of the late labeling DNA depends on synthesis of new RNA and protein. Similarly, the synthesis of new RNA and protein appears to be required for the mitotic process. In addition, the antibiotic phleomycin was found to selectively block the mitotic process prior to the time of colchicine action.

Synthesis of macromolecules in synchronized cultures of yeast was discussed by H. O. Halvorson (University of Wisconsin). Synchronization was accomplished by repeated cycles of starvation and sizing of cells. In such synchronized cells RNA and protein synthesis occurs throughout the cell cycle, whereas DNA is replicated in the first two-thirds of the cell cycle. Halvorson showed that specific enzymes are produced only at discrete intervals of the cell cycle and that the time of synthesis varies with each enzyme. He showed further that enzymes which are closely linked on the genetic map are produced at the same period of the cell cycle. The results were interpreted to suggest that transcription of the yeast genome is ordered and a given genetic region is transcribed only during a brief interval of the cell cycle. The paper was followed by a vigorous discussion of the oscillatory nature of various enzymes and the occurrence and disappearance of various enzymes during the cell cycle. The utilization of synchronized systems to describe such oscillatory processes was discussed.

Cell division in the synchronized culture of *Astasia longa* was reported on by J. R. Sommer and J. J. Blum (Duke University). Detailed ultrastructural studies of the dynamics of division were presented on these flagellates, synchronized by the repetitive temperature previously described. It was found that during early prophase the adult complement of pellicle complexes (36 per cell) is doubled by the appearance of 36 newly formed presumptive ridges which alternate with the adult ridges. Such ridges also exist in the cytostome region of the interphase cell where they alternate with larger ridges. The presumptive ridges gradually reach adult size, a process which may not be completed until the cell completes cytokinesis. Sommer and Blum also described the existence of a hitherto unrecognized spindle-like apparatus in the nucleus of *Astasia*. In addition, a discussion of the involvement of the cell nucleus during division was presented. It was shown that the nucleus becomes closely associated with the basal body of the flagellum and that there are breaks in the nuclear envelope during division.

P. A. Van Dreal and N. G. Anderson (Oak Ridge National Laboratory) succeeded in isolating ribosomes and

polysomes from *Astasia longa* by means of the zonal ultracentrifuge. They also analyzed the TCA soluble nucleotides during the repetitive synchronized cycle of *Astasia longa*. The mono-, di-, and triphosphates are much reduced in concentration at the end of the cold period. They appear, however, to increase in amount during the warm period. Their involvement in the initiation of the division process was suggested.

The regulation of cell division and chromosome replication in *Bacillus subtilis* was discussed by N. Sueoka (Princeton University). Synchronization of chromosome replication was obtained either by releasing the growth from stationary phase or by germinating spores. Sequential replication of the *Bacillus subtilis* chromosome from one end (origin) to the other end (terminus) was demonstrated by comparing relative frequencies of various transforming markers in DNA preparations obtained from exponentially grown cells and stationary cells. In contrast to strain W26 of *B. subtilis*, strain W168 does not show polarity of chromosome replication. The apparent nonpolar behavior of strain W168 arises from poor regulation of chromosome replication rather than from a basic difference in the mode of replication with regard to origin and sequence. Evidence was presented for multifork replication of chromosomes during rapid growth of the cells.

G. L. Whitson, G. M. Padilla, and W. D. Fisher (Oak Ridge National Laboratory) presented a paper on the inhibition by actinomycin D in *Tetrahymena pyriformis* synchronized by the method of Padilla and Cameron. It was found that the cells are sensitive to actinomycin D (10 μg/ml) added 1 hour before the temperature shift from the cold to the warm period. More than 90 percent of the cells failed to divide. Partial inhibition of cell division occurs if actinomycin D is added later in the temperature cycle. Stomatogenesis, which is confined to the warm period, does not occur in the cells treated with actinomycin D. Zonal centrifugation of cells treated with actinomycin D showed a decrease in the 110*S* ribosome fraction and an increase in the 70*S* fraction. In addition, pulse-labeling experiments with RNA precursors show a marked inhibition of uptake after treatment with actinomycin D. Studies of long-term incorporation indicate that the fraction of RNA synthesized during actinomycin D treatment is not a short-lived messenger. Relations between development and maintenance of differentiated structures and RNA inhibition were discussed.

In a complementary presentation D. S. Nachtwey and W. J. Dickinson (U.S. Naval Radiological Laboratory, San Francisco) discussed the effects of actinomycin D treatment on *Tetrahymena* synchronized by the method of Scherbaum and Zeuthen. They showed that *Tetrahymena* synchronized by the heat-shock method are much more resistant to actinomycin than *Tetrahymena* synchronized by the repetitive cold temperature cycle. The variables studied in this presentation were the concentration of the drug, duration of exposure after the end of the synchronization treatment, and the type of medium on which exposure occurs. It was found that the critical time at which the drug must be added to block division in 50 percent of the cells yields a saturation-type curve. In nonnutrient medium the cells are much less sensitive whether they are exposed to actinomycin D continuously or for shorter periods of time. It was suggested that the relative sensitivity of the cells in the two media is correlated with the relative rate and extent of food vacuole formation. This interpretation does not exclude the dependence of inhibition on the extent of saturation at the target site.

Cytological studies on the cortical organelle development of *Tetrahymena pyriformis*, as related to the cell cycle, were noted by J. Frankel (State University of Iowa). He used exponentially growing *Tetrahymena* or *Tetrahymena* synchronized by the Scherbaum-Zeuthen method. Frankel was able to demonstrate a stabilization point about two-thirds of the way through the cell cycle, beyond which addition of numerous RNA and protein inhibitors had no effect on the ensuing cortical development, that is, on the development of oral cilia. He concluded that new RNA and protein synthesis are necessary during each cell cycle to support cortical organelle development.

Some of the participants in this conference suggested that a synchrony conference of this nature be held in two years. Plans for such a conference are being formulated by the organizing committee which consists of G. M. Padilla, chairman; I. L. Cameron; and G. L. Whitson (Oak Ridge National Laboratory).

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Correlations of Particles Emitted in Nuclear Reactions

The three-body problem is the most celebrated of all dynamical problems and has troubled physicists from the earliest days. In a few well-known systems, such as the sun, earth, and moon and the helium atom, accurate numerical solutions have been obtained by successive approximations. The approximations which make these examples *relatively* simple cannot be made in the three-body problem of nuclear physics, because here the interacting particles have comparable masses and the forces acting between them are extremely complex, since the particles are often so close together that their intrinsic structure becomes important. The hope, however, is the same: that the nuclear three-body system is capable of description in terms of two-body forces.

The bound three-body systems in nuclear physics, namely the H^3 and He^3 nuclei, have only static properties, such as binding energy, that can be used to test a theory. The dynamical structure of the system cannot be discovered from its static properties. Three-body systems in excited states, if they have sufficient energy, can decay into three free particles; the kinematics of the decay reflects the internal dynamics of the system so that experiments can be performed which give information about specific dynamical configurations of the system.

Problems connected with such systems were studied at a conference on "Correlations of particles emitted in nuclear reactions" held at Gatlinburg, Tennessee, 15–17 October.

C. Zupancic of the J. Stefan Institute in Ljubljana, Yugoslavia, opened the conference with a talk in which he pointed out that most of the phenomena under study here have analogues in atomic physics, where better approximations can be used. As an example