References and Notes

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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5. C. S. Pittendrigh, "Perspectives in the study of biological clocks," in *Perspectives in Marine Biology* (University of California Press, Los Angeles, 1959); S. K. deF. Roberts, "Circadian activity rhythms in cockroaches," Ph.D. thesis, Princeton University (1959).
6. V. G. Bruce and C. S. Pittendrigh, *Am. Naturalist* **92**, 295 (1958).
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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*).

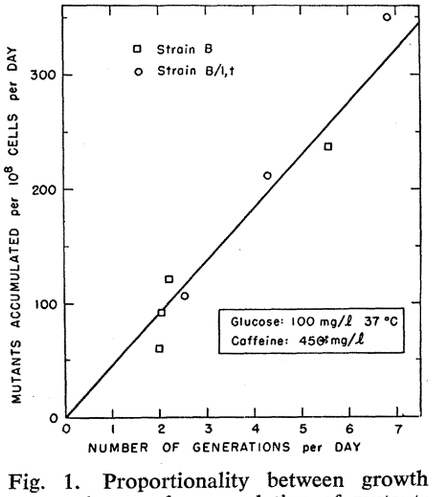

Fig. 1. Proportionality between growth rate and rate of accumulation of mutants to T5 resistance in glucose-limited chemostat cultures.

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References and Notes

1. See, for example, R. Y. Stanier, M. Douderoff, E. A. Adelberg, *The Microbial World* (Prentice-Hall, Englewood, N.J., 1957), p. 391.
2. A. Novick and L. Szilard, *Proc. Natl. Acad. Sci. U.S.* **36**, 708 (1950).
3. Unpublished observations, in collaboration with H. E. Bendigkeit.
4. A. Novick and L. Szilard, *Nature* **170**, 926 (1952).
5. H. E. Kubitschek and H. E. Bendigkeit, manuscript in preparation.
6. This work was performed under the auspices of the U.S. Atomic Energy Commission. It is a pleasure to acknowledge discussion with, and advice of, David A. Yphantis.

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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