preceding each experiment from Lidyhites Pond, New Haven, Conn. In the laboratory, leaves with healthy, functional traps were cut from the plant stem and cleaned of entangling filamentous algae. Secondary or small bladders were removed from the leaf periphery, leaving ten large traps near the midvein of each leaf. Distinctions of trap type, small or large, were made according to the scheme of R. L. Wallace [*Freshwater Biol.* 7, 301 (1977)].

16. Mean number of captures per antenna or bristle-set is based on treatment comparisons. For example, the contribution of 1B was calculated from the summation of the subtractions of the mean of treatment 2 (T2) from T1, T4 from T3, T5 from T4, and T7 from T6, and the resultant divided by 4 (Table 1). The variance between the compared treatments was significant for the contribution of 1B ($P < .05$), 2B ($P < .01$), 1A ($P < .005$), and 2A ($P < .005$) (Bonferroni multiple comparison t statistic). Bonferroni inequality was applied to the levels of significance of Student's t test according to R. J. Harris [*A Primer of Multivariate Statistics* (Academic Press, New York, 1975), pp. 98-101].
17. Antenna length, including branches and bristle-sets, was measured on 258 randomly chosen traps: antenna, 2.92 mm ± 0.14 mm; bristle-set, 2.29 mm ± 0.11 mm (mean ± standard error).
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25 July 1978; revised 28 August 1978

Radiation from Tritiated Thymidine Perturbs the Cell Cycle Progression of Stimulated Lymphocytes

Abstract. *Tritiated thymidine was found to affect the cell cycle progression of phytohemagglutinin-stimulated human lymphocytes. By means of flow cytometry a statistically significant increase in the G_2 and M phases of the cell cycle was observed in cultures with low concentrations of tritiated thymidine added 18 hours before the cultures were harvested.*

Tritiated thymidine is widely used for labeling newly synthesized DNA in proliferating cells. The incorporated radioisotope can be rapidly measured by liquid scintillation counting and autoradiography. However, in the late 1950's evidence was obtained that incorporated [^{3}H]thymidine suppressed cell proliferation (*1*). Colony count inhibition (*2*), quantitation of chromosome aberrations (*3*), inactivation of transforming DNA (*4*), production of mutations (*5*), and the induction of DNA strand breaks (*6, 7*) have all been used to demonstrate the deleterious effects of [^{3}H]thymidine, and have shown that these effects are a function of specific activity, concentration, and exposure time.

Flow cytometry (FCM) has become a popular method for quantitating DNA content on a per cell basis (*8*). Cells or nuclei stained with a fluorescent dye such as propidium iodide (PI) are suspended in a fluid stream that passes through an argon-ion laser beam (488 nm). As the cells pass through the laser, at rates of 100 to 3000 cells per second, a photomultiplier tube detects the amount of fluorescence emitted per particle, and this information is collected on a multichannel analyzer or computer. When a relatively specific dye for double-stranded nucleic acid, such as PI, is used, the resultant histogram displays the number of cells on the ordinate against the fluorescence intensity (proportional to DNA content) on the abscissa. These histograms are therefore a "snapshot" representation of the proportion of cells distributed about the phases of the cell cycle and can be used to estimate the percentages of cells in G_0+G_1, S, and G_2+M (*9*). Also, groups of histograms (for example, quadruplicate cultures of two test groups) can be averaged and compared statistically (*10*). This statistical comparison shows areas of significant differences between the averaged histograms.

We have used FCM to study cell cycle changes caused by the addition of [^{3}H]thymidine to phytohemagglutinin-stimulated lymphocyte microcultures. These 0.2-ml cultures were made up of human peripheral blood lymphocytes separated by Ficoll-Paque (Pharmacia). Each well contained 1×10^5 cells suspended in Medium 199 buffered with 0.23 percent bicarbonate and supplemented with 2m*M* L-glutamine, 20 percent heat-inactivated fetal calf serum, and a 1× antibiotic-antimycotic mixture (Gibco). Lymphocytes were stimulated with optimum PHA concentrations (PHA-P, Difco) for 90 hours and [^{3}H]thymidine was added 18 hours or 20 minutes before the culture was harvested. Cultures were treated with 0.03 ml of medium with or without [^{3}H]thymidine of specific activity 1.9 Ci/mmole (Schwarz/Mann), or 50 Ci/mmole (New England Nuclear) or $2.6 \times 10^{-7}M$ unlabeled thymidine (Sigma) (see Figs. 1 to 3). Flow cytometric analyses were performed on PI-stained nuclei (*11*). Each FCM analysis shown (Figs. 1 to 3) is a statistical comparison between two groups of quadruplicate smoothed, translocated, and averaged histograms (*10*). Figure 1 shows the differences observed when 0.1 μCi of [^{3}H]thymidine (1.9 Ci/mmole) was added per culture, or when medium alone was added for the last 18 hours. The 18-hour labeling period was used because labeling times of 12 to 24 hours are commonly used with PHA-stimulated lymphocyte cultures. The cultures exposed to [^{3}H]thymidine showed a marked buildup of cells in G_2+M with a reduced proportion of cells in S. These differences in S and G_2+M were significant to $P < .01$. To determine whether the effect elicited by [^{3}H]thymidine was due to the concentrations of thymidine employed, we tested unlabeled thymidine at the same concentration ($2.6 \times 10^{-7}M$).

Figure 2 compares the effects of adding unlabeled thymidine with the effects of adding medium containing no thymidine. Only two points were significantly

Table 1. The incorporation of [^{3}H]thymidine by cultures exposed to either 0.1 μCi of [^{3}H]thymidine (1.9 Ci/mmole) for 18 hours or 1.0 μCi (50 Ci/mmole) for 20 minutes. At 90 hours the microcultures were harvested onto glass fiber filters by means of a Titertek Cell-Harvester (Flow Labs). Dried filters were counted (3255 Packard Scintillation Spectrometer) in 5 ml of scintillation fluid containing 8.0 g of butyl-PBD [2-(4′-tertiarybutylphenyl)-5-(4″-biphenyl)-1,3,4-oxadiazole] and 0.5 g of PBBO [2-(4′-biphenyl)-6-phenylbenzoxazole] (Beckman) per liter of Scintanalyzed Toluene (Fischer). The stimulation index (SI) was calculated by the formula: SI = [cpm (with PHA) − cpm (no PHA)]/cpm (no PHA), where cpm is counts per minute.

Amount of [^{3}H]thymidine added (μCi per culture)	PHA added	Radioactivity (count/min)*	Stimulation index
	Cells exposed for 18 hours		
0.1	−	132 ± 27	
0.1	+	42,190 ± 1,500	319
	Cells exposed for 20 minutes		
1.0	−	158 ± 54	
1.0	+	42,825 ± 2,377	270

*Mean ± standard deviation.

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different between the two averaged histograms. This indicated that unlabeled TdR was not responsible for the block (*12*) in G_2+M.

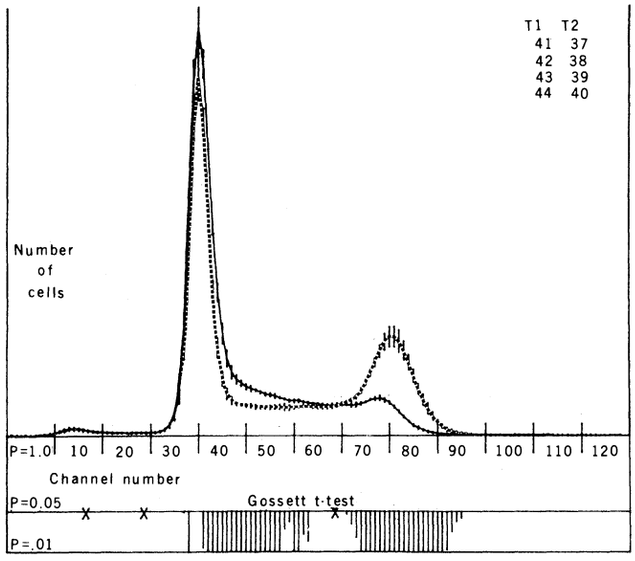
Figure 3 shows that short-term exposure to 1 μCi of [^{3}H]thymidine per culture (50 Ci/mmole) does not significantly perturb cycling lymphocytes when added 20 minutes before the culture is harvested. Cultures exposed to [^{3}H]thymidine for a very short period are compared with cultures to which only medium was added. The FCM analysis shows that such short-term exposure to [^{3}H]thymidine of high specific activity does not significantly alter lymphocyte cell cycle progression. Unstimulated cultures without PHA showed only sparse numbers of cells in S and G_2+M and were therefore not included in the figures.

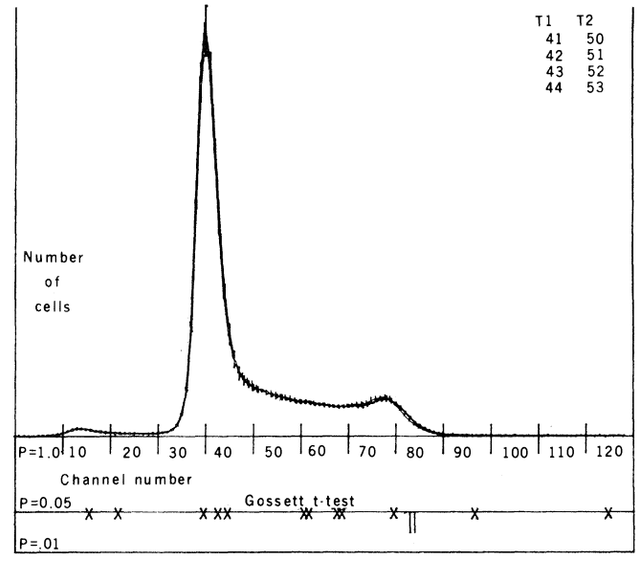
Table 1 is a summary of the [^{3}H]thymidine incorporation data. When 0.1 μCi of [^{3}H]thymidine (1.9 Ci/mmole) was added to cultures for the last 18 hours, an average of 42190 count/min were incorporated per culture. By exposing cultures to 1 μCi of [^{3}H]thymidine (50 Ci/mmole) for 20 minutes prior to culture termination, similar amounts of tritium were incorporated (42825 count/min). Thus, although the same quantities of radioactivity were incorporated in both tests, an extended exposure time was necessary for the expression of a block in G_2+M.

Our results show that the block in G_2+M was caused by [^{3}H]thymidine and not unlabeled thymidine at the same concentration. This inhibition of lymphocyte cycling in the G_2+M phases was also significant ($P < .01$) when as little as 0.01 μCi of [^{3}H]thymidine (1.9 Ci/mmole) was added per culture for the 18 hours before the cells were harvested (*13*). Similar significant changes ($P < .01$) in the FCM analysis were evident in 72-hour cultures when 0.1 μCi of [^{3}H]thymidine was added for the last 18 hours. (*13*).

The incorporation of [^{3}H]thymidine into the DNA of cycling cells results in strand breaks (*6, 7*). This form of radiation damage is caused largely by ^{3}H β particle emission and is dependent on the location of ^{3}H in the cell. Intranuclear ^{3}H results in more damage to DNA than ^{3}H located in the cytoplasm or media (*7*).

We believe the block observed in G_2+M by the FCM analysis was caused by intranuclear [^{3}H]thymidine. The possibility that a contaminant in the [^{3}H]thymidine was responsible for the block in G_2+M is highly unlikely. Different lots of [^{3}H]thymidine from different companies have been tested (*13*) and the same effect has always been found. Also, we have tested up to 10 μCi of ^3H_2O per culture and have not observed any significant perturbation of





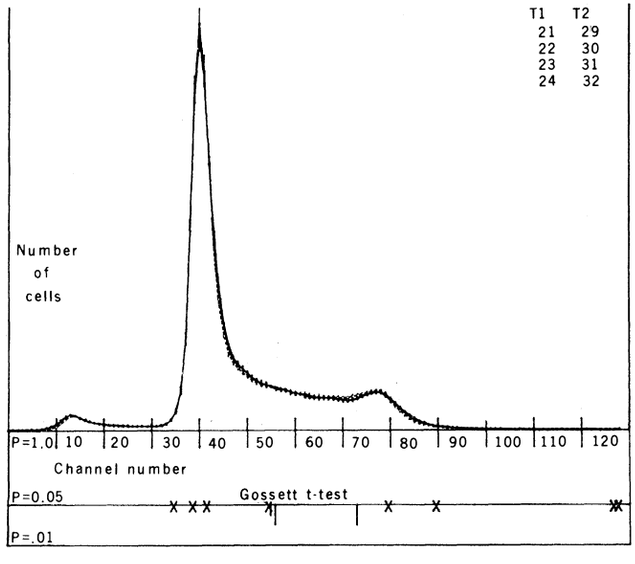


Fig. 1 (top left). Flow cytometric analysis of cultures to which either 0.1 μCi [^{3}H]thymidine per well (1.9 Ci/mmole) (broken line) or medium alone (solid line) was added 18 hours before the cells were harvested at 90 hours. Each histogram shown is an average of four histograms from a set of quadruplicate samples. One standard deviation above and below the averaged points are represented by vertical lines. A channel-by-channel Gossett Student's *t*-test was performed in which areas of significance (from $P = .05$ to $P = .01$) are shown by the vertical lines underneath the histograms being compared. Fluorescence intensity is displayed on the abscissa and number of cells (nuclei) on the ordinate. Fig. 2 (top right). Statistical comparison of cultures analysed by FCM. Either $2.6 \times 10^{-7}M$ thymidine (broken line) or medium alone (solid line, superimposed) was added to the cultures 18 hours before the cells were harvested at 90 hours. See Fig. 1 for other details. Fig. 3 (bottom left). Flow cytometric analysis of cultures exposed to 1μCi of [^{3}H]thymidine (50 Ci/mmole) for 20 minutes before the cells were harvested (broken line) and cultures to which medium alone was added for the last 20 minutes (solid line, superimposed). Cultures were harvested at 90 hours. See Fig. 1 for other details.

lymphocyte cell cycle progression (*13*). This is 1000 times the [^{3}H]thymidine radioactivity per culture necessary for the observed radiation effects. The fact that ^3H_2O did not block lymphocytes in G_2+M at the high concentrations used lends further support to the concept that the block was caused primarily by intranuclear tritium. The question of whether the β particles emitted from intranuclear tritium would interfere with PI intercalation into DNA is answered by the results in Table 1. The fact that the amounts of intranuclear tritium were similar for both test groups, namely, cultures exposed to 0.1 μCi of [^{3}H]-thymidine (1.9 Ci/mmole) for 18 hours or to 1.0 μCi of [^{3}H]thymidine (50 Ci/mmole) for 20 minutes before being harvested, whereas the FCM analyses differed, suggests that the incorporated radioisotope does not affect dye intercalation.

Ehmann *et al.* (*14*) have also shown by FCM that radiation from [^{3}H]thymidine causes a G_2+M block in various tissue culture lines. Their results in combination with ours demonstrate the consistency with which the perturbation of cell cycle progression by [^{3}H] thymidine is observed.

A block in G_2+M such as the one we describe is evidence of cellular injury and should be taken into account in the testing of compounds that inhibit proliferating cells. The radiation damage promoted by intranuclear tritium combined with the inhibitory action of another agent (for example, chemotherapeutic drugs, chalones), might promote a synergistic or antagonistic effect, thereby distorting the results. In cell cycle studies and in investigations of agents perturbing cycling cells it might be possible to avoid these radiation effects by exposing the cells to [^{3}H]thymidine for short time periods.

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12. The words block and inhibition of lymphocytes in the G_2+M phases refer to the observed elevation in the G_2+M peak when [^{3}H]thymidine is added. Since the FCM analysis provides only a static representation of the distribution of cells about the cell cycle phases, the elevation in the G_2+M peak might reflect some phenomenon other than a block (for example, cells in G_1 and S could have been accelerated relative to the cells in G_2+M). However, the possibility that the increase in the number of cells traversing G_2+M, when exposed to [^{3}H]thymidine, was not due to a block is remote. For this reason the terms block and inhibition are used in the text to denote the elevation in the G_2+M peak.
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26 June 1978; revised 21 August 1978

Retinoblastoma with 13q− Chromosomal Deletion Associated with Maternal Paracentric Inversion of 13q

Abstract. *A girl with sporadic unilateral retinoblastoma and mental retardation has an interstitial deletion in the long arm of chromosome 13. Her mother has a paracentric inversion of one chromosome 13; the deleted chromosome 13 in the daughter is derived from the mother's normal chromosome 13.*

Although retinoblastoma is an uncommon eye tumor in humans, it has proved to be of exceptional interest as a model system for understanding possible genetic implications for human tumors (*1*). In some instances the tumor is sporadic, whereas in others it is the result of dominant inheritance. A third small group has been found to have a partial deletion of the long arms of chromosome 13 (13q−). While evaluating a patient who falls into this last group, we found that her mother has a paracentric inversion of the long arm of one chromosome 13. To our knowledge, this is the first instance in which a parent of a child with the 13q− deletion has been found to have a chromosome abnormality affecting a chromosome 13. This inversion of the chromosome in the mother appears to have resulted in the partial deletion of the long arm of chromosome 13 in her

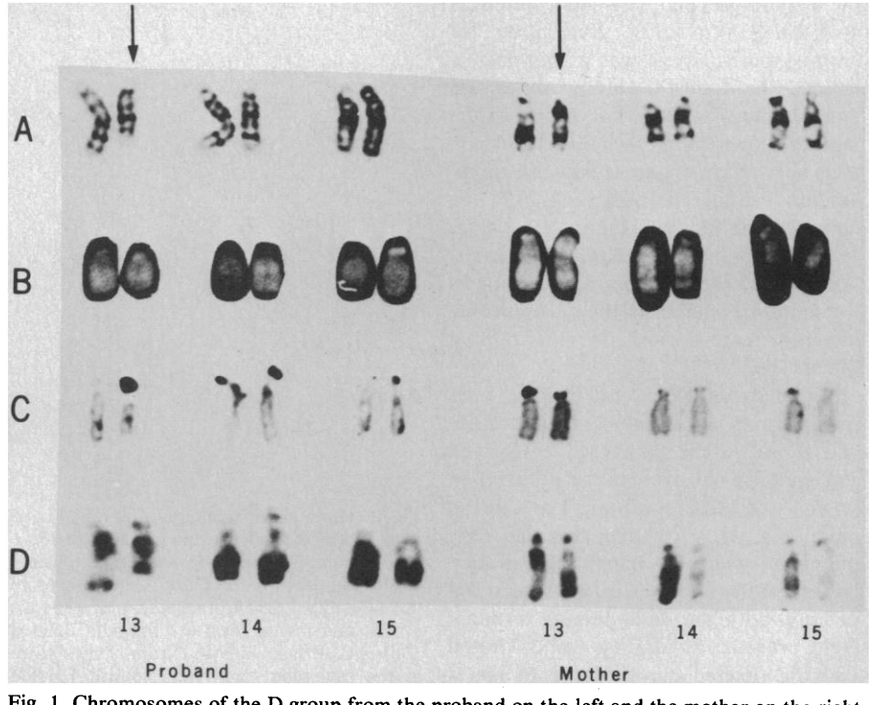

Fig. 1. Chromosomes of the D group from the proband on the left and the mother on the right. Note the deleted chromosome 13 in the proband (arrow) and the inverted chromosome 13 in the mother (arrow). Chromosome stains used are: (A) trypsin-Giemsa banding; (B) quinacrine fluorescence; (C) silver stain; (D) reverse (R) banding. Comparison of the mother's chromosomes 13 with those of the daughter indicates that the daughter's 13q- is derived from the mother's normal chromosome 13 rather than the inverted 13.

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