The accumulation of radon in the sealed cans was scrutinized more closely by taking daily counts on a sealed sample from the lower B horizon (19- to 25-in.) during a 3-week period. The rise in count rate followed very closely an inverted decay curve with the characteristic half-life of radon (3.8 days).

A series of chemical analyses of total potassium as a function of depth were made by Donald Coonrod (values lay within the range 1.48 to 1.83 percent). A close correlation was found between total potassium content and gamma activity versus soil depth (the confidence limits were 0.986). We therefore calibrated the counting equipment for potassium. Our method was suggested by Gustafson, Marinelli, and Brar (*3*) of the Argonne National Laboratories. A "mock soil" was made by mixing enough KCl into uncontaminated sodium phosphate to give the same mass of potassium as that contained in one of the 1-kg soil samples and to give the same counting geometry. The counts obtained in this way were interpreted as counts from soil potassium only. By this method we concluded that the radioactive isotope of potassium (K^{40}) accounts for about 21.3 percent of the total count in the A horizon, 19.3 percent in the B, and 18.5 percent in the C. It seems highly probable that the remaining 78.7 to 81.5 percent of the count arises from radioelements of the uranium and thorium series. The emanation of radon (Rn^{222}), previously mentioned, can arise only from radium, (Ra^{226}), but because of soil leaching and weathering the radium will not necessarily be in equilibrium with its longer-lived parents.

The increased count rate as one goes from the A through the B horizon would appear to be accounted for partly by the greater concentration of potassium, and therefore of K^{40}, in the lower soil horizons and partly by a similar trend for radium. The greater accumulation of radon in the B horizon must be accompanied by a higher radium concentration. This was not expected, since radium is known to behave chemically somewhat as calcium does, and calcium (in these soils) has been leached from the B horizon but occurs at higher concentrations in the underlying glacial till (C horizon). It is possible that the leaching of uranium from the A horizon and its adsorption on clay surfaces in the B horizon are important here.

In order to learn something about the absorption of radioelements on clay surfaces, a study of particle size was made. Clay, silt, and sand-size particles were separated by wet-sieving followed by sedimentation (the U.S. Department of Agriculture's mechanical analysis procedure was adapted for studying large samples). The gamma activities, in counts per minute per gram, were found to be as follows: clay, 6.95; silt, 5.40; sand, 1.38. The fact that the silt and clay activities are of the same order of magnitude would seem to indicate that a substantial portion of the radioelements are held in mineral form in the silt range. This is in agreement with the findings of Hoogteijling and Sizoo (*8*). Some adsorption of radioelements on clay surfaces may also occur.

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14 October 1959

Resetting the Sporulation Rhythm in Pilobolus with Short Light Flashes of High Intensity

Abstract. The "clock-controlled" endogenous sporulation rhythm in the fungus *Pilobolus sphaerosporus* has been investigated as part of a comparative study aimed at elucidating characteristic common features of circadian (*1*) rhythms. *Pilobolus* was chosen for inclusion in this study because of its demonstrated rhythm and sensitivity to light, and because it is a relatively simple plant. It has been shown that a single, high-intensity, 1/2000 second light flash will completely reset (shift the phase of) a rhythm persisting in continuous dim red light at constant temperature, and that one or more transient cycles occur before the phase shift is complete. The significance of these results is discussed.

The fungus *Pilobolus sphaerosporus* ejects spores at periodically timed intervals. The organism possesses an endogenous, temperature-compensated, rhythmic system which can be synchronized by appropriate light-dark cycles or temperature cycles but which persists with approximately a 24-hour period in continuous darkness or continuous dim red light (*2*). This "clock-controlled" sporulation rhythm is especially sensitive to light, and we have therefore examined the phase-shifting characteristics of the rhythm in response to light flashes of short duration and high intensity. This investigation (*3*) is part of a comparative study in which the general characteristics of the biological clocks of such diverse organisms as mammals (*4*), insects (*5*), and microorganisms (*6*) have been investigated.

From the comparative point of view it is desirable to know whether the properties of the "clock" system in microorganisms are qualitatively similar to those in higher organisms. As a tool for the comparative study, we have attached considerable significance to the way in which the phase of a persistent rhythm is shifted in response to single light stimuli (*7*). The fact that a new steady state is not achieved immediately, but only several cycles after a phase-shifting light signal, has been interpreted by us in terms of a generalized coupled-oscillator model. The transient approach to new phase is interpreted in terms of a gradual re-entrainment of one oscillator (not reset by the light signal) by another oscillator which is reset by the light signal. These transients, which may continue for seven or eight cycles in hamsters and three or four cycles in *Drosophila*, are difficult to detect in microorganisms and plants. The present demonstration of their occurrence in *Pilobolus*, together with the previous claim for their existence in *Euglena*, is evidence that the underlying features of the clock system which they reflect require neither the complexity of multicellular organization nor the presence of a nervous system.

Pilobolus sphaerosphorus was cultured on Bovung-oatmeal-agar of the following composition: 200 gm of Bovung (Walker-Gordon dried cow manure) boiled in 1 liter of water for 20 minutes and filtered through cheesecloth; 60 gm of oatmeal boiled 60 minutes in 1 liter of water and filtered through cheesecloth; 1.2 gm of K_2HPO_4; 1 gm of KH_2PO_4; and 40 gm of agar. The total volume was brought to 2 liters with water, and the medium was autoclaved for 20 minutes and poured into petri dishes 50 mm in diameter.

Agar-block transfers were made at 3- to 4-day intervals, and the plates were kept in a 25°C cabinet maintained on a light cycle with 12 hours of white fluorescent light per 24 hours. Sporulation started 6 or 7 days after inoculation. Ejected spores were collected with a specially constructed device consisting of a moving carriage holding eight petri plates which are slowly (56 mm/hr) pulled beneath eight glass strips just above the petri plates. The ejected spores adhere to the glass strips. Every 24 hours the glass strips were removed and replaced

with clean ones, and the carriage was reset for another 24-hour run. Ejected spores were counted against a grid calibrated in hourly intervals. The whole apparatus is in a room at 25°C in continuous dim red light.

Experiments were performed as follows: Plates were transferred from the light-cycle cabinet 6 or 7 days after inoculation to the carriage of the recording device. The transfer was performed at the very end of a dark period before the lights were turned on, and the carriage was set in motion at the time of the "expected dawn" when the lights would have been turned on. This time was considered to be hour zero of the cycle. At each of the hours 0, 3, 6, 9, 12, 15, 18, and 21 a plate was removed to a dark room, exposed to a single, high-intensity 1/2000 second flash from a stroboscopic lamp, and replaced in the carriage. The plates continue to eject spores for at least 4 days and in some cases for 5 or 6 days. In Fig. 1*C* the absence of data for some of the cultures on the 5th day reflects the fact that these particular cultures had ejected all of their spores in 4 days.

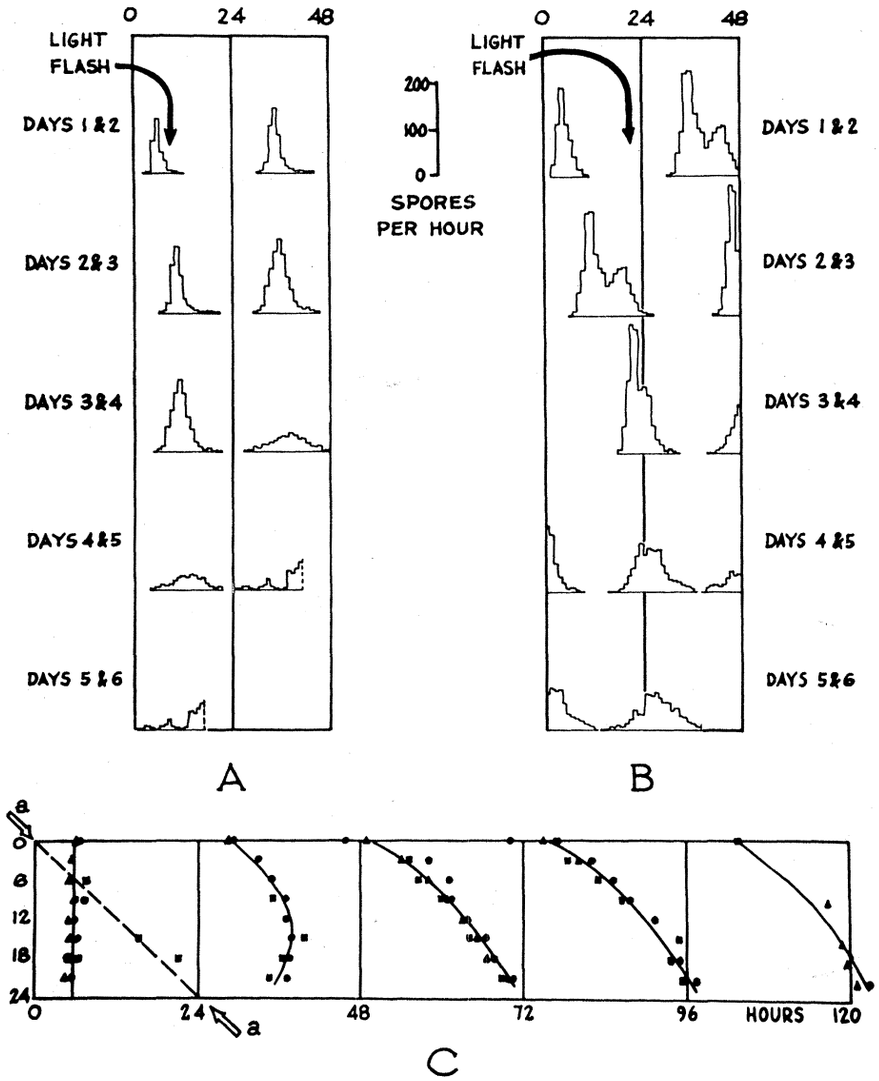

Fig. 1. *A, B,* Histograms of the number of spores discharged in hourly intervals from a petri plate culture of *Pilobolus sphaerosphorus.* Six days' record are shown with each day's record (except the first and last) being repeated once below, and once to the right of, the previous day's record. The sporulation rhythms of the cultures were previously synchronized by a light-dark cycle. At hour 0 of day 1 (24 hours after the last "dawn") the cultures were placed in continuous dim red light and constant temperature and remained in these conditions for the six days shown here. Nine hours (*A*) and 21 hours (*B*) after hour 0 (the "subjective dawn") a high-intensity, short-duration light flash was given to each culture. The histograms illustrate the way in which the rhythm controlling sporulation was reset in phase. *C,* Medians of the sporulation peaks of a number of similar experiments. The horizontal time scale (5 days) represents the number of hours elapsed after the cultures were transferred from the light cycle to constant dim red light. Hour zero is 24 hours after the last dawn of the previous light cycle. The dashed line *a-a* and the numbers on the vertical scale indicate the time in the cycle (measured from subjective dawn) at which each culture received a strobe flash. The circles, triangles, and squares correspond to replicate experiments.

Figure 1 summarizes the results of three replicate experiments of this type. Two histograms, corresponding to experiments in which the strobe flashes were given at hours 9 and 21, show the way in which the rhythm controlling sporulation was reset in phase. Part *C* of the figure, in which the medians of the sporulation peaks are shown, summarizes the results of all of these experiments. It may be pointed out that by the second or third day after the strobe flash the cultures had been almost completely reset—that is, the line through the medians of the peaks is essentially parallel to the line *a-a* which indicates the time at which the strobe signal was given. The reset is not immediate, however, as might be expected from some of the simplest oscillatory schemes which could be visualized as models for the system. There is a gradual approach to new phase involving one or, in some cases, more transient cycles.

This combination of transients with ultimate determination of phase by a single signal is what we reported earlier for *Drosophila.* It is what led to the coupled-oscillator scheme we described (*7*). In the previously reported experiments with *Drosophila* the duration of the light signal was either 12 hours or 4 hours, and the pattern of transients in the *Drosophila* experiments cannot be compared with the pattern of transients in the present experiments. Unpublished experiments with *Drosophila,* in which a strobe light was used to reset the eclosion rhythm, indicate that the details of the pattern of transients are not the same in the two organisms. The phase of the rhythm in *Drosophila* can be shifted only a few hours at the most, and both short-period ("advancing") and long-period ("delaying") transients are observed. In *Pilobolus,* on the other hand, large phase shifts are observed and only long-period ("delaying") transients are observed. A more detailed analysis of the *Pilobolus* system might reveal the existence of "advancing" transients when strobe signals are given in the vicinity of hours 0 and 24. In any event, this constitutes a detail of the system and the essential point which is demonstrated by the *Pilobolus* experiments is the indication that light operates on an oscillatory system distinct from that which proximally regulates spore-ejection time.

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1. The term *circadian*, which was introduced by F. Halberg, includes all persistent endogenous biological rhythms with periods of about 24 hours. It is introduced in preference to the somewhat confusing terms *diurnal rhythm, daily rhythm, 24-hour rhythm*, and others.
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The Error Hypothesis of Mutation

Abstract. Accumulation of mutants in glucose-limited chemostats is proportional to growth rate, while in tryptophan-limited chemostats it is independent of growth rate. This behavior, which implies the failure of the error hypothesis, may be explainable on the basis of a unitary hypothesis: the results with glucose may be due to reversion or loss of latent mutants.

The most common hypothesis of gene mutation has been the error hypothesis, which assumes that mutations arise as a result of an "error" in gene replication [that is, the "copying error" (*1*)]. According to this hypothesis, rate of mutation would be expected to be proportional to rate of gene replication, which in turn is proportional to division rate under constant growth conditions. However, Novick and Szilard (*2*) demonstrated that the rate of spontaneous mutation to resistance to bacteriophage T5 was independent of growth rate in tryptophan-limited chemostat cultures of *Escherichia coli* strain B/1, *t* for generation times varying from 2 to 12 hours. Their result appeared to be contradictory to the error hypothesis of mutation, suggesting that the rate of gene replication might be independent of the growth rate of the cell.

In contrast to the above response, when growth is limited with glucose the rate of accumulation of mutants is proportional to growth rate (Fig. 1) for caffeine-induced mutations in the same strain and in the related strain B. These contrasting results would be easily understood if the process of spontaneous mutation were different from that for caffeine-induced mutation. Instead, evidence supports their similarity: work in this laboratory (*3*) indicates that the rate of accumulation of caffeine-induced mutants also is independent of growth rate in tryptophan-limited cultures. Furthermore, the rates of both spontaneous and caffeine-induced mutations decrease in the presence of the antimutagen guanosine, although not to the same extent (*4*).

It is possible to regard these divergent responses in glucose- and tryptophan-limited cultures as arising in a common manner by assuming that the results with glucose-limited growth are due to a secondary process. In this unitary hypothesis, the first step is the induction of the latent mutant, a cell with wild phenotype which will later exhibit the mutant character in itself or in its progeny. The induction rate is presumed to be relatively independent of growth rate. The second step is the transition of the cell from latent to expressed mutant. During this transition or prior to it, some latent mutants may be lost by death or reversion. In glucose-limited cultures the fraction of latent mutants surviving this transition is, according to the data of Fig. 1, proportional to growth rate; in tryptophan-limited cultures the loss would be constant, perhaps negligible. Evidence supporting this hypothesis has been obtained from study of the kinetics of accumulation of mutants upon the addition of caffeine to glucose-limited chemostats (*5*): the fraction of latent mutants that reach phenotypic expression appears to diminish as growth rate is decreased.

The major difficulty of the error hypothesis is that it cannot explain the time-independence of the mutation rate in tryptophan-limited cultures without further assumptions. This is true also of other hypotheses which are dependent on metabolic rate, such as "errors" arising in the synthesis of genic precursors, or the enzymatic inhibition of these. If the unitary hypothesis is correct, then mutation must result from a rate-independent process, as, for example, a rare alteration or substitution in already-formed genetic material due to a process which is relatively independent of metabolic rate (*6*).

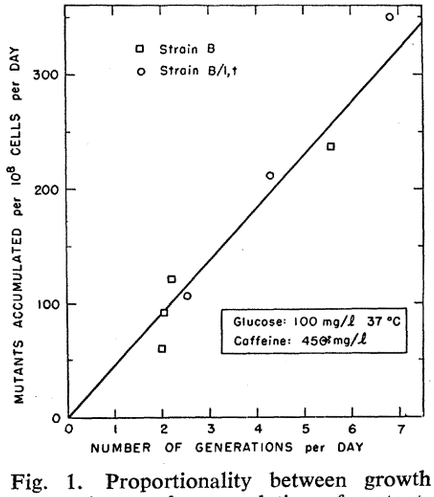

Fig. 1. Proportionality between growth rate and rate of accumulation of mutants to T5 resistance in glucose-limited chemostat cultures.

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Cytological Instability in Tumors of Picea glauca

Abstract. Smear preparations of cells taken from primary explants of normal and adjacent tumor wood of *Picea glauca* showed completely regular mitotic behavior in the normal cells, with the great majority of cells diploid (22 chromosomes), a few tetraploid, but almost none aneuploid. Tumor tissue was extremely unstable, with numbers ranging from 3 to more than 70, with a high proportion of aneuploids but otherwise normal-appearing mitoses. The relation of this mitotic instability to other data on these tumors is pointed out.

Picea glauca and its western equivalent, *Picea sitchensis*, in certain limited areas on the coasts of North America and in a few inland locations, is subject to a massive type of tumorous growth which has occupied the attention of this laboratory for a number of years (*1–3*). The growths are distinguished from most "burls" by their smooth, subglobose character (*4*). No causal organism has been identified. Tumors occur singly or in great numbers on trunks, branches, and roots (*5*). In section they always extend to the pith, indicating that they originate in the bud (*2*). Apparently single cells in the procambium or primary vascular cambium undergo some profound and irreversible change, giving rise to single files of tumor cells which subsequently expand to form chimeric sectors of tumor wood (*2*, *5*). Such transformations are frequently multiple in a particular bud, the resulting adjacent sectors fusing to produce the massive growths observed.

We have concentrated much of our attention on defining the physiology of