EFFECT OF X-RADIATION ON THE GROWTH AND DIVISION PROCESS OF TETRAHYMENA PYRIFORMIS

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The inhibitory effect of X-irradiation on DNA synthesis is well established (Lajtha, 1960). However, little is known how this inhibition relates to cell growth. In experiments reported here cell replication in Tetrahymena pyriformis was blocked by sublethal doses of X-irradiation shortly after the beginning of irradiation. However, small (initially young) cells grew at the same exponential rate as large (old) cells and the volume distribution remained lognormal indicating that cell growth is significantly independent of the division cycle. In contrast, DNA synthesis ceased at the beginning and was resumed during irradiation with exponential kinetics indicating an adaptation of unknown character.

Tetrahymena pyriformis GL were cultured in a sterile medium at 28°C as described previously (Schmid, a, 1966). Ten ml fractions of a mass culture were transferred to plastic flasks with wide orifice pipettes and the flasks returned to a 28.0°C incubator. Plastic flasks were irradiated at 28.0°C in a plastic constant temperature chamber at a dose rate of 250 R/min, X-rays were generated by a 250 KVP Westinghouse X-ray machine operated at 15 mA (HVL 0.35 mm copper). Tritiated thymidine (^{3}H-TdR 1.9 c/mMole) was purchased from Schwarz Bioresearch, Inc., Orangeburg, New York. In experiments in which incorporation of tritiated thymidine into DNA was measured, the precursor was added to the mass culture at 1μc per ml. Incorporation of ^{3}H-TdR was measured by liquid scintillation counting on DNA extracted by a Schmidt-Tannhauser method (Schmid, 1963). Counting was

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performed on a Packard liquid scintillation spectrometer (Model 4322). A minimum of 5000 counts above background per sample was recorded. Tritiated toluene was used as an internal standard. At frequent time intervals before, during and after irradiation, cell numbers and volume distributions were measured with an electronic cell counter. Evaluation of volume distributions were performed as reported previously (Schmid, a,b, 1966).

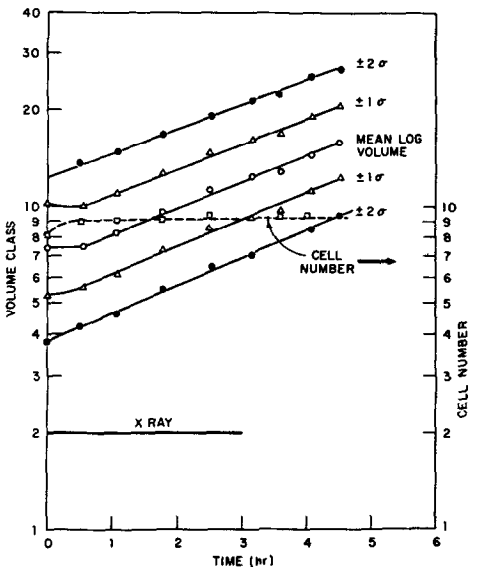

Fig. 1. Cell volumes and cell numbers of Tetrahymena pyriformis. Between 0 and 3 hrs the culture was irradiated at 250 R/min. ● volume class for plus and minus two standard deviation from the mean log volume. Δ plus and minus one standard deviation from the mean log volume. 0 mean log volume.

As shown in Fig. 1, on exposure to X-rays, cell replication ceased within 30-60 min. During the period of inhibited cell replication individual cells grew exponentially like cells whose division processes were blocked by a change in temperature. (Schmid, a, 1966). Furthermore, the rate of growth of small and large cells was the same and the volume distribution remained lognormal as can be seen from the parallel nature of the lines for plus and minus 2 standard deviation from the mean log volume. Fig. 1 suggest that no control mechanisms for limitation of cell growth exists, since after division block initially small cells, which originated from recently divided cells grew at the same rate as

large cells, which were initially close to division. However, in a replicating culture, cell growth is limited by division. These experiments also indicate that the growth rate during X-ray exposure is equal to that in the period following radiation exposure. Since Scherbaum(1959) and Christensson(1959) have shown that increase in volume in Tetrahymena is reflected in an increase of dry weight and protein content with time, the results suggest that X-rays, at the dose rate used, do not significantly interfere with overall growth processes of Tetrahymena cells. Formation of large cells following exposure to radiation has been documented repeatedly and various mechanisms have been proposed (Kohn and Fogh, 1959, Painter and Robertson, 1959, Whitmore, et.al. 1958). Our data demonstrate that the formation of large cells is due to interference with cell replication caused by sublethal doses of radiation. However, formation of large cells is not a specific effect of radiation since any agent which inhibits cell replication for a significant time interval will cause formation of giant cells. Our observations thus demonstrate a substantial independence of growth processes from division processes.

When incorporation of tritiated thymidine into DNA was used as a measure of nuclear metabolic activity the tracer was added to the culture 3 hours prior to irradiation (Fig. 2). This was necessary because more than 1.0 generation time was required to establish a steady state concentration of radioactivity in DNA, indicated by the horizontal position of the curve, although the rate of incorporation was relatively constant in less than 1 hour. As shown on the lower part of Fig. 2, radioactive thymidine had no effect on the rate of cell replication of the control culture in that the cell number continued to increase exponentially.

In cultures exposed to X-rays, cell division ceased within 30 minutes and the rate of DNA synthesis decreased to zero within the same time interval and remained inhibited for about 1.6 hours. At this time the tracer was again rapidly incorporated even though cell division was still blocked. The character of the curve for thymidine incorporation into irradiated cells indicates expo-

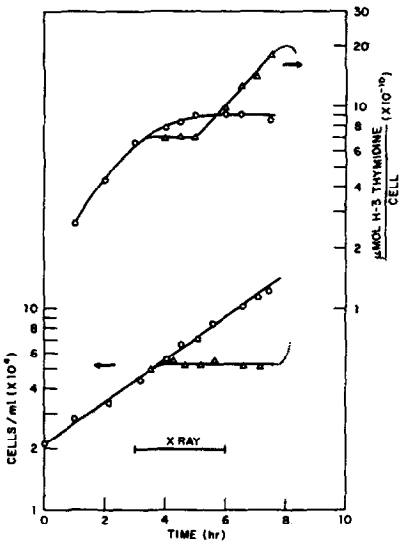

Fig. 2. Incorporation of ^{3}H Thymidine per cell and cell number before, during and following X-irradiation at 260 R/min. O control culture. Δ irradiated culture.

nential kinetics unlike the curve for increase of radioactive thymidine in the DNA of the unirradiated control culture which curve is of a saturation type. At approximately 8 hours, a synchronous burst of cell division was observed during which more than 70% of the cells divided within less than 30 minutes (unpublished data). During this period the rate of DNA synthesis decreased to zero and the amount of DNA per cell decreased.

Inhibition of the synthesis of DNA as a result of X-irradiation has been reported repeatedly (Lajtha 1960), although in most cases the relatively large doses of radiation used would have killed a significant number of cells. Our observations indicate that with sublethal doses of radiation DNA synthesis is inhibited temporarily for a time interval corresponding to about 1/2 generation time during which no inhibition of cell growth can be demonstrated. This may indicate that, with sublethal doses of X-irradiation, nuclear oxidative phosphorylation is temporarily inhibited while mitochondrial oxidative phosphorylation is not. Inhibition of nuclear ATP synthesis has been shown previously by

(Klouwen (1965) who reported on the early inhibition of ^{32}P incorporation into DNA in cells which underwent interphase death. However, he could not demonstrate an inhibition before 12-24 hours after 1000 R in cells which underwent mitotic death.

Our data indicate that DNA synthesis recommences during irradiation and ^{3}H-TdR incorporation increases from 7 to 18 x 10^{-10} µMol per cell prior to the next division. Once biosynthesis of DNA is resumed, the rate of synthesis corresponds to a doubling time of about 0.7 generation time. This is longer than the value of 0.4 generation time for the duration of the S-phase of Tetrahymena pyriformis HSM and 0.35 for GL reported by Cleffman (1965) and Cameron and Nachtwey (1966), respectively. Several explanations can be advanced to explain the low rate of DNA synthesis corresponding to the long doubling time for DNA: a) Once resumed, synthesis is depressed and lower than normal due to damage which cannot be repaired prior to the next cell division. b) Once resumed, the rate of synthesis is not affected by radiation, but if a broad distribution of starting time for the synthesis existed, the low rate would be the result of the asynchronous onset of DNA synthesis in the culture. However, with an asynchronous onset of DNA synthesis the rate would be expected to increase to a maximal value and then decrease to zero. c) If radiation caused only a temporary block in the transition of cells from G_1 into S phase, synthesis of DNA per se would be inhibited but since the S phase is only a small part of the generation time, cells in S phase at the beginning of irradiation would rapidly move into G_2 and incorporation of thymidine would then stop temporarily. After removal of the temporary block, even if the rate of DNA synthesis returned to normal but was higher than the rate of transfer of cells from G_1 to S, then the rate of transfer from G_1 to S would determine the observed low rate of DNA synthesis.

At this time we do not have the data to distinguish between these various explanations but clearly the effect of irradiation on the synthesis of DNA is not simply an inhibition. However, cell growth, that is enlargement of cells

is not inhibited by sublethal doses of radiation during inhibited or accelerated DNA synthesis in Tetrahymena pyriformis.

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