JUL 26 1965

MASTER

BNL 9331

CONF-650302-23

H^3 THYMIDINE (H^3 TdR) INCORPORATED INTO
DNA; DOSIMETRIC AND RADIOBIOLOGICAL
CONSIDERATIONS

V.P. Bond* and L.E. Feinendegen**

Presented at the I.A.A.E. Panel on the "Biophysical Aspects of Radiation
Quality". Held in Vienna, from March 29 to April 2, 1965.⁴

Research supported by the U.S. Atomic Energy Commission

PATENT CLEARANCE OBTAINED. RELEASE TO
THE PUBLIC IS APPROVED. PROCEDURES
ARE ON FILE IN THE RECEIVING SECTION.

*Medical Research Center, Brookhaven National Laboratory,
Upton, L.I., New York

**Institute du Radium, Laboratoire Pasteur, 26 Rue d'Ulm,
Paris, Ve., France

⁴The papers presented at the panel meeting are to be published by the IAEA

LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

INTRODUCTION

Tritium can be selectively incorporated into DNA in vivo by administration of tritiated thymidine (H^3 TdR), and this labeled precursor is used widely and effectively in investigations on the mechanism of DNA synthesis, in studies on the kinetics of cell proliferation, and for the purpose of selective cell destruction. It is important, of course, to be aware of the "threshold" for toxicity of the isotope used in this manner. In addition, the unique dosimetric features involved present theoretical and practical problems, the solutions of which will shed light on mechanisms of radiobiological effect. The highly selective irradiation of the nucleus of those cells which incorporated H^3 TdR at the time of DNA synthesis constitutes not only partial-body irradiation, but partial cell irradiation, and homogeneity of exposure can be defined only in terms of subcellular dimensions. The toxicity of H^3 TdR and the associated problems have been reviewed extensively (1).

It is the object of the present paper to a) evaluate from data in the literature and from data to be presented, whether observed effects from intranuclear H^3 can be accounted for on the basis of calculated absorbed dose in rads to the cell nucleus, or, alternatively, whether additional factors deriving from the presence of the H^3 in the DNA molecule must be invoked. In the latter case the biological effects would not be generally predictable on the basis of absorbed dose; and b) to discuss the implications of the findings with respect to radiobiology and radiation protection. The conclusion is reached that while data are as yet inadequate for firm evaluation, biological effects of H^3 TdR are those predictable on the basis of the average absorbed dose to the nucleus.

The applicability of the absorbed dose concept can be evaluated satisfactorily only if adequate comparisons of the effect of incorporated H^3 TdR can be made with a suitable "standard" external radiation such as x-rays, or

possibly with the same isotope (H^3) distributed essentially randomly in tissue, as is tritiated water. The low dose rate from the incorporated H^3 must be taken into account in practically all experiments, as must the possibility of an "intrinsic" relative biological effectiveness (RBE) of H^3 beta radiation, compared to x- or gamma-rays. The possibility of "transmutation" effects cannot be ignored

In evaluating the effects of intranuclear tritium the following approach will be used: The method of calculating the absorbed dose for the nucleus will be presented first. This is followed by a discussion of three factors required in the evaluation of the relative effectiveness of tritium disintegrations, 1) The "intrinsic" relative biological effectiveness (RBE) of H^3 beta rays; 2) possible "transmutation" effects; 3) asynchronous chromosome labeling. Dose-effect relationships for H^3 incorporated in the nucleus from administered H^3 TdR will then be discussed in the following order: 1) cell killing effects in tissue culture; 2) cytogenetic effects in mammalian cells; 3) mitotic delay in tissue culture; 4) cell killing effects in the intact mammal; 5) genetic effects and cancerogenesis.

Calculation of absorbed dose: "edge effects". Calculation of the average absorbed dose (or dose rate) to the nucleus from incorporated H^3 TdR is in principle simple, and the dose can be estimated from the energy emitted by using the well-known formulation

$$\frac{(A) \times (E) \times (k)}{(m) \times (100)} = \text{Dose (or rate) in rads} \\ \text{(per unit time)}$$

where:

A = Disintegrations per nucleus (per unit time) This value may be obtained by radiochemical and autoradiographic analysis, or by autoradiographic considerations alone (2).

E = Average value for energy of H^3 beta ray (5.7×10^{-3} MeV)

$k = 1.602 \times 10^{-6}$ (ergs/Mev)

m - Mass of nucleus, usually obtained from volume determinations assuming unit density.

100 - Factor to convert ergs/gram to rads.

The formula carried the assumption that the H^3 is located essentially entirely in the nucleus, and that all energy from the beta rays is absorbed in the nucleus. The first assumption is not seriously questioned. The second assumption is true except for "edge effects" i.e., the escape of beta rays from the nuclear surface into the cytoplasm. The range-dose relationships for H^3 beta rays have been reviewed by Robertson (3) and Goodheart (4). The beta rays have a maximum range in water of approximately 6 microns and an average range of 0.8 - 1 micron.

The magnitude of the edge effect, calculated for unit-density spheres containing uniformly-distributed H^3 , is indicated in Fig. 1 (5). For instance, in a sphere of 2μ radius, 37 per cent of the total energy escapes and 63 per cent is retained; the corresponding values are 20 and 80 per cent for a sphere of 4μ radius. The nuclei particularly of growing tissue culture cells undoubtedly are not spherical, a factor that will be commented on below. Also, the nuclear volume increases during DNA synthesis. Average values for nuclear size may overcome this factor in longer term experiments; however, it may have to be considered in short-term experiments.

Relative Effectiveness of H^3 Beta Rays

"Intrinsic" RBE of H^3 beta rays. It might be expected that under conditions in which the absorbed energy from H^3 beta rays and from x-rays are uniformly distributed in the biological tissue volume of interest, the beta rays would show a greater degree of effect for a given absorbed dose ("intrinsic" RBE greater than unity). This could perhaps be expected on the basis that each of the low energy beta rays (E_{max} 18 kev; E_{ave} 5.7 kev) might be considered

as the end portion of higher-energy beta "tracks", where the LET is relatively high. On the average, the tritium beta has been calculated to produce approximately 100-200 ion pairs per 0.5 micron of water and approximately this in tissue; however, it is conceivable that on the average the energy from the H^3 -beta rays may be delivered in significantly larger "packages" than is that from high energy beta rays or x-rays.

The toxicity of tritiated water has been studied in simpler biological systems (6-9), and one recent study (10) allows direct comparison with the effects of Co^{60} gamma radiation. Tritiated water was added to a culture of Chinese hamster cells, and the number of chromosomal aberrations was scored after a 10 hour exposure. Comparison cultures were exposed to Co^{60} gamma rays at similar dose rates. The results are shown in Fig. 2 (data from reference 10 combined and replotted; H^3 TdR data in Figure to be discussed later). The biological effectiveness of the tritium beta rays from tritiated water appeared to be somewhat greater than that of Co^{60} , of the order of 1.1. Thus the relative biological effectiveness (RBE) of the beta rays compared to 250 KVP x-rays probably is not different from unity in this system.

Attempts to determine an RBE in the intact mammal (11-13) have been made, and in two studies (12,13) the dose rate from the comparison Co^{60} radiation was adjusted to approximate that from the injected tritiated water (dose given over several days). A variety of end points was studied, including mortality, and spleen-thymus weight loss. The RBE values obtained at the relatively low and changing dose rates employed ranged from 1.3 to 1.7. These values would correspond to approximately 1.0 and 1.4 if 250 KVP x-rays instead of Co^{60} gamma rays were used as the standard. Also, since the calculations were made on the basis of uniform distribution of the tracer in total body water rather than on the basis of inhomogeneity of distribution (minimal uptake of H^3_2O in bone), the RBE values calculated would become further reduced. It is thus unlikely that the true RBE value of tritium beta rays exceeds unity,

compared to 250 KVP x-rays, and thus an RBE of 1 may be assumed to be correct.

Possible transmutation effects. Transmutation* effects from the disintegration of isotopes such as P^{32} are well demonstrated and transmutation effects from H^3 disintegration have been reported for bacteria. Person (14-16) suspected such effects, but indicates they have no role in lethality (17). Such effects appear to have been shown in the experiments of Rachmeler and Pardee (18). These investigators labeled the DNA of E. Coli uniformly with tritium, or, in such a manner that tritium was distributed throughout the chromosome except for the region responsible for production of the enzyme beta galactosidase (accomplished by mating procedures). Killing from ionizations occurred with both types of labeling. However, effects on the enzyme occurred only if the enzyme "locus" were labeled. These results were confirmed by RNA label as well. Transmutation effects may be invoked to explain the data of Kaplan (19) who labeled drosophila with H^3 TdR and mapped the distribution of sex-linked recessive lethals along the chromosomes. The distribution was non-random but different from that seen with x-rays. Transmutation effects thus may be important as far as genetic mutations are concerned; probably of little or no importance as far as observable cell damage and cell lethality is concerned (see below).

Asynchronous chromosome labeling. Asynchronous labeling of chromosomes occurs (20) and would conceivably result in "hot spots" of localized activity in the nucleus. The degree to which this factor may have to be taken into account is discussed below under dose-effect relationships, cytogenetic effects in mammalian cells.

*Transmutation effects refer here to possible biological damage resulting from the replacement of the H^3 atom by an He atom as a result of decay of H^3 . While the recoil energy of the He atom, a maximum of 3ev, is considered inadequate to disrupt chemical bonds, the sudden alteration of charge as a result of transmutation may conceivably cause molecular excitation adequate for chemical consequences.

Dose-Effect Relationships for
Intranuclear H^3 Beta Rays

Cell Killing Effects in Tissue Culture. Cell killing in tissue culture represents a relatively clearcut situation for comparison of the effects of H^3 beta rays from H^3 TdR, and x-radiation. Marin and Bender (21,22) exposed HeLa (21) and Chinese hamster cells (22) to H^3 TdR and H^3 -uridine with the primary objective of determining the shape of the dose-survival curve. Little effort was made to calculate radiation dose (22) to the flat cells in monolayer on glass surfaces and a conversion factor that applies directly only to a smaller nuclear volume (4) was used to obtain absorbed dose from disintegrations per nucleus. The specific activity of the thymidine was varied rather than the total amount added to the medium, which was kept at $0.001\mu M$ per ml., and the autoradiographic grains/cell as a function of the amount of H^3 TdR in the medium was determined. The exposure to H^3 TdR for 16 hours resulted in the labeling of nearly all cells in the cultures while the cells were still in the single-cell dispersed state. It was recognized that the total radiation dose was delivered while single cells were giving rise to colonies, i.e., over a period of several cell generation times.

The survival curve obtained was clearly of the "multi hit" type with an extrapolation number greater than unity for both HeLa cells and Chinese hamster cells. The authors attempted to correct for possible masking of a simple exponential dose relationship as a result of the killing of some cells after one or two divisions within a growing cell colony. A model was constructed and analysis was made by means of a computer. The conclusion was that the extrapolation number almost certainly exceeded one. External irradiation to the whole cells resulted in the same type of survival kinetics.

The nuclear dose from H^3 TdR at a given level of effect is compared with that from x-radiation required to produce the same degree of effect, as follows: The slopes of the "exponential" part of the curves (21,22) are

not readily determinable, and thus the LD_{37} values are used for comparison. For the HeLa cell, the LD_{37} was found at an H^3 TdR concentration of $0.21 \mu\text{C/ml}$, corresponding to 4.1 gr/cell/hr . No figure for autoradiographic efficiency for the HeLa experiments were given; however, efficiency measurements in this laboratory with the same strain of HeLa yielded a value of approximately 11 d/gr , with a range of approximately 9 to 13 (Kodak AR10 film). Thus the LD_{37} value corresponds to $4.1 \times 14 = 57 \text{ d/cell/hr}$. Cell or nuclear size was not given. Estimates of nuclear size from the literature have been of the order of 1000 to $1500^{3/4}$. Recent measurements in this laboratory under conditions of early growth of a culture indicated average nuclear dimensions of approximately $18 \times 11 \times 5 \mu$, corresponding to a nuclear volume of approximately $1000^{3/4}$, or a mass of 1×10^{-9} grams, assuming unit density. The amount of H^3 TdR per nucleus is halved on the average with each generation, assumed to be about 24 hours in duration for those cells. Thus the "average" duration of exposure can be taken as $24/\ln 2$, or 35 hours.

The total accumulated dose to the nucleus at the LD_{37} level can then be calculated (edge effects not included):

$$\frac{(57) (35) (5.7 \times 10^{-3}) (1.59 \times 10^{-6})}{(1.0 \times 10^{-9}) (100)} = 178 \text{ rads}$$

A dose rate factor must be considered for comparison with x-radiation delivered in a few minutes. The magnitude of this factor is not known; however, an estimate can be made. From data available principally on small mammals (23) about double the dose of x-rays is required if delivered over 24 to 36 hours, rather than over a few minutes. However, since actually the major portion of the dose from incorporated H^3 TdR is delivered during the first half of the 35 hour exposure, the degree of dose rate effects tends to be lower than that of x-irradiation with constant dose rate. Thus the appropriate factor is taken to be approximately 0.75. For correction of the edge effect, a factor of approximately 0.9 was chosen (Fig. 1). Consequently,

the LD_{37} for the selective intranuclear radiation is estimated to be approximately $178 \times 0.9 \times 0.75 = 113$ rads. The LD_{37} value for x-radiation of the HeLa strain used by Marin and Bender (21) was not determined and the value differs depending on the conditions employed. In this laboratory, irradiation of HeLa cells in the log phase of growth (newly plated cells) under conditions similar to those chosen by Marin and Bender (21) yields an LD_{37} of approximately 140 rads; exposure of an 18-hour culture (about 1.5 cells/clone) yields an LD_{37} of 200 rads or more. Values for HeLa S-3 from the literature (24) are of the order of 200 rads. Thus the dose from x-rays and the calculated dose from intranuclear H^3 TdR, that will yield an LD_{37} , agree for HeLa cells within a factor of approximately 2, the H^3 TdR seemingly being more effective.

The data of Drew and Painter (25-27) lend themselves to similar calculations; however, these data were obtained under different culture conditions in that all cells were labeled by exposure to H^3 TdR for 24 hours starting after commencement of clone growth, i.e., after at least two divisions had taken place. The LD_{37} value obtained was approximately 0.07-0.08 μ C/ml (Sp. Act. 1.9C/mM) of medium. The amount of tracer incorporated was not determined in these experiments but from Painter et al (26), it can be determined by interpolation that one hour's exposure of these cells to this concentration of H^3 TdR yields 180 grains per nucleus from a 3 day film exposure (Kodak AR-10). Assuming a DNA synthesis time of 6 hours, this corresponds to $180 \times 6 / 72$, or 15 grains/hr. With an autoradiographic efficiency of approximately 11 disintegrations per silver grain in AR-10 (Kodak) film from HeLa cells labeled with H^3 TdR, as determined in this laboratory, 15 grains/hour correspond to about 165 d per nucleus per hour to reduce clone survival to 0.37. 165 d/hr per nucleus (1.0×10^{-9} g) is equivalent to about 15 rads/hour, without consideration of "edge-effects". Assuming a factor of 0.9 for edge effects, and for these experiments a period of 35 hours of effective

exposure (generation time of HeLa-S-3 cells is about 24 hours) the accumulated dose becomes about 470 rads. If, for comparison with single x-ray exposure the dose rate factor of 0.75 is applied, the value becomes about 350 rads. The LD₃₇ value (x-rays) for irradiation of cells that have undergone 2-3 divisions is about 200 to 250 rad (28), indicating that the H³ffrom thymidine may be less effective than x-rays. This figure for the effects of H³TdR is more uncertain than that calculated from the data of Marin and Bender. However, these sets of data on the HeLa cell indicate that the doses required for cell killing, from x-radiation or from selective intracellular irradiation from H³TdR, probably do not differ appreciably.

Similar reasoning may be applied to the data on the Chinese hamster cell (22). An LD₃₇ of 5.7 gr/cell/hr was obtained corresponding to 80 d/cell/hr derived from the autoradiographic efficiency of 14d/cell per grain that was determined. The nuclear volume was not given. A nuclear radius of approximately 3.8μ and a nuclear volume of 225μ³, considerably less than that for the HeLa, has been reported (10) for one strain of Chinese hamster cells. Those used by Marin and Bender were described as "fibroblast-like," and the nucleus may well have been larger. The generation time of the hamster cell is approximately 16 hours; average exposure time is thus 16/0.69 = 23 hours. The maximum dose at the LD₃₇ is thus

$$\frac{80 \times 23 \times 5.7 \times 10^{-3} \times 1.60 \times 10^{-6}}{225 \times 10^{-12} \times 100} = 750 \text{ rads}$$

The edge effect correction of 0.8 (Fig. 1) reduces this dose to about 590 rads. While no comparison was made with x-irradiated hamster cells, the value of 590 rads is above the value reported for the x-ray LD₃₇ of Chinese hamster cells (29). (See under cytogenetic effects below for further discussion of work with the hamster cell).

Only two studies were found in which an effort was made to compare directly the cell killing effects of intranuclear irradiation from H³TdR with

that from tritiated water. Painter et al (7) attempted a rough comparison using HeLa cells and concluded that, within a factor of two, the same amount of intranuclear radiation from the two compounds leads to the same degree of effect. These authors held it unnecessary to postulate additional factors besides ionizations from H^3 incorporated into DNA to explain the killing effects observed. Sanders et al (9) compared the effects of H^3_2O and H^3Tdr on HeLa cells and concluded that the nuclear dose from H^3Tdr was considerably more effective than the same dose from H^3_2O . Mechanisms in addition to damage from the beta rays were postulated. However, the low specific activity of the H^3Tdr used undoubtedly caused saturation of the cellular thymidine pool and metabolic toxicity (30). Moreover, effects of incorporated H^3 were not evaluated until 48 and 72 hours. But the comparison of intranuclear H^3 from H^3Tdr and H^3_2O was made at 30 hours. The cells in medium containing H^3Tdr undoubtedly continued to incorporate and concentrate H^3 beyond the 30 hour sampling time. Thus analysis at later times as well would be necessary to establish a greater effectiveness of dose from H^3Tdr . Ideally, a short lasting (less than 24 hours) exposure of the cells to H^3Tdr and H^3_2O should be made, followed by culturing in regular medium without H^3Tdr but with decreasing concentrations of H^3_2O to match approximately the rate at which intranuclear H^3Tdr decreases by cell division.

Cytogenetic effects: Cytogenetic effects (chromatid and chromosome type aberrations) have been observed in mammalian (and plant) cells. Bender et al (31) and Marin and Prescott (32) noted definite abnormalities in cultured human blood lymphocytes and in hamster cells exposed to H^3Tdr . Autoradiograms were made after abnormalities were scored (31). The sites of abnormalities did not correspond to sites of grain concentration. The authors concluded that "transmutation" apparently played no part in this phenomenon (and probably not in cell lethality): this would indicate that ionizations along the beta "track" alone are responsible for the damage. But, it was also observed that certain types of chromatid aberrations seen after labeling with H^3Tdr (interstitial

deletions, achromatic lesions) occur very rarely or not at all with x-rays. Further, H^3 -uridine (mainly in RNA) showed a still different pattern. These findings are not explained as yet. Hsu and Zenzes (33), using a similar technique and exposing Chinese hamster cells for various periods of time, reported a difference between expected and scored number of chromosome aberrations in late-labeling x and y chromosomes of cells exposed to H^3 TdR late in the DNA synthesis period. These findings were interpreted as indicating that breaks may be accounted for largely on the basis of H^3 disintegrations within the affected chromosome. Other results were not consistent with this interpretation, i.e., expected vs scored aberrations in the late-labeling chromosomes numbers 9, 10 and 11. More evaluation is required, however, insufficient evidence exists at present to establish the relative contribution in the production of chromosome aberrations, of H^3 disintegrations originating within or outside of the affected chromosome. Perhaps more pertinent to an evaluation of the usefulness of absorbed dose to the nucleus from H^3 TdR are studies in which the absolute numbers of aberrations per cell are compared following exposure to H^3 TdR, or external radiation or H^3_2O . One such study is discussed below.

Chromosomal aberrations have been employed (10) in a quantitative study comparing the effects of H^3 TdR, H^3_2O and Co^{60} gamma radiations in Chinese hamster cells in culture. The cells were pulse labeled for 1 hour with H^3 TdR, colcemid was added at 10 hours, and the cells were prepared at 11 hours, by the hypotonic method, for scoring of chromosomal breaks and exchanges. Grain counts were done on the same cells scored, and autoradiographic efficiency (10.8 d/cell per grain) as well as nuclear dimensions (cells assumed to be spherical during exposure, radius and volume 3.8μ and $224\mu^3$, respectively) were determined. Comparable cultures were exposed to beta radiation from H^3_2O , or to Co^{60} gamma radiation at similar dose rates. In calculating the dose rate from H^3_2O , a correction factor of 0.8 was introduced to correct for the 20 per cent of protoplasm that is not water.

The results are shown in Fig. 2 (data replotted and combined). The authors indicate that no significant difference among the three radiations was detectable at the level of approximately 1 aberration per cell, but that radiation from H^3TdR at the level of 2 breaks per cell was less effective than that for H_2^{18}O beta rays or Co^{60} gamma rays (doses of 820, 490 and 520 rads, respectively). Tentative explanations for the differences are suggested, including possible differences in energy absorption in the nuclear membrane. The results with H^3TdR were considered to be best described by a straight line; the aberration frequency with radiation from H_2^{18}O or Co^{60} could be described in terms of the 1.8 or 1.9 power of the dose. Transmutation effects were considered to be minimal or negligible.

The experiments were carefully done and a number of factors possibly affecting the results were taken into account (eg, edge effects, change in nuclear size with stage of cell cycle, mitotic delay and changes in radio-sensitivity with progression through the cell cycle). However, the difference between the results with H^3TdR and the two "standard" radiations is not large, as with cell killing (factor of approximately 1.6 at 2 aberrations per cell, no difference at 1 aberration per cell). A similar low degree of effectiveness of H^3TdR was noted for cell killing effects with Chinese hamster cells (see under "cell killing effects" above), while cell killing effects with HeLa cells showed no such reduced effectiveness. In the calculations of dose for cell killing effects in the hamster cells, the same nuclear volume given by the authors determining chromosome aberrations in these cells (10) was employed. The trypsinized spherical cell was used in the determination of nuclear size, which may not apply to the attached growing cell. The attached growing cell is flatter, and the edge effects for the smaller hamster cell in this configuration may be considerably greater than for the layer HeLa cell. The incidence of chromosomal breaks has been reported to depend on the stage of the cell cycle in which radiation occurred (34). These careful studies of Dewey et al (10) comparing

cell aberrations are indicative; however the possibility remains that the apparently somewhat reduced effectiveness of beta rays from H^3 TdR (factor of about 0.6) may be due to technical difficulties. It also remains to be shown that H^3 TdR incorporated at other times during DNA synthesis would be as effective per unit exposure (only the "flash" label, with harvest at 11 hours was used).

Mitotic delay in tissue culture. A small amount of data on mitotic delay in HeLa cells (27) allows a rough comparison with x-ray effects. The cells were exposed to H^3 TdR, $2\mu\text{C}/\text{ml}$, Sp. Act. $1.9\text{C}/\text{min}$. for 0.5, 3, 6, 9, 12, 15 and 24 hours, after which "cold" thymidine was added. Grains per labeled cell were determined on samples taken at the end of exposure. Labeled mitotic figures were seen first among cells harvested after the 9 hours exposure. Since with tracer amounts of thymidine, ($0.02\mu\text{C}/\text{ml}$ for 30 minutes) labeled mitotic figures are clearly in evidence by two to three hours and all mitotic figures are labeled at 6 hours after exposure (35), mitotic delay must have occurred in the above experiments. Thus, the 3 hour exposure might have delayed mitosis by perhaps 1 hour; however, the 6 hour exposure had delayed mitoses by at least 4 hours, the 9 hours exposure by at least 7 hours. The average grains per labeled cell per hour were 25 in the 3 hour sample, 34 and 35 after 6 and 9 hours exposure, corresponding to 275, 375, and 385 d per nucleus per hour. The absorbed dose for the 3 hour sample is

$$\frac{(275) (5.7 \times 10^{-3}) (1.60 \times 10^{-6})}{(1.45 \times 10^{-9}) (100)} = 17 \text{ rad/hour}$$

The dose for the 6 and 9 hour samples is approximately 23 and 24 rads per hour respectively. Assuming these doses on the average to be representative for calculating accumulated dose during the premitotic "rest-period", the total dose becomes 51 rads for the 3 hour sample, 138 rads for 6 hours, 216 rads for 9 hours. The average grain count does not necessarily reflect the actual dose to those cells that were delayed in entering mitosis (grain counts for mitotic figures not available); however, these doses though lower, are consistent with those obtained from x-rays for the same degree of mitotic delay (36, 37).

This approach, if refined, would yield an excellent method for accurate comparison of intranuclear H^3 TdR effects with those from x-rays or tritiated water, since the problem of dose rate and dose accumulation becomes minimal.

Cell killing in the intact mammal. Dose calculations for mammalian cells in vivo are less satisfactory than for simpler systems. Generally, one is not dealing with a homogeneous population of cells, and frequently it is most difficult to classify various cell categories and thus to count accurately those labeled within a given class of cells. Mammalian cell nuclei in vivo in general are smaller than is the HeLa cell nucleus and greater "edge effects" must be expected. Moreover, it is in most instances difficult to arrive at an "effective time" over which the dose of beta radiation is delivered. Nonetheless it is important and instructive to attempt calculations even though such parameters are presently still uncertain. In approaching these problems, new data to be published (38) will be presented briefly, following which material from the literature will be evaluated.

The object of the experiments to be presented (38) was to determine the smallest amount of intravenously-injected tritiated thymidine that would result in a detectable effect in the bone marrow of the Sprague-Dawley rat, evaluated principally on the basis of the disappearance rate of acid insoluble H^3 from the bone marrow cells after injection of H^3 TdR in amounts of 0.1, 0.5 and 1.0 μ C/g body weight, and C^{14} TdR in amounts of 0.01 μ C/g (Sp. Act. H^3 TdR, 1.9C/mM; C^{14} TdR, 30mC/mM). Animals were sacrificed serially, the marrow was isolated from bones of the extremities and the total count of nucleated bone marrow cells per unit volume of suspension was ascertained. The amount of isotope per cell nucleus was determined radiochemically and autoradiographically.

A definite diminution in the turnover rate was seen in rats given 1.0 μ C/g H^3 TdR; a questionable change was seen with 0.5 μ C H^3 TdR. (The results with 0.1 μ C H^3 TdR and 0.01 μ C C^{14} TdR were nearly identical, and were taken as the physiological rate as measured by tracer levels of activity.) At no dose level was there a detectable

effect on the bone marrow differential counts, nor was there unequivocal evidence of cell death. However, occasional abnormal (bi- and tetranucleated) bone marrow cells were seen in animals at day 3 after injection of 1.0 μ C/g. It thus appears that definite changes in DNA- H^3 turnover rate are observable after injection of 1.0 μ C H^3 TdR/gram weight and that such changes represent a more sensitive index of effect than do conventional histo-cytological techniques. The alteration in turnover of total marrow DNA- H^3 may be due to a slowing down of cell proliferation, to killing of younger cells with consequently increased availability of labeled breakdown products for reutilization, or a combination of the two. The possibility of DNA repair in vivo with reutilization of H^3 -catabolites by viable cells should also be considered.

From the radiochemical data, the activity of the marrow of rats receiving 1.0 μ C/g of H^3 TdR and sacrificed at 1 to 12 hours was approximately 6000 disintegrations per minute (dpm) average per 10^6 total nucleated bone marrow cells. Autoradiographic studies on the one hour samples from animals receiving 1.0 μ C/g H^3 TdR indicated that 27 per cent of all nucleated bone marrow cells were labeled, and thus each labeled bone marrow cell had on the average an activity of $6000/0.27 \times 10^6$ or 0.022 dpm. The mean grain count (all labeled cells) was 26 after 14 days autoradiographic exposure corresponding to 0.0013 grains per cell per minute. The autoradiographic efficiency can thus be calculated, and is $0.0013/0.022$, or 0.06. Thus on the average, about 16 to 17 intranuclear disintegrations are required per silver grain. In another autoradiographic preparation of labeled rat bone marrow the same calculation resulted in 14 to 15 d. to cause 1 silver grain.

Detailed autoradiographic findings are shown in Table I. It is seen that while the grain count per cell varies markedly (from less than 10 to greater than 150), considerably less variation is seen in the average count for different cell types. The megakaryocytes label heavily; however, the averages for other cell types are within a factor of two of the overall average. It is

clear, however, that the autoradiographic efficiency in different individual cells and in cell types is influenced appreciably by self absorption factors, and that hence considerable variation exists around the average figures used above for calculation.

The average absorbed dose to the cell nucleus can be calculated as follows ($1.0 \mu\text{Ci } ^3\text{H-TdR}$ injected per gram body weight, average activity per cell nucleus of 0.022 dpm, or 1.2 d per hour per nucleus at one hour after $^3\text{H-TdR}$ administration: An average nuclear diameter can be taken as 8 microns, corresponding to a mass of 270×10^{-12} g. It is difficult to decide on an effective time of dose delivery for cell killing; however, 24 to 36 hours appears reasonable (the generation time of most rat bone marrow cells is about 10 to 15 hours). Thus the dose to the average nucleus can be approximated:

$$\frac{(1.2) \times (20) \times (5.7 \times 10^{-3}) \times (1.60 \times 10^{-6}) \times P}{(2.7 \times 10^{-10}) \times (100)} \text{ rads}$$

Corrected for edge effects, this corresponds to approximately 1 rads. Apparently, some 20 d per nucleus (average) are required for minimally-detectable effects.

A scan of Table I will indicate that some cells would have received as much as 4 times the average dose, namely of the order of 25 to 35 rads over a period of 24 hours. This is consistent with the x-ray dose levels at which mitotic delay (36, 37, 39), cell abnormalities and some cell death become apparent. Definite changes in the DNA content of the marrow of mice were seen at doses as low as 46 rad of x-radiation (40). Thus, changes in the rate of DNA- ^3H turnover might well be expected in rat bone marrow after injection of $1 \mu\text{Ci } ^3\text{H-TdR}$ per g body weight. Only a few cells selectively killed by a high content of ^3H would be sufficient to contribute enough ^3H -DNA

TABLE I

Grain Count per Labeled Bone Marrow Cell, One Hour
After Injections of H^3 TdR, 1.0 μ c/gram

Cell Type	Grains/labeled Cell	
	Average	Range
Erythrocytic, all cell types	18	2 - 97
Basophilic normoblast	25	9 - 51
Myelocytic, cell types that label	23	2 - 131
Myelocytes	19	2 - 62
"Large" lymphocytes	34	20 - 73
"Medium" lymphocytes	18	3 - 66
Megakaryocyte, young	106	51 - 175

catabolites to the reutilization pathway to account for the alterations seen.

In other experiments in the literature, directly observable cytological manifestations or cell killing after H^3 TdR injection (41-46) were questionable at $1.0\mu\text{c/g}$ weight, definite at $10\mu\text{c/g}$. Cell killing effects were seen in one leukemic patient given $0.25\mu\text{c/g}$ in each of 10 injections over a 5 day period (47). Thus the evaluation based on the turnover rate of DNA- H^3 in the total marrow cell population appears to be more sensitive than are the more standard histocytological techniques.

Kisielewski et al (43) studied H^3 TdR toxicity in mammals in vivo and attempted to determine the amount of H^3 per cell and to estimate the radiation dose received. The decrease in the number of resting primary spermatocytes per tubule in the mouse testis was studied. Questionable effects were seen at $1.0\mu\text{c/gram}$ injected dose; significant effects at 10 and $50\mu\text{c/gram}$. From data at $10\mu\text{c/gram}$ evaluated at 4 to 6 days, and from the group receiving $50\mu\text{c/g}$ and evaluated at 2 to 4 days, the authors estimate that the "threshold" dose for demonstrable effects occurs at a level of about 125 total d per nucleus. It was then calculated that this amount of activity delivers 300 rads to the nucleus, taken to be 4.2μ in diameter.

These values of absorbed dose are much higher than those calculated above for a "threshold" effect of H^3 TdR on the rat bone marrow, and are at least ten times higher than x-ray doses required to produce the degree of effect described. Thus from studies in the laboratory (48) and elsewhere, although direct comparisons are difficult, the degree of irradiation of primary resting spermatocytes reported by Kisielewski would be expected at x-ray dose levels of 25r or below. Further the effects per amount of injected H^3 TdR differ markedly from those reported by Johnson and Cronkite (41).

The method of evaluating the beta counts per cell nucleus in these experiments was of necessity quite indirect, and was based on total activity per testis and on an estimate, from histological sections, of the total number of primary spermatocytes. The error involved in this estimate is difficult to evaluate. The disposition of DNA-H^3 from killed cells is not known, but its retention in the testis perhaps by reutilization would give increased values for H^3 content per total organ. Also, total disintegrations required to kill a cell were calculated up to the time of accomplished cell death and the number of dead cells was determined by their absence. Thus fewer disintegrations per nucleus than that reported for accomplished death were actually required for initiating cell death and this factor could be appreciable, perhaps 0.5 or less. Furthermore, the authors assume that all the liberated energy is deposited in the nucleus taken to have a diameter of 4.2μ ; however, only some 60 percent or less of the liberated energy is deposited in a nucleus of this size (Fig. 1), and the dose at the nuclear membrane is approximately one-half that at the center. This inhomogeneity factor may also reduce the calculated value of effective dose absorbed. The nuclear diameter of unfixed primary spermatogonia is larger in the unfixed tissue than indicated by the 4.2μ diameter used in the calculations (48). The nucleus is a spheroid with axes of approximately 5.9 and 7.3μ and a volume over 4 times that of a 4.2μ diameter sphere, which leads to a significantly lower calculated dose. Thus the actual "effective" dose absorbed by the nucleus could well be as little as one-tenth or less of that calculated by Kisilewski (43), i.e., about 30 rads or less, close to the dose range known from x-rays to cause the effects.

Genetic effects and cancerogenesis. It might be expected that genetic effects would derive in part from ionizations and in part from transmutation, and it would be instructive if the ratio from the two sources could be

determined for specific situations. Mutations have been reported from injected H^3 TdR in *Drosophila* (19, 49) and mammals (50,51). Meaningful calculations of dose are most difficult, as are comparisons with results from x-ray exposure. Bateman (50) reported that one dominant lethal per sperm (mice) resulted from a dose of H^3 TdR that caused 25 autoradiographic grains after a 3 week film exposure, on sperm harvested 4 to 5 weeks after $7.5\mu c H^3$ TdR per g weight, injected intraperitoneally in 6 fractions over 2 days. The time from injection to mating was approximately 5 weeks. Assuming an autoradiographic efficiency of 9 per cent, the total disintegration/nucleus over the first 5 weeks is at least 450. A greater H^3 TdR concentration in the nucleus over the first 4 to 6 days when the initially-labeling young cells are dividing would add another 200 to 300 d/nucleus, yielding a total of about 700 disintegrations per nucleus per lethal mutation. Assuming a 25 per cent loss of H^3 beta energy from the nucleus (size 7, 3 x 5.9 μ , diameter), the absorbed dose is calculated to be approximately 300 rads. The dose of x-rays to produce a comparable degree of effect has been estimated to be 462 r (52). This comparison, admittedly rough, indicates that the dominant lethals seen resulted largely, if not entirely, from the ionization produced by the beta rays, and that it is unnecessary to invoke other effects such as those from transmutation. It is pertinent also that in the work of Rachmeler and Pardee (18), approximately the same number of β dose integrations (and thus the same absorbed dose) was required for enzyme interaction as for cell killing, although different mechanisms are involved.

An increased incidence of tumors in mice after injections of as little as $1.0\mu c$ /gram of H^3 TdR has been reported (53). However, depending on the age of the animals at the time of injections, in some of the control groups of animals the incidence of tumors was higher than for the injected mice. In other experiments (54), within a limited period of observations of 250 days

after H^3 TdR injection, no tumors were observed in mice after a dose as high as 30 μ c/gram. Dose calculations involve large uncertainties, indeed, and data thus far are insufficient for this purpose.

In considering the possibility of genetic or late somatic effects from H^3 TdR, it is important to point out that proliferating cells do not retain tritium bound to DNA indefinitely. The activity is lost exponentially in units as chromatids transfer to daughter cells with each division for several generations until at last only one labeled chromatid is left to be transmitted (55). Thus, except for the increment from reutilization of breakdown products, the proliferating cells and essentially all mature cells would become devoid of H^3 within several generations. Prolonged retention in cells of the adult would be expected only in a rare mature non-proliferating cell stemming from cells or tissues with a slow turnover rate, that happened to be in the process of DNA synthesis at the time of H^3 TdR administration. Further, there is evidence that the bulk of cells in the stem cell pool of at least some tissues normally are not actively proliferating and do not incorporate H^3 TdR (56). It is also pertinent that once a given chromatid is labeled and replication is complete, that chromatid will not take up label with subsequent injections of H^3 TdR. From these considerations one might expect minimal absorbed dose to cells, and thus minimal chance of long-term somatic effects from even a high dose of H^3 TdR given over a short period of time.

DISCUSSION

Although data available allow reasonable characterization of cell responses to intranuclear H^3 TdR completely satisfactory experiments and data for direct comparison of absorbed dose from H^3 TdR with x-rays (or with H^3 ₂₀) have not as yet been done. Sources of error lie on the uncertain values for autoradiographic efficiency for the different cell types, an inability "to correct" rigorously for the low and changing dose rates encountered in

most experiments involving exposure to H^3 TdR, "edge effects" encountered particularly in small nuclei, and perhaps principally in the lack of precise knowledge of nuclear size and shape while the cells are actually being exposed. Some quantitative experiments have indicated an effect from H^3 TdR in excess of that expected on the basis of ionization from the H^3 beta rays ;and other mechanisms have been invoked. Other experiments have indicated a lesser degree of effect and still other explanations have been postulated. Since almost all experiments show agreement within a factor of two of results expected, it seems probable that the degree of biological effect from H^3 TdR deposited in the cell nucleus is predictable on the basis of absorbed dose to the nucleus; however, further experiments to prove this point to be generally valid in mammalian cells are necessary. Establishment of dose effect relationship thus far strengthens several theories and hypotheses as follows:

- 1) The absorbed dose to the mammalian cell nucleus determines quantitatively the degree of effect; the dose to the cytoplasm contributes little or nothing. This concept is supported particularly by the work of Marin and Bender (22), who compared the effects of H^3 TdR (intranuclear only) with the effects of H^3 -uridine (nuclear plus cytoplasmic exposure). The cell killing effect from H^3 -uridine could be accounted for solely on the basis of the intranuclear component of the H^3 irradiation with no observable contribution from cytoplasmic exposure. Results from the literature (57-61) on non-mammalian systems also support the considerably greater sensitivity of the nucleus. It should be recalled, however, that apparently definite differences in the types of cytogenetic effects were obtained with x-rays, H^3 TdR and H^3 -uridine (31,32).

- 2) The ability of H^3 TdR to kill selectively those cells that incorporated the labeled precursor strongly confirms the thesis that the basis of early

radiation injury in the mammal lies in cell injury and death in the proliferating cell systems (39). Cell destruction from the localized irradiation from intranuclear H^3 in proliferating cells leaves little ground for arguing that the cell depletion observed is the result of abscopal effects rather than the sole result from the radiation actually delivered to the individual cell. Integral dose to the entire body or to large body regions is not a precise dose parameter to be used alone for explaining and interpreting early effects in the mammal.

3) The results on H^3 -dose-effect cited above have implications with respect to the influence of the micro-distribution of the beta "tracks" and ionizations. Definite effects are noted at doses as low as 25 to 50 rads to the HeLa cell cultures, corresponding to a total of the order of 400 to 800 H^3 disintegrations per nucleus, or of the order of two hundred thousand ion pairs per nucleus. The distribution of packages of energy sufficiently large to produce a "hit" appear to be equally random with respect to the "target", whether the events derive from H^3 originating in the DNA molecule, or from an external source of x-rays. Although the beta "tracks" originate in DNA (origins presumed to be randomly located in the interphase nucleus), this fact appears to have no significance with respect to cell killing, and probably little or none with respect to other effects in mammalian cells.

4) If the beta radiation from intranuclear H^3 TdR is in fact no more effective than x-rays (random distribution groups of ion pairs), the conclusion can be drawn that there can be no significant fraction of the nuclear volume which is both insensitive to radiation and which lies beyond the range of the H^3 β rays from the disintegrating H^3 nucleus. If there were such a fraction of the volume, then the energy delivered by x-rays would be partly wasted and proportionately more dose would have to be delivered for

equivalent damage. Furthermore, if the beta radiation from intranuclear H^3 TdR is in fact no less effective than x-rays, one can say that all of the sensitive volume lies within the beta ray range of the chromatids, i. e., the radiobiological "targets" in the nucleus lie less than the effective track length of the H^3 beta rays from the chromatids. If a portion of the total "target" volume lay beyond this range, the beta radiation from the H^3 TdR would be less effective than the randomly-distributed ion pairs from x-rays.

5) If absorbed dose to the nucleus can be used to predict the degree of somatic biological effect, then the problems of possible H^3 TdR toxicity and health protection could be put on a more systematic basis and some fears could be allayed. Concern has been variously expressed on the basis that irradiation from H^3 TdR is intranuclear and in close juxtaposition to the DNA. It has been stated that a single H^3 disintegration may result in a large localized dose (9,42,43,62-65) well in excess of 100 rads, with the implication that even tracer doses may lead to serious consequences in anyone so exposed. The present studies indicate that early somatic effects can be predicted on the basis of the absorbed dose. At tracer doses, of the order of $0.3\mu C$ H^3 TdR/gram, the average dose to bone marrow cells "labeled" would be of the order of 3 rads. While this dose level would be expected to produce no detectable early somatic effects, nevertheless cell killing may occur rarely, undetected by the methods presently available. The evidence is that long-term somatic and genetic effects do not exceed those expected on the basis of absorbed dose; however, further work is needed for adequate evaluation. Thus it remains prudent not to use H^3 -DNA-precursors in young individuals, and in those of child-bearing age.

SUMMARY

It appears that the degree of early somatic biological effect from intranuclear H^3 is that anticipated on the basis of calculated absorbed

dose to the cell nucleus, at least down to doses of 5 rads or less. This indicates: a) the absorbed dose concept holds down to the order of 10^{-11} grams or less, and somatic effects can be predicted on this basis; b) the distribution of H^3 atoms incorporated into DNA as H^3 TdR (and ion pairs from the beta particles) are randomly distributed as far as dose calculations for the purpose of predicting somatic effects are concerned; c) every part of the cell nucleus lies within one H^3 beta range of some part of a chromatid, and the nucleus contains no contiguous "insensitive" volume of a radius exceeding the effective range of H^3 beta rays (1 to 2μ); d) the origin of H^3 beta tracks in, or their close juxtaposition to the DNA molecule does not appear to enhance the degree of somatic effect.

"Transmutation" effects of the disintegrating H^3 nuclide in DNA appear to have been demonstrated to produce genetic mutations in microorganisms, and data suggestive of this mechanism in *Drosophila* are available. The degree to which this factor in addition to absorbed dose must be taken into account in estimating possible genetic effects in the mammal cannot be assessed adequately at this time, but appears to be small. From available data, it appears that the degree of genetic effect in mammalian cells can be approximated closely from the absorbed dose to the nucleus.

Acknowledgements

The authors are indebted to the following individuals for their help and review in the preparation of this manuscript: J.S. Robertson, C. Robinson, E.P. Cronkite and J. Bateman.

References

1. Feinendegen, L. Tritium Labeled Molecules in Biology and Medicine. Monograph for the American Institute of Biological Sciences. In Press.
2. Levi, H. The Interpretation of Autoradiograms, Especially when Using Tritium as a Tracer. *Scand. J. Haemat.* 1: 138-149, 1964.
3. Robertson, J.S. and Hughes, W.L. Intra-nuclear Irradiation with Tritium-labeled Thymidine. *Proceed. of the First National Biophysics Conf.* (H. Quastler and H. Morowitz, Eds.) pp 278-283, Yale University Press, New Haven, 1959.
4. Goodheart, C. R. Radiation Dose Calculation in Cells Containing Intra-nuclear Tritium. *Rad. Res.* 15: 767-773, 1961.
5. Robertson, J. S. Personal Communication. Calculations by J. S. Robertson.
6. Powers, E. L. and Shefner, D. Tritium-Induced Effects in *Paramecium Aurelia*. *Proc. Soc. Exptl. Biol. and Med.* 78: 493-497, 1951.
7. Painter, R.B., Drew, R.M. and Hughes, W.L. Inhibition of HeLa Growth by Intracellular Tritium. *Sci.* 127: 1244-45, 1958.
8. Nias, A.H. W. and Lajtha, L.G. Continuous Irradiation with Tritiated Water of Mammalian Cells in a Monolayer. *Nature* 202: 613-614, 1964.
9. Sanders, P.C., Peterson, D.F. and Langham, W.H. The Influence of Intra-nuclear Irradiation on the Growth of HeLa Cells in Agitated Fluid Medium. *Annals N.Y. Acad. Sci.* 95: 969-974, 1961.
10. Dewey, W.C., Humphrey, R.M. and Jones, B.A. Comparisons of Tritiated Thymidine, Tritiated Water, and Co^{60} Gamma Rays in Inducing Chromosomal Aberrations. *Rad. Res.* 24: 214-238, 1965.
11. Brues, A., Stroud, A. and Rietz, L. Toxicity of Tritium Oxide to Mice. *Proc. Soc. Exptl. Biol. and Med.* 79: 174-176, 1952.
12. Furchner, J.E. Relative Biological Effectiveness of Tritium Beta-Particles and Co^{60} Gamma-Rays Measured by Lethality in CFI Mice. *Rad. Res.* 6: 483-490, 1957.
13. Storer, J.B., Harris, P.S., Furchner, J.E. and Langham, W.H. The Relative Biological Effectiveness of Various Ionizing Radiations in Mammalian Systems. *Rad. Res.* 6: 188-288, 1957.
14. Person, S. and Lewis, H.L. Effects of Decay of Incorporated H^3 -Thymidine on Bacteria. *Biophysical J.* 2: 451-63, 1962.
15. Person, S. Comparative Killing Efficiencies for Decays of Tritiated Compounds Incorporated into E. Coli. *Biophysical J.* 3: 183-187, 1963.
16. Person, S. and Bockrath, R.C. Jr. Differential Mutation Production by the Decay of Incorporated Tritium Compounds in E. Coli. *Biophysical J.* 4: 355-365, 1964.

17. Person, S., Funk, F.D. and Bockrath, R.C. The Mechanism of Lethality by Tritium Decay in T4 Coliphage. Abstracts, Biophysical Society. Meeting of 24-26 Feb., 1965.
18. Rachmeler, M. and Pardee, A.E. Loss of Viability and Beta-galactosidase-forming Ability as a Consequence of Tritium Decay in *Escherichia coli*. *Biochem. et Biophysica Acta* 69:62-67, 1963.
19. Kaplan, W.D., Gugler, H.D., Kidd, K.K. and Tinderholt, V.E. Nonrandom Distribution of Lethals Induced by Tritiated Thymidine in *Drosophila Melanogaster*. *Genetics* 49:701-714, 1964.
20. Taylor, J.H., Asynchronous Duplication of Chromosomes in Cultured Cells of Chinese Hamster. *J. Biophys. and Biochem. Cytol.* 7:455-463, 1960.
21. Marin, G. and Bender, M.A. Survival Kinetics of HeLa S-3 Cells after Incorporation of H^3 -Thymidine or H^3 -Uridine. *Intl. J. Rad. Biol.* 7: 221-233, 1963.
22. Marin, G. and Bender, M.A. A Comparison of Cell Killing by Incorporated H^3 -Thymidine and H^3 -Uridine. *Intl. J. Rad. Biol.* 7:235-244, 1963.
23. Bateman, J.L., Bond, V.P. and Robertson, J.S. Dose-rate Dependence of Early Radiation Effects in Small Mammals. *Radiology* 79:1008-1014, 1962.
24. Elkind, M.M. Radiation Responses of Mammalian Cells. Brookhaven Symposia No. 14. Fundamental Aspects of Radiosensitivity BNL 675 (C-31) (Biology and Medicine) TID 4500, 16th Edition, 1961, pp 220-245.
25. Drew, R.M. and Painter, R.B. Action of Tritiated Thymidine on the Clonal Growth of Mammalian Cells. *Rad. Res.* 11:535-544, 1959.
26. Painter, R.B., Drew, R.M. and Glauque, E.G. Further Studies on DNA Metabolism in Mammalian Cell Cultures. *Exptl. Cell Res.* 21:98-105, 1960.

27. Drew, R.M. and Painter, R.B. Further Studies on the Clonal Growth of HeLa S-3 Cells Treated with Tritiated Thymidine. Rad. Res. 16:303-311, 1962.
28. Archambeau, J. Personal Communication.
29. Elkind, M.M. and Sutton, H. Radiation Response of Mammalian Cells Grown in Culture. I. Repair of X-ray Damage in Surviving Chinese Hamster Cells. Rad. Res. 13:555-593, 1960.
30. Painter, R.B., Drew, R.M., and Rasmussen, R.E. Limitations in the Use of Carbon-labeled and Tritium Labeled Thymidine in Cell Culture Studies. Rad. Res. 21:355-366, 1964.
31. Bender, M.A., Gooch, P.C. and Prescott, D.M. Aberrations Induced in Human Leukocyte Chromosomes by H^3 -Labeled Nucleosides. Cytogenetics 1:65-74, 1962.
32. Marin, G. and Prescott, D.M. The Frequency of Sister Chromatid Exchanges Following Exposure to Varying Doses of H^3 -thymidine or x-rays. J. Cell Biol. 21:159-167, 1964.
33. Hsu, T.C. and Zenzes, T. Chromosome Aberrations Induced by Incorporation of Tritiated Thymidine. Cell Rad. Biol. A collection of papers presented at the eighteenth annual symposium on fundamental cancer research. William and Wilkinson Co., Baltimore, 1965.
34. Hsu, T.C., Dewey, W.C. and Humphrey, R.M. Radiosensitivity of Cells of Chinese Hamster In Vitro in Relation to the Cell Cycle. Exptl. Cell Res. 27:441-452, 1962.
35. Painter, R.B. and Drew, R.M. Studies on DNA Metabolism in Human Cancer Cells (HeLa). Lab. Invest. 8:278-285, 1959.
36. Kim, J.H. and Evans, T.C. Effects of X-Irradiation on the Mitotic Cycle of Ehrlich Ascites Tumor Cells. Rad. Res. 21:129-143, 1964.
37. Elkind, M.M., Han, A. and Volz, K.W. Radiation Response of Mammalian Cells Grown in Culture. IV. Dose Dependence of Division Delay and

- Post Irradiation Growth of Surviving and Non-surviving Chinese Hamster Cells. J. Nat. Cancer Inst. 30:705-721, 1963.
38. Feinendegen, L.E. et al, In preparation.
39. Bond, V.P., Fliedner, T.M. and Archambeau, J. Mammalian Radiation Lethality, a Disturbance in Cellular Kinetics. Monograph for the Amer. Inst. of Biological Sciences. In Press.
40. Davis, W.E. and Cole, L.J. Comparative Effects of Fast Neutrons and X-Rays on Marrow DNA Content in Mice. Rad. Res. 14:104-116, 1961.
41. Johnson, H.A. and Cronkite, E.P. The Effect of Tritiated Thymidine on Mouse Spermatogonia. Rad. Res. 11:825-831, 1959.
42. Samuels, L.D. and Kisielleski, W.E. Toxicological Studies of Tritiated Thymidine. Rad. Res. 18:620-632, 1963.
43. Kisielleski, W.E., Samuels, L.D. and Hiler, P.C. Dose-Effect Measurements of Radiation Following Administration of Tritiated Thymidine. Nature 202:458-459, 1964.
44. Griesham, J.W. Inhibitory Effect of Tritiated Thymidine on Regeneration of the Liver in the Young Rat. Proc. Soc. Exptl. Med. and Biol. 105: 555-558, 1960.
45. Post, J. and Hoffman, J. Some Effects of Tritiated Thymidine as a DNA Label in the Rat Liver, Rad. Res. 14:713-720, 1961.
46. Cronkite, E.P., Fliedner, T.M., Killman, S.A. and Rubini, J.E. Tritium Labeled Thymidine (H^3 TdR): Its Somatic Toxicity and Use in the Study of Growth Rates and Potentials in Normal and Malignant Tissue of Man and Animals, from "Tritium in the Physical and Biological Sciences". Symposium of the Intl. Atomic Energy Agency, Vienna 1961, Vol. II, p. 189-207.
47. Greenberg, M.L., Chanana, A.D., Cronkite, E.P., Schiffer, L.M. and Stryckmans, P.A. Detection of Cytocidal Effects of Tritiated Thymidine (H^3 TdR) During a Study of Cell Proliferation in a Case of Acute Leukemia. (Abstract). Presented at American Federation for Clinical Res. Atlantic City, May 2-4, 1965.

48. Bateman, J. Personal Communication.
49. Strömnes, O and Kuelland, I The Induction of Minute Mutations in *Drosophila* with Tritium Labeled Thymidine. *Genetics* 48:1559-65, 1963.
50. Bateman, A.J. and Chandley, A C Mutations Induced in the Mouse with Tritiated Thymidine. *Nature* 193:705-6, 1962.
51. Greulich, R C. Deleterious Influence of Orally Administered Tritiated Thymidine on Reproductive Capacity of Mice. *Rad. Res.* 14:83-95, 1961.
52. Bateman, A.J. Personal Communication.
53. Bazerga, R., Lisco, H and Kisieleski, W. Further Observations on Induction of Tumors in Mice with Radioactive Thymidine. *Proc. Soc. Exptl. Biol. and Med.* 110:687-690, 1962.
54. Cottier, H, Cronkite, E P, Tonns, E.A. and Nielsen, N.O. Leukemogenic Effect of Whole-body ^{60}Co Gamma Irradiation Compared with H^3 Thymidine and H^3 Cytidine: Preliminary Report on the Development of Thymic Lymphocytes in C57 BL/6 g mice from "Cellular Basis and Actiology of Late Somatic Effects of Ionizing Radiation:", Academic Press, New York, 1962, p. 27-34
55. Cronkite, E.P., Greenhouse, S W., Brecher, G. and Bond, V.P. Implication of Chromosome Structure and Replication on Hazard of Tritiated Thymidine and Interpretation of Data on Cell Proliferation. *Nature* 189:153-4, 1961.
56. Becker, A.J, McCulloch, E A., Siminovitch, L. and Till, J.E. The Effect of Differing Demands for Blood Cell Production on DNA Synthesis by Hemopoietic Colony-forming Cells of Mice. *Blood*, In Press.
57. Von Borstel, R C. and Wolff, S. Photoactivation Experiments on the Nucleus and Cytoplasm of the *Habrobracon* Egg. *Proc. Natl. Acad. Sci.* 41:1004-1009, 1955.
58. Ulrich, H Radiation Effects on Nucleus and Cytoplasm and Their Dependence Upon Oxygen. *Rad. Res.* 9:196-197, 1958.

59. Von Borstel, R.C. and Rogers, R.W. Alpha-particle Bombardment of the Habrobracon Egg II. Response of the Cytoplasm. Rad. Res. 8:248-253, 1958.
60. Zirkle, R.E. and Bloom, R.E. Irradiation of Parts of Individual Cells. Science 117:487-493, 1953.
61. Munro, T.R. Alpha Radiation of Parts of Single Cells in Tissue Culture IV. Irradiation of Chick Fibroblasts During Metaphase and Anaphase. Exptl. Cell Res. 18:76-99, 1959.
62. Lajtha, L.G. and Oliver, R. The Application of Autoradiography in the Study of Nucleic Acid Metabolism. Lab. Invest. 8:214-221, 1959.
63. Plaut, W. The Effect of Tritium on the Interpretation of Autoradiographic Studies on Chromosomes. Lab. Invest. 8:286-293, 1959.
64. Oliver, R. and Lajtha, L.G. Hazard of Tritium as a DNA Label in Man. Nature 186:91-92, 1960.
65. Guild, W.R. Hazards from Isotopic Tracers. Science. 128:1308, 1958.

FIGURE CAPTIONS

- Fig. 1. A comparison of the effectiveness of H^3 TdR, H^3_2O and Co^{60} gamma radiations in producing chromosome aberrations in Chinese hamster cells in culture (redrawn and combined from data given in ref. 12)
- Fig. 2 Relative amount of total energy emitted that is absorbed within small unit-density spheres (cell nuclei) of varying diameter, from H^3 uniformly deposited within the sphere. The curve indicates the magnitude of the "edge effect" (loss of energy to the cytoplasm) as a function of the radius of the cell nucleus.

FIGURE 1

ENERGY FROM TRITIUM BETA RAYS, RELATIVE DEPOSITION IN SPHERICAL VOLUMES

FIG. 2