hours was instituted to determine whether DNA replication would still occur at the usual time (hours 8 to 14 after "dawn"), even though now this time would fall within the lengthened dark period, and to see if cell division would occur although the light energy available for cellular growth had been reduced by 6 hours. After the 24-hour dark period, a 6-hour exposure to light was given in an attempt to compensate for the previous loss of 6 hours of light; this 6-hour light period was followed by a normal dark period (10 hours) and then light; the cell number was monitored throughout the experiment.

The results demonstrated that doubling of DNA did indeed occur in the *dark* (2.3 to 4.6 $\mu\mu$g per cell); however, the increase in total protein ceased as soon as darkness began. Cell division did not occur, the concentration of 7.5 $\times$ 10^4 cells per milliliter being maintained throughout the entire reduced light period and lengthened dark period. Actually, a small increase (5 percent) in cell number did occur after 6 hours of dark, the time in previous cycles that represented the usual light-dark transition and onset of cell division. This almost negligible and perhaps transient increase may reflect the division of those cells which were metabolically "precocious" after only 8 hours of light and required only DNA replication in order to divide. Or perhaps this small increase was caused by sampling errors due to differential cell motility. After the 6-hour exposure to light, however, a substantial division burst occurred with a step size of 1.46 representing a 44 percent cell number increase. These results indicate that 8 hours of light is insufficient for cell division to occur, even though DNA replication has occurred, and that only when 6 hours more of light was obtained after a 24-hour dark period did significant fission take place. That the step size of this last burst was much less than 2.00 suggests that some of the cells had depleted their metabolic pools because of starvation during the extended dark period to such a degree that even the additional 6 hours of light was not enough for normal cellular growth to have been completed. It is also evident that this latter experiment introduced a G_2 phase of no net DNA synthesis between the normal period of synthesis (S) and mitosis (M), whereas under the usual 14:10 cycle the G_2 phase is almost entirely absent.

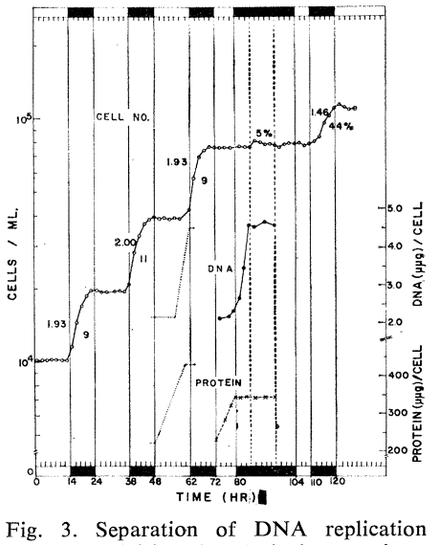

Fig. 3. Separation of DNA replication and cell division by dark interruptions. For the first three cycles *E. gracilis* (Z) was grown in a 14:10 light-dark cycle under described conditions. During the next cycle only 8 hours of light were given, followed by periods of 24 hours dark, 6 hours light, 10 hours dark, and then continuous light. At intervals of 2 to 4 hours, DNA, cell growth (total protein), and cell number were determined across a portion of this extended fourth cycle as indicated. The percentage increase in cell number is given for the last two smaller fission bursts, and step size for all major bursts. The dotted lines represent DNA and protein synthesis during a normal cycle.

A further experiment, in which the light period was reduced to only 2 hours, demonstrated that protein synthesis once more ceased abruptly with the onset of dark, and that *neither DNA synthesis nor cell division occurred*, even after 24 hours of dark. These results suggest that DNA replication is dependent on events occurring within the cell 2 to 8 hours after the onset of light, as, for example, the synthesis of a specific enzyme.

In conclusion, the results corroborate the generalization that DNA replication is a necessary but not sufficient condition for cellular division. The problem of the initiation of DNA replication remains. It may depend on prior protein or RNA synthesis or both and perhaps direct energy input by way of the light (*14*). Alternatively, the onset of light may serve as a phasing synchronizing agent (*17*). It may well be that these aspects of the cell cycle can be attacked profitably through dark blocks and brief intervals or "pulses" of darkness during the cycle of *Euglena* in synchronous division.

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24 April 1964

Automatic Sampling Device for Study of Synchronized Cultures of Microorganisms

Abstract. *A versatile automatic culture-sampling system is described and data are presented from experiments in which this device was used to study growth and synchronized cell division in* Euglena gracilis.

The desirability of synchronized cultures of microorganisms for studying events in the cell cycle has created a need for following the time course of division in the population. This report describes a sampling system designed to fulfill the requirements encountered in a study of growth characteristics of *Euglena gracilis* under varying culture conditions. The device is reliable, ac-

curate, relatively simple, and inexpensive. It has been used to sample cultures in a number of studies ranging from experiments on the environmental control of synchronized division in *Euglena* and the effects of nutrilites on synchrony, to studies of periodic variations of mating reactivity of *Paramecium* (*1*). This report describes the mechanics of the sampling system, along with data obtained from typical experiments (*2*).

A schematic diagram of the device, which consists of four components, is shown in Fig. 1.

1) The culture vessel is a cotton-stoppered flask containing a teflon-coated, magnetic stirring bar and three glass tubes. One tube serves for aeration; another is closed at one end by a rubber diaphragm through which cell suspension, fresh medium, or nutrilites can be injected aseptically; and a third contains a length of "spaghetti" tubing which serves as a sampling tube.

2) The turntable (fraction collector) has three functions. The head of the turntable is a circular piece of lucite with 36 symmetrically spaced holes. The small inner holes are tapped and fitted with 1-inch (2.5 cm) brass screws (activator pins) which close a microswitch (MS). The base of the table, a circular piece of bakelite, has a number of brass support rods, three of which are fitted with roller bearings on which the turntable head rides. A synchronous motor (1 rev/day) supported on four central rods drives the turntable head. Another rod supports a microswitch (MS) which, when closed by one of the activator pins, initiates events leading to a sampling of the culture. Finally, one brass rod supports the needle holder which swings freely between two fixed positions directly above the centers of two adjacent vials. This movement is solenoid operated. Thus, the turntable serves as a vial holder, as a timer determining the frequency of sampling, and as a selective dispensing mechanism.

3) The automatic pipette is controlled by two microswitches and a relay. These operate on signal from MS, and the pipette delivers a predetermined volume of cell suspension. Volume of delivery is regulated by the number of complete strokes accomplished by the pipette during the time interval when the system is activated. This time can be varied and is controlled by the interval timer. The pipette can also be controlled manually by a toggle switch.

4) The interval timer consists of a three-ganged percentage timer. The first gang (MS_1) operates in tandem with a normally closed, 5-second, time-delay relay and controls current flow to the motor of the interval timer. This is adjusted so that the cams of the timer complete one revolution each time MS is closed. The second gang (MS_2) controls current flow to the solenoid of the needle holder. When the solenoid is activated the needle is held above a waste vial and when inactivated it is held above an adjacent sample vial. This feature allows clearing of the tubing and valve assembly of cell suspension from previous samplings. The third gang (MS_3) controls current flow to the normally open pipette relay. When this relay is closed, the pipette is activated and continues to operate until both MS_3 and MS_4 open. MS_3 is set to open during the mid-point of the pipette's stroking cycle. Since MS_4 is a shunt circuit in parallel with MS_3, the pipette relay will remain closed until MS_4 opens. MS_4 is fixed to the chassis of the pipette machine and the point at which it opens relative to the stroking cycle is determined by its position.

By synchronizing events during the period of activation of the timing mechanism, it is possible to adjust the sampling device to clear automatically the lines of cell suspension remaining from the previous sampling and to deliver a known volume of material to a sample vial. The amount of clearing required, the volume and number of samples desired, and the frequency at

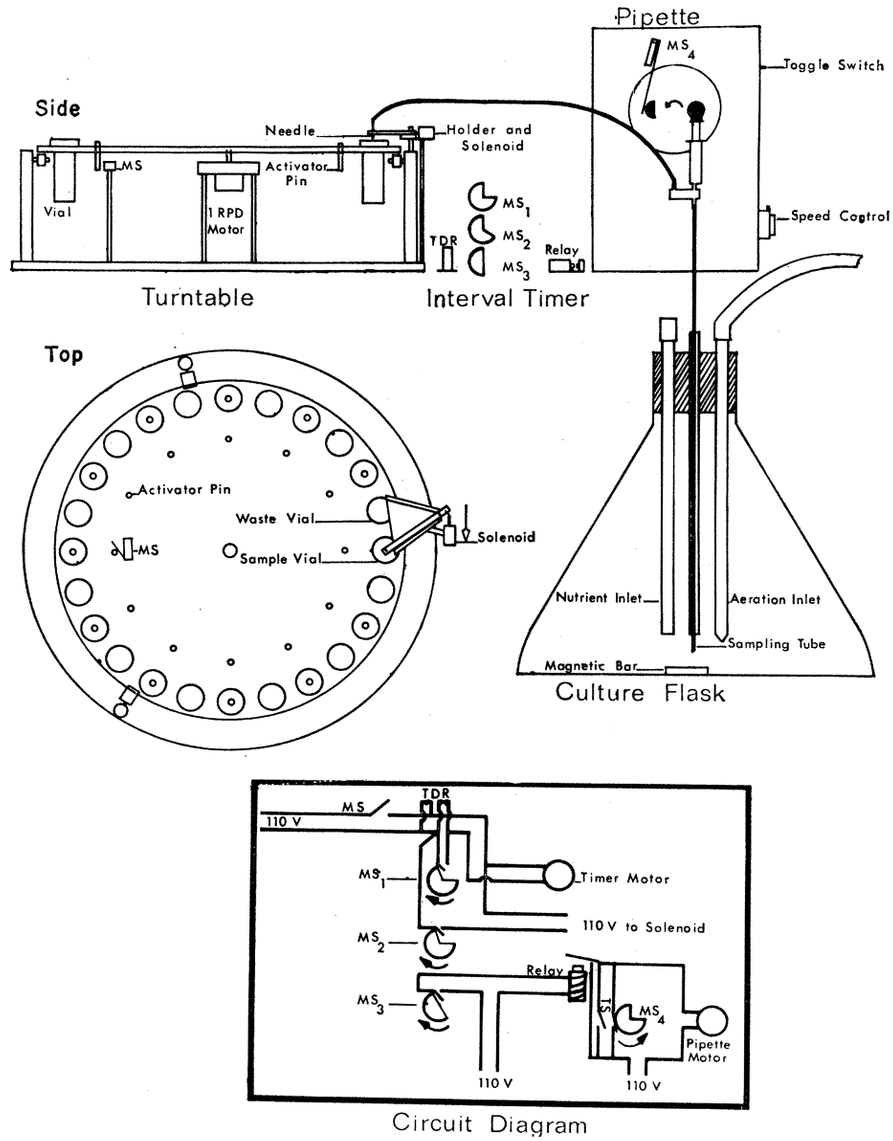

Fig. 1. A schematic diagram of the culture sampling system and a circuit diagram. MS, microswitch; TDR, time delay relay; TS, toggle switch; 1 RPD, 1 revolution per day, synchronous motor.

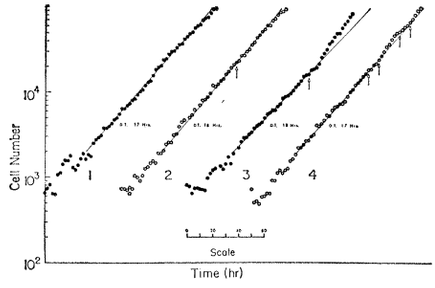

Fig. 2. Growth, plotted as cell number per milliliter against time, of four subcultures of *Euglena gracilis*, strain Z, maintained in separate light and temperature controlled growth chambers, sampled simultaneously every 2 hours by individual sampling systems operated by the same timer. Dark lines through the circles have a slope which gives the doubling times (D.T.), shown at the right of each curve, for the cultures. Arrows indicate the points at which light intensities were changed using six 40-watt General Electric cool white fluorescent bulbs instead of the usual three. In culture No. 4, the first arrow indicates an increase, and the second arrow a decrease (and so forth) in light intensity.

which samples are to be taken will depend upon the design of the experiment. These features can be varied and the desired program obtained by simple adjustments of the timing mechanism, the turntable head, the speed of the synchronous driving motor, and the pipette speed. Also, more than one fraction collector can be operated by the same timing mechanism.

Figure 2 is from an experiment illustrating a "bioassay" of the sampling system. Four subcultures of *Euglena*

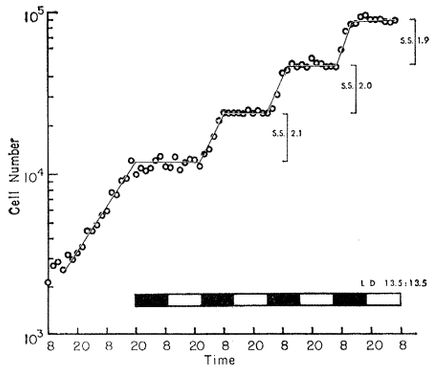

Fig. 3. Growth, plotted as in Fig. 2, of *Euglena* maintained a light cycle of 13.5 hours light and 13.5 hours dark. Alternating blocks of light and dark along the abscissa represent the cycle. In the step portion of the curve, figures are given for step size (S.S.), defined as the average cell number in the plateau after a burst divided by the average cell number in the plateau before division.

gracilis, strain Z (*3*) were grown in 1500 ml of inorganic medium (*4*) in constant light (*5*) at 25°C. Each subculture was placed in a separate temperature-controlled growth chamber equipped with a sampling device regulated by the same timing mechanism. The figure shows that the doubling times (D.T.) for the four cultures ranged from 16 hours for culture No. 2 to 18 hours for culture No. 3. In culture No. 3, which had the longest doubling time, an increase in light intensity (initiated at the time indicated by the arrow) caused an increased division rate, whereas in culture No. 2, which had the shortest doubling time, a similar increase in light intensity had no effect on the rate of division. Light conditions in the cabinets housing cultures No. 2 and No. 3 were, therefore, different before the changes in intensity. Thus, culture No. 2 was at saturating light intensities prior to the increase in intensity and the doubling time was unaffected, whereas culture No. 3 was at subsaturating intensities and the increase in intensity brought about an increased division rate. Culture No. 4 was exposed to a cycle of two differing intensities, namely, 8 hours of higher intensity followed by 16 hours of lower intensity. The initial effect of the higher intensity was to inhibit division slightly.

Figure 3 shows synchronously dividing *Euglena gracilis* in a light cycle (LD 13.5 hours light, 13.5 hours dark). After an initial 2 days in continuous light, the culture was placed on this light schedule. Division ceased during the first cycle and began in the dark period of the second cycle. Thereafter, divisions were confined to the first 10 hours of the dark periods. In any division period there was an approximate doubling in cell number—a twofold increase in cell number giving a step size (S.S.) of 2.

The sampling system has been efficiently used as a monitoring device for studies of biochemical events during the cell cycle (*6*) and for studies of ultraviolet-induced bleaching in synchronized *Euglena* (*7*).

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Serological Evidence of Arbovirus Infection in the Seminole Indians of Southern Florida

Abstract. *A serological survey of the Seminole Indians in south Florida for evidence of exposure to arbovirus infection indicated not only past infection with the recognized North American encephalitis viruses—a high incidence for St. Louis encephalitis—but exposure to a Bunyamwera group agent and to Venezuelan equine encephalitis virus, activity of which had not been previously recognized north of Panama.*

Exotic arboviruses may be defined as those which were originally isolated abroad or are otherwise epidemiologically related to a geographical area outside the continental United States. Indigenous arboviruses are those originally isolated and described within the United States.

While working with R. M. Taylor in the Nile Delta of Egypt in 1954, we isolated a number of virus strains from *Argas* ticks which were intimately associated with breeding colonies of the cattle egret, *Bubulcus ibis* (*1*). In 1958, we initiated a search in the U.S. for exotic *Argas* tick viruses that might be linked with rookeries of this Old World egret species which had recently become established in southern Florida. Although this investigation produced no evidence of a similar tick-egret-virus ecology, it brought us into contact with the indigenous Seminole Indians of the Brighton and Big Cypress reservations (Fig. 1).

These Indians are lifelong residents of palmetto, sawgrass, hammock, swamp, and everglade, which are habitats characteristic of south Florida, the area of most extensive and variable tropical attributes in the continental United States. Just as military sentries are posted to signal unusual or sig-