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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2 November 1959

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*).

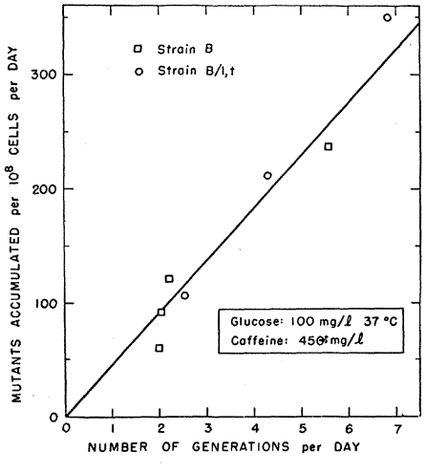

Fig. 1. Proportionality between growth rate and rate of accumulation of mutants to T5 resistance in glucose-limited chemostat cultures.

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2 November 1959

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

tumor tissues as contrasted with the normal. One possible origin of physiological instability is to be sought in the mitotic behavior. Rapid-growing, friable tissue cultures are well adapted to investigation of mitoses by squash methods. We have initiated such studies, using a modified Feulgen method followed by Belling's acetocarmine. We have used newly formed cells arising on primary explants grown for 1 to 2 months in culture. Tumor wood and normal wood for initiation of cultures was taken from adjacent areas on the same tree; several affected trees were used. This report presents the results of a cytological study of 1000 somatic nuclei from dividing callus cells. Three hundred and twenty were from cultures of normal wood, 680 from tumor wood.

The diploid chromosome number for most conifers (*Cupressus, Juniperus, Metasequoia, Sequoia, Taxodium, Torreya*) is given by Delay (*6*) as 22 or a multiple thereof (44, 66). Seitz (*7*) and Sax (*8*) give 24 ($= 2 \times 12$) for several species of *Picea*.

The chromosomes are long and slender, making counts difficult. (Fig. 1, *A,B,C*). Of the normal cells of *Picea glauca* examined, 51 percent clearly had 22 chromosomes (Fig. 1*B*, Fig. 2) and an additional 15 percent were close enough to this number to be within the range of probable counting errors. Another 5 to 8 percent were clearly tetraploids with 44 chromosomes. This proportion of tetraploids is not unusual in many normal tissues. Only a very small percentage appeared to be aneuploids, and these counts might have been due to errors. The mitotic figures were regular, without lagging chromosomes or bridges (Fig. 1*D*). No pycnotic nuclei were observed. The cultures from normal wood thus appear to be made up of cells which are essentially uniform and regular in their behavior. We are justified in drawing conclusions from a relatively small number of such cells.

The behavior of the tumor cultures was different. While no pycnotic nuclei were observed and while there was no evidence of bridges, there was nevertheless a very high percentage of mitoses which were aneuploid. Gymnosperm cells, even in the meristematic state, have a tough pellicle and are thus not subject to the tearing which in more fragile materials permits the loss of chromosomes and renders counts suspect. Mitoses which seemed to be intact gave numbers ranging from as low as *three* recognizable chromosomes to uncountable complexes of more than 70. The chromosomes appear to be somewhat thicker than in normal wood, although in the absence of precise measurements this impression remains sub-

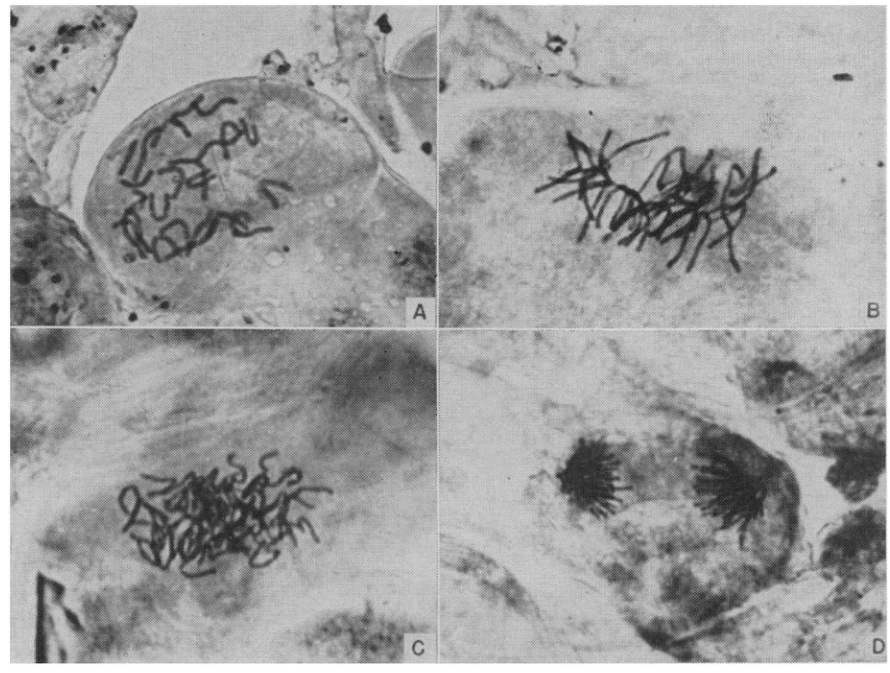

Fig. 1. Mitoses in cells of primary tissue cultures from *Picea glauca* (about ×200). *A*, Diploid metaphase, 22 chromosomes (tumor). *B*, Early anaphase, two sets of 22 chromosomes (normal). *C*, Metaphase, tetraploid, 44 chromosomes (tumor). *D*, Telophase (normal); note the completely regular grouping, and the absence of lagging chromosomes or bridges.

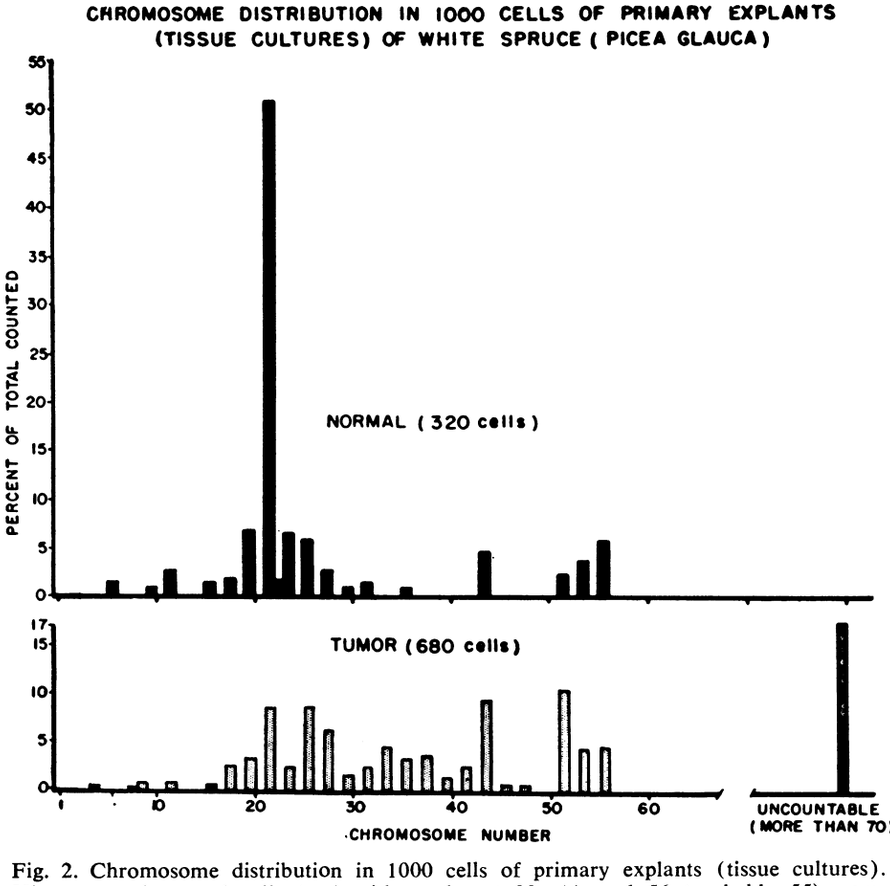

Fig. 2. Chromosome distribution in 1000 cells of primary explants (tissue cultures). Histogram of normal cells (top) with modes at 22, 44, and 56 (probably 55), compared to tumor cells (bottom) with major modes at 22, 26, 44, and 52 and minor modes at 28, 34, 54, and 56 chromosomes.

jective. While tumor cells do not appear to be significantly larger than normal, nor more irregular in size, the preparations give the impression of many *more* mitoses in tumor cultures than in normal.

The difference in mitotic behavior between normal cells and adjacent tumor cells is certainly marked. Figure 2 shows that in normal tissue the modes at 22 (diploid) and 44 (tetraploid) are clear-cut. The same modes reappear in the tumor tissue, but there are additional modes at 26 and 52 and minor concentrations at 28, 34, 54, and 56. Statistical analysis of our data by the usual *t*-test shows that the differences between the two sets are certainly significant at the 95:5 level and probably so at 99:1 (*9*). The modes mentioned are all real since they reappear whether we plot the 680 tumor nuclei as a group or divide them chronologically, according to dates of counting, into two groups of 349 and 331, respectively. Even the minor variations at 18, 20, 34, 36, and 38 fall outside the band of statistical uncertainty. The extreme cytological instability of tumor wood as contrasted to the high degree of uniformity in adjacent normal tissue emerges from this study with great clarity.

These findings are entirely consonant with our earlier results on the nutritional behavior of normal versus tumor cells in tissue culture (*3*). It will be recalled that normal wood was consistent in its behavior on a given nutrient while tumor wood was variable in growth rate, growth pattern, degree of solidity or friability, and color, often throwing irregular sectors in a single culture.

The variable cytology of the spruce tumors is in marked contrast to the stable cytology of the best known of other plant tumors, the "crown gall." Levine (*10*) found both chromosome number and mitotic behavior in crown gall to be quite normal. Although tetraploids and octoploids were fairly common, there was no aneuploidy, polyploidy evidently resulting from simple failure of cell division to follow mitosis. Kupila (*11*) found the same to be true of crown gall of sunflower, pea, and tomato. She concluded that only normal diploid cells took part in propagation of the tumors. Partanen (*12*) also found, by photometric measurements of deoxyribonucleic acid, no evidence of aneuploidy in crown gall of *Helianthus* and even less polyploidy than in normal tissues.

The results also differ from those described by Torrey (*13*) in normal pea-root cultures in which there appears to be a progressive polyploidy with selection of the tetraploids on certain culture media, but again without aneuploidy.

The cytology of these tumors most closely resembles the later stages noted by Partanen in cultures of fern gametophyte tissue (*14*) and by Hauschka (*15*) and others in animal tissue cultures and ascites tumors. These authors have noted a gradual loss of euploidy, that is, a spreading of chromosome number with time. This deviation they have attributed to the "abnormal" conditions involved in growth in vitro and in the ascites form, to the removal of the selective mechanisms which tend to eliminate those deviations which may occur in the body. This explanation can scarcely apply to spruce callus maintained for only brief periods as primary explants and in which normal and tumor tissues grown under identical conditions behave so differently.

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30 October 1959

Radiometric Analysis of Tritiated Organic Compounds by Means of Vapor Phase Chromatography

Abstract. An analytical method, involving the gas chromatographic separation and the quantitative measurement of tritiated volatile compounds, was studied. The method has been successfully employed to detect traces of carrier-free tritiated substances.

Identification of trace constituents in a mixture of radioactive compounds and the measurement of their radioactivity has become increasingly important in the preparation and application of labeled substances. Volatile compounds can be efficiently fractionated by means of vapor phase chromatography, and the radioactivity can be determined in the effluent gas.

This method has the basic advantage of detecting all the radioactive components of the mixture to be analyzed, and hence constitutes a powerful means for the separation and dosage of carrier-free compounds.

In the case of tritium-labeled substances, the low energy of β-particles precludes the use of Geiger counters or scintillation heads immersed in the effluent gas, which have been employed, for example, in the dosage of volatile compounds containing Br^{80} and Br^{82} (*1*), or thin-walled Geiger counters, useful in the case of C^{14}-labeled substances (*2*). The continuous condensation of emergent vapors in a cooled solution of organic scintillator, satisfactory for C^{14} (*3*), would result in low efficiency and high background in the use of tritiated compounds. In view of these drawbacks, efficient use can be made only of proportional counters (*4*) and ionization chambers (*5*, *6*). This paper describes a technique, based on the use of a flow ionization chamber, suitable for the separation and determination of the radioactivity of tritiated compounds having boiling points up to 150°C.

The purpose of the present investigation was to develop a technique for the measurement of radioactivity, independent, within a wide range, of the particular gas chromatographic conditions such as the flow rate of the carrier gas, the column temperature, the nature and the amount of the compounds to be analyzed, and so forth.

According to this technique, the effluent gases from the chromatographic column, having been passed through a conventional thermoconductivity cell for the usual analysis of compounds present in macroscopic quantities, are diluted with a current of the carrier gas in an appropriate mixer. The dilution is effected in such a way that the total flow rate may be adjusted to a certain fixed value at which the ionization chamber is calibrated. The radioactivity measurements are consequently independent of the carrier gas flow rate in the chromatograph. Besides, the relatively large volume of the gas in which the vapors leaving the column are diluted eliminates the necessity of heating the ionization chamber, which is generally required at low flow rates in order to prevent the condensation of compounds with high boiling points. Substances such as chlorobenzene and anisol (boiling points 132° and 155°C, respectively) have been, in fact, satisfactorily analyzed. The possibility of maintaining the cham-