deficiency may be explained by accepting the hypothesis of either a complete precipitation of all α-chains in vivo, or their combination with free γ-chains after lysis of the cells. Indeed, a small excess of γ-chains has been demonstrated in hemolysates of β-thalassemia homozygotes (*11*). This excess is probably present in only a portion of the total number of red cells. Rapid formation of complete hemoglobin molecules has been observed after the addition in vitro of β_4- or γ_4-hemoglobin to hemoglobin-containing α-chains (*12*). Therefore the release of any free γ-chains after lysis is expected to lead to the formation of $\alpha_2\gamma_2$ molecules, hemoglobin F, resulting in reduction or complete disappearance of the α-chain zone. In spite of these exceptions, there appears to be a gradation in the number of α-chains detectable by electrophoresis, samples from splenectomized homozygous β-thalassemias exhibiting usually the largest amounts, from nonsplenectomized cases smaller quantities, and from hemoglobin S–β-thalassemias only traces. A similar gradation occurs in the number of cells carrying inclusion bodies (*8*). In simple β-thalassemia trait the excess of α-chains may be below the limits of sensitivity of our methods, but other interpretations could also apply.

Our findings lend support to the hypothesis that the α-chain may be synthesized or released (or both) independently of the presence of β-, γ- or δ-chains. However, the degree of this independence cannot be estimated unless the total excess of uncombined α-chains, precipitated and nonprecipitated, is accurately measured. The observations also suggest that under certain conditions the hemoglobin pattern of hemolysates may be altered by the combination of hemoglobin fractions occurring in different lines of cells; they further emphasize the importance of alterations that arise during the process of obtaining hemoglobin solutions or during storage.

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9. Benzidine base (100 mg) in 80 ml of distilled water, 10 ml of 1.5*M* acetic acid, 10 ml of 1.5*M* sodium acetate, and 0.5 ml of 30 percent hydrogen peroxide were added just prior to use.
10. We have observed an analogous deleterious effect when chloroform, instead of toluol, is used for the clarification of hemolysates; it causes complete precipitation of hemoglobin β_4 (hemoglobin H) as well as of the α-chain component.
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Somatic Mitoses in Cells of Picea glauca Cultivated in vitro

Abstract. *The cytology of one strain of tumor tissue from the spruce tree,* Picea glauca, *grown in vitro for more than a year was examined. The cells of this strain are characterized by a uniform chromosome number of 24, and the strain appears to be quite stable. The implications of the results of this study and previous studies on similar material are discussed.*

In 1960 de Torok and White (*1*) reported that cells of a tumor of *Picea glauca* showed an extreme cytological instability; the chromosome numbers varied between 4 and more than 70 without showing clear modes. Cells of corresponding normal tissues, on the other hand, were quite stable, with most mitoses showing 22 chromosomes, a few tetraploids, and no more aneuploids than one might expect from unavoidable counting errors.

This result appeared to be consistent with the evidence of nutritional and morphological instability reported earlier by Reinert and White (*2*) for a corresponding tumor strain. The chromosome number of 22 differed from the number 24 reported for *Picea glauca, P. pungens,* and *P. abies* by Sax and Sax (*3*). And the strain of tumor tissue currently grown in this laboratory (1961–1963) has been quite stable both morphologically and nutritionally (*4*). In view of these discrepancies it has seemed desirable to reexamine the question.

The materials and methods used by de Torok and White differed somewhat from those used here. Their chromosome counts were made on primary explants, that is, on cells emerging as callus from the cambium of bits of wood transferred directly from the tree to a nutrient substratum while still remaining partially dependent on nutrients drawn from the explants. None of the explants had been out of the tree for more than 2 months; they might reasonably be expected to represent the conditions in the tree itself. They were, however, treated with dichlorobenzene before squashing and were subjected to mild hydrolysis with HCl before staining. What cytological effects this treatment may have had, other than the presumed arrest of mitoses at the metaphase, is not known. The material described in this report was drawn from stock cultures of tumor cells of an isolation which had been cultivated for 1½ to 2 years (40 to 50 passages) and was thus far removed from the parent tree and stabilized on a completely defined nutrient (*4*). The cells were treated briefly with colchicine, without hydrolysis, and were compared with untreated controls. The strain, unlike other strains studied previously, appeared to be morphologically and nutritionally stable.

Picea chromosomes are long, and are difficult to examine unless they are shortened and spread by the initial treatment. After testing several fixatives that would shorten the chromosomes before the cells were killed, under a variety of conditions, colchicine was chosen as most satisfactory. The procedure described here consistently yielded good results. The tumor tissue was placed in 1 percent colchicine and kept in the dark for 6 hours; it was transferred to acetic alcohol, 3:1, at room temperature for 24 hours, and then hydrolyzed in 1*N* HCl for 20 minutes at 60°C. It was stored in 50 percent alcohol and stained by the squash method in acetocarmine.

The tumor tissue used had been in cultivation for a minimum of 40 passages or approximately 1½ years. The individual cultures for counting were chosen at random from stocks which were routinely subcultured at 2-week intervals. Preliminary work indicated that the greatest percentage of cells were dividing during the hours of 4:00 A.M. to 8:00 A.M. during the first 6 days of the 14-day passage. For optimum counting efficiency most of the cultures examined were taken from the medium

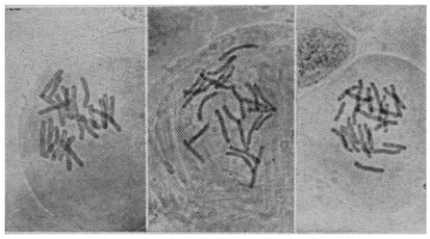

Fig. 1. Metaphase plates from three typical cells of *Picea glauca* tumor grown in vitro.

during these periods. Later work by Kessel (*5*), however, based on much larger numbers, failed to substantiate any preferred time of day or any date earlier than about 21 days.

When properly squashed, individual chromosomes were easily distinguishable (Fig. 1), and only occasionally was there difficulty in counting. To insure that the colchicine was not altering the normal cell structure, several mitotic figures were obtained from untreated material. In some of the controls, and occasionally in the colchicine-treated cultures, obscurities made the use of a camera lucida desirable. In these cases the fiugres were drawn and the chromosomes were then counted from the drawings.

The chromosome numbers are shown in Fig. 2. Of the 300 cells counted, 270 (90 percent) had a (diploid) chromosome number of 24. This is the number given by Sax and Sax for *Picea glauca, P. pungens,* and *P. abies* (*3*). There were seven tetraploid nuclei (48 chromosomes) which is probably not an abnormally high number of polyploids (2.3 percent). The remaining 23 were distributed among 9 aneuploid numbers and may represent counting errors.

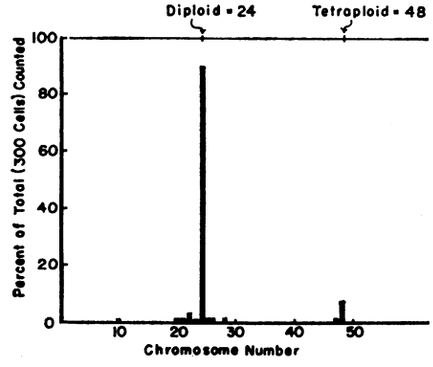

Fig. 2. Distribution of chromosome numbers expressed as percent of the 300 cells counted. Ninety percent of the total have the diploid number 24.

The one cell with ten chromosomes remains somewhat of an anomaly. The obvious explanation would be that the cell wall was broken in the process of squashing and some of the chromosomes were lost from the cell. Since this cell was so unusual, it was examined with special care. No detectable break in the wall was found.

The divisions appeared normal with no bridging or lagging of chromosomes. During the late anaphase and early telophase, one to four chromosomes would frequently spread out ahead of the rest of the migrating chromosomes. This spreading is not peculiar to the tumor cells, but it is also found in microspore cells and in cells from vegetative buds taken from normal-appearing spruce trees.

The mitoses in this strain of spruce tumor cells appear to be completely normal, uniform, and stable, showing none of the irregularities noted in another strain by de Torok and White (*1*). This stability matches the physiological stability of the strain. It also corresponds to the stability noted in strains of crown gall tumors reported by Levine (*6*), Kupila (*7*), and Partanen (*8*), and the diploid number of 24 is the same as that reported by Sax and Sax (*3*) for normal tissues of this genus.

Torrey (*9*) has reported a polyploid drift in cultures of normal pea root tissue grown in a complex nutrient. He attributes this drift to selective influences by the nutrient. Similar drifts have been reported repeatedly in animal tissue cultures (Ford *et al., 10*) and they are suspected of playing some role in the emergence of neoplasia. In this respect the instability reported by de Torok and White was not unexpected.

The preparations made by de Torok and White were from primary explants which had been in culture for no more than 1 to 2 months and which had not been subcultured. Their results should represent as nearly as possible the conditions within the tree. The strain considered in this investigation had been under cultivation for more than 40 passages. If a drift toward polyploidy, either innate or under the impact of nutrient influences or selection, had been a factor in the development of this strain, some indication would be expected in this older tissue. There has been none. Either this strain was innately stable or whatever selection there may have been was in favor of the diploids, not in favor of polyploids or aneuploids. This tissue is growing rapidly, uniformly, and apparently in quite normal fashion cytologically. Neither cytological instability nor polyploidy is an essential characteristic of the tumorous state in this tree. Such instability as that observed by de Torok and White was fortuitous, not general.

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Bile Duct Restoration in Rana pipiens after Ligation of the Hepatoduodenal Ligament

Abstract. *A single cotton ligature closed the common bile duct, the hepatic artery, and the hepatic portal vein. The continuity of the bile duct was restored in 14 of 69 cases, the restoration process being associated with mitosis in the bile duct epithelium. There was evidence that the continuity of the blood vessels was also restored.*

The data for this report emerged from a projected experiment on liver homografting. Williams (*1*) described successful grafts made in *Triturus viridescens.* I had proposed transplanting a liver lobe to an ectopic site in the hope that it would acquire vascular adhesions with the host tissues. In order to assess the survival value of such a graft, it would be necessary to determine the average life expectancy in