The Uptake, Excretion, and Radiation Hazards of Tritiated Thymidine in Humans

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SUMMARY

Nine patients with malignant disease were given i.v. injections of tritiated thymidine, 0.2 mCi/kg, for tumor cell kinetics studies. Serial plasma, urine, saliva, and air vapor samples were collected variously for up to 79 days, and tritium activity was measured. The initial half-life of plasma activity was rapid. After 1 day, the activity decayed with a half-life of 10.8 days, indicating equilibration of activity with the total body water. Urine activity was over 100 times the plasma activity within 1 hr, with equilibration approaching the plasma activity after 2 days, and then decayed at a similar rate. Saliva and air vapor activity increased to plasma levels and then decayed at the same rate as did plasma activity. In the first 24 hr, appreciably one-third of the total injected activity was excreted in the urine. During the first 12 days there were 54.2% urinary and 10.6% insensible losses. Maximum losses determined by extrapolation of observed data were 68% urinary and 19.5% insensible losses, or a total of 87.5%. Approximately 7% of the injected activity may represent material initially incorporated into DNA but later metabolized and excreted.

The radiation dose from total body water is estimated at 0.69 rad. The estimated dose absorbed by cell nuclei from incorporated material is a maximum of 20.5 rads. These radiation doses would not seem to contraindicate injection of 0.2 mCi tritiated thymidine per kg to patients in this clinical and experimental setting. Measurements of activity in personnel and room air indicate that the use of such doses is not hazardous if appropriate precautions are followed.

INTRODUCTION

The radioactive DNA precursor [3H]thymidine is rapidly and selectively incorporated into DNA (7). The accumulation of activity in DNA is essentially complete within 1 hr following the i.v. or i.p. injection of [3H]thymidine into experimental animals (36) and humans (33). [3H]Thymidine has a maximum β energy of 18 keV and an average range in nuclear emulsions of 1 to 2 μm, making possible high resolution radioautographs of labeled cell nuclei (4, 7). These attributes have resulted in the widespread and effective use of [3H]thymidine in the study of normal and tumor cell kinetics in animals (17) and in humans (10).

Direct estimates of the radiation hazard due to tritium activity in the body water or in the cell DNA have primarily been made in rodents (7, 23, 29, 34, 41, 43), with extrapolation of the results to humans. Wade and Shaw (43) estimated from mice that a single p.o. ingestion of 1.0 mCi of [3H]thymidine by man would be required to deliver a dose of 1.25 rads over a period of 13 weeks to the nuclei of a significant fraction of cells in the total body.

In kinetic studies of patients with leukemias and solid tumors (2, 5, 6, 15, 21), i.v. doses of 10 to 40 mCi of [3H]thymidine have been necessary for adequate radioautographs. There has been, however, only 1 report, to our knowledge, in which radiation hazards were directly estimated from metabolic studies of [3H]thymidine in humans. Two patients received i.v. [3H]thymidine. Measurements of tritium activity in plasma, urine, stool, and spinal fluid were performed. It was estimated that over 50% of the injected dose was incorporated into DNA (33).

In this report, frequent collections of various body fluids from 9 patients with lung or head and neck cancer were analyzed for tritium activity for up to 79 days following the i.v. administration of [3H]thymidine. Quantitative 24-hr urine collections and frequent expired water vapor specimens permitted an accurate accounting of excreted activity. Single or serial tumor samples were obtained after [3H]thymidine injection and were processed for radioautographic cell kinetics studies. Based on the metabolic studies in conjunction with cell kinetics parameters, we have attempted to define more specifically the radiohazard of [3H]thymidine to the patient. Corollary data defining the radiohazard to personnel are included.

MATERIALS AND METHODS

Patient Selection. All patients in this study were afebrile and had advanced lung cancer or head and neck cancer with normal renal and hepatic function and had tumor accessible for cell kinetics investigations (Table 1). Patients received an undiluted i.v. injection of [3H]thymidine, 0.2 mCi/kg body weight, specific activity, 1.9 Ci/m mole; and sterile pyrogen-free aqueous solution 1 mCi/ml (Schwarz/
Metabolism and Dosimetry of [3H]thymidine

The door of the patient’s room was closed over 99% of the study period. Two portable Hankscraft cold water humidifiers operated continuously starting 1 day before injection and delivered approximately 4 liters of water each day. A Sears dehumidifier collected approximately 3 liters of the room vapor each day. The activity in triplicate aliquots of collected water was determined as above.

Cell Kinetics. Tumor samples were obtained by direct visualization with flexible fiberoptic bronchoscopy. In other patients with accessible masses, the tumor was removed by surgical biopsy, by skin punch, or by aspiration of the lesion through a 25-gauge needle. Solid tissue was placed in Bouin’s solution for 24 hr and cleared with 70% ethanol. Tissue was sectioned onto gelatin-subbed slides and overstained with eosin. Aspirates were smeared directly onto slides and air dried. The slides were prepared for radioautography in a darkroom with controlled temperature and humidity. The slides were dipped in NTB2 emulsion diluted 1:1 with distilled water. These were dried and incubated in the dark for varying exposure durations and later were developed (Kodak D-19 developer) and fixed in Kodak fixer. Special procedures adapted for background analysis and grain counting will be described elsewhere.

Analysis of radioautography data involves the following parameters (6, 7). The LI is defined as the fraction of a population of cells that incorporates detectable amounts of radioactive label. The grain count distribution indicates the frequency of cells with various grain counts. The grain count of a given cell is proportional to its DNA synthesis rate. The PLM curve is the ratio of labeled mitotic cells to total mitotic cells as a function of time. PLM curves are used to derive the time required for cells to traverse the phases of the cell cycle. The DNA-synthetic phase (S phase) (16) is determined from the PLM curve as the time between the midpoints ascending and descending the 1st wave of the labeled mitosis curve. The generation time or mean cell cycle time ($T_c$) is defined as the time interval between the initiation of 2 sequential mitoses. $T_c$ is operationally determined by measuring the time between equivalent points of sequential mitoses in a PLM curve.

Radiation Dosimetry. The total body dose delivered during a time interval $t$, to $t_2$ is given as (18)

$$D_{t_2} (\text{rads}) = F \int_{t_1}^{t_2} q_{v}(\tau) \, d\tau$$  \hspace{1cm} (A)$$

where

$$F (\text{rads/day}) = (A \cdot E \cdot CF)/(M \cdot 100 \text{ ergs g}^{-1} \text{ rad}^{-1})$$  \hspace{1cm} (B)$$

and 1 $\mu$Ci is $3.2 \times 10^6$ disintegrations/day, $A$ = activity at initial time $t$ (disintegrations/day), $E$ = mean energy/disintegration = $5.7 \times 10^6$ eV, $M$ = mass under consideration (g), $CF$ = conversion factor = $1.6 \times 10^{-12}$ ergs/eV, $\tau$ = biological half-life of tritium, and $q_{v}(\tau) = e^{-0.693/\tau}$. 

Equation A gives the body dose due to the presence of tritium activity which is decaying with a half-life equivalent

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to that of the body water. Energy deposited within the cell nucleus due to the incorporation of activity into DNA is given by Equation B (4). For cell nuclei energy, deposition is assumed to be constant until cell division, at which time energy deposition persists at one-half the former rate.

**Data Analysis.** Statistical analysis and regression formulas were calculated with a Honeywell 516 computer time sharing system (DataComp, Inc., Silver Spring, Md.).

**RESULTS**

**Uptake and Distribution of [³H]Thymidine**

**Plasma.** Following the i.v. administration of [³H]thymidine (0.2 mCi/kg), the plasma activity was determined. Maximum activity was observed within 2 to 3 min in both patients studied during the 1st min postinjection (Chart 1, A and B). Equal distribution of the administered activity throughout the total body water, assuming an average of 55% of body weight as total body water for male patients over age 40 (12), would result in an expected activity of 0.354 μCi/ml. Plasma levels approaching 3 times this activity were observed during the 1st 5 to 7 min. This indicates that uniform mixing of the injected dose throughout the total body water was not attained until after the 1st several min. Initial clearance of plasma activity was very rapid, with a half-life of 3 to 4 min associated with equilibration of the dose with the body water. Thereafter, clearance was much slower and approached a uniform rate of decay by the end of the 1st day.

Plasma activity was determined at intervals during 79 days following the administration of [³H]thymidine to 8 patients in 9 individual experiments. Representative observations of plasma activity are plotted for 3 patients in Chart 2.

A least-squares regression analysis (Chart 3 A) performed on 216 data points collected in the 9 experiments indicates that mean plasma activity may be expressed during the 1st day by Equation C, although we recognize that this is a composite expression representing a series of distinct components.

\[
\text{Plasma activity (Day 1)} = 0.22e^{-0.33t} \quad (C)
\]

For subsequent days, plasma activity is described by Equation D.

\[
\text{Plasma activity (day > 1)} = 0.16e^{-0.84t} \quad (D)
\]

The latter equation indicates that, after the 1st day, plasma activity decays with a half-life of 10.8 days.
Urine. Within 2 to 3 min after i.v. administration of [\(^3\)H]thymidine, tritium activity was detected in urine specimens (Chart 1, A and B). Urine activity rapidly increased to a concentration as high as 115 times plasma activity within the 1st 60 min. Tritium activity excreted in the urine up to 79 days postinjection was determined for 9 patients in 10 experiments. Representative observations of urinary activity for 3 patients are shown in Chart 4. The least-squares regression analysis of 224 data points from all patients indicates that the urine activity may be described by Equation E (Chart 3B).

Urine activity (all days)  

\[
\text{Urine activity} = 8.0e^{-2.63t} + 0.033e^{-0.47t} + 0.17e^{-0.056t}
\]  

(E)

The cumulative renal excretion for the 1st 12 days was determined by measuring the activity of the total 24-hr urine output collected. Mean cumulative urinary losses for 9 patients are plotted in Chart 5. Data points are fitted by integration of Equation E. A mean of 54.2 ± 2.4% (S.E.) of the total administered dose was excreted in the urine in the 1st 12 days. Integration of Equation E predicts the cumulative urinary loss of 67.4% of the injected dose within 60 days with a theoretical limit of 68% cumulative loss. Most of the injected dose, then, is lost by renal excretion.

Insensible Losses. Additional loss of administered activity occurred insensibly in the form of expired water vapor, sweat, saliva, and stool. The water vapor activity in 30 air samples collected from Patient W. S. in 2 experiments demonstrated the rapid appearance of activity in expired air. The concentration of the activity remained slightly lower than plasma activity but, after the 1st 2 to 3 days, decayed at the same rate (Chart 6A).
I A instantaneous total body water activity predicted in Equations C and D indicates that the minimum of 1.4% of the administered dose is lost insensibly during the 1st day, 10.6% loss during the 1st 12 days, and 19.5% ultimate loss.

**Total Excreted Activity.** Total losses, then, include those accounted for by urinary and insensible excretion. During the 1st 12 days, 54.2% urinary and 10.6% insensible losses account for 64.8% of the administered dose. Integration of Equations C, D, and E predicts a total of 68% urinary and 19.5% insensible losses, or 87.5% of the initial dose.

It has been demonstrated that the incorporation of [3H]thymidine into cell DNA predominantly occurs within the 1st several min following i.v. administration and is essentially complete by the end of the 1st hr (33, 36). Residual activity in the body water at the end of the 1st day is, therefore, unavailable for incorporation into DNA and will eventually be excreted. Table 2 presents the plasma activities and cumulative urine losses in the study group at the end of the 1st and 12th days. The total body water activity, as reflected by the mean plasma activity of 0.164 μCi/ml after the 1st day, represents a pool of 45.1% of the administered dose.

Observed urinary and insensible losses for the same period represent 29.6% of the injected dose (6.5% difference). Furthermore, projected urinary and insensible losses following Day 12 account for the excess loss of an additional 0.8% of injected activity beyond that anticipated from the total body water activity. The activity balance would indicate that, particularly during the 1st 12 days, more

**Chart 6.** A, tritium activity in plasma, urine, saliva, and air vapor in a patient monitored 12 days; B, tritium activity in plasma, urine, and saliva in a patient monitored over 60 days. [3H]TdR, tritiated thymidine.

**Table 2.** Plasma activity and cumulative urine losses

<table>
<thead>
<tr>
<th>Patient</th>
<th>[3H]Thymidine administered (mCi)</th>
<th>Day 1</th>
<th>Day 12</th>
<th>Cumulative urine activity excreted (mCi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. A.</td>
<td>12</td>
<td>0.165</td>
<td>0.101</td>
<td>4.83</td>
</tr>
<tr>
<td>J. C.</td>
<td>15</td>
<td>0.173</td>
<td>0.078</td>
<td>3.25</td>
</tr>
<tr>
<td>H. D.</td>
<td>16</td>
<td>0.180</td>
<td>0.099</td>
<td>5.76</td>
</tr>
<tr>
<td>K. H.</td>
<td>12 NA*</td>
<td>NA</td>
<td>NA</td>
<td>6.76</td>
</tr>
<tr>
<td>C. P.</td>
<td>14</td>
<td>0.120</td>
<td>0.060</td>
<td>4.69</td>
</tr>
<tr>
<td>D. S.</td>
<td>9</td>
<td>0.100</td>
<td>0.056</td>
<td>2.85</td>
</tr>
<tr>
<td>W. S.</td>
<td>14</td>
<td>0.108</td>
<td>0.074</td>
<td>5.17</td>
</tr>
<tr>
<td>P. T.</td>
<td>15</td>
<td>0.255</td>
<td>NA</td>
<td>3.73</td>
</tr>
<tr>
<td>F. V.</td>
<td>13</td>
<td>0.280</td>
<td>0.099</td>
<td>2.48</td>
</tr>
</tbody>
</table>

Mean activity 0.164 0.080

* NA, data not available.

‡ Dose administered on 2 occasions.

Seventy-one saliva (and/or sputum) specimens were obtained from 3 patients at various times following [3H]thymidine injection. Tritium activity in the saliva is plotted for 2 patients (Chart 6, A and B). The data demonstrate the equilibration of saliva activity with the total body water during the 1st day.

Pleural fluid was obtained by thoracentesis from Patient D. S. Activity in the malignant effusion 14 days after i.v. administration of [3H]thymidine was determined to be 0.050 μCi/ml, identical to the plasma activity of the same day.

Reasonable estimates of insensible losses can be made assuming that such losses occur with an activity equal to that of blood plasma, an assumption well supported by the collective expired air, saliva, and pleural fluid data. Conservatively, a volume equivalent to 2.8% of the total body water (1078 ml/day for a 70-kg man) is lost insensibly each day in a nonfebrile individual. Integration of 2.8% of the instantaneous total body water activity predicted in Equations C and D indicates that the minimum of 1.4% of the administered dose is lost insensibly during the 1st day, 10.6% loss during the 1st 12 days, and 19.5% ultimate loss.
The incorporation of \(^{3}H\)thymidine into cell DNA. Serial biopsies taken at intervals following i.v. administration of \(^{3}H\)thymidine were analyzed by radioautography. PLM curves, LI's, and grain count distributions were obtained. Detailed analysis of these results will be the subject of a future publication. Data obtained from aspirates of 3 tumor sites of 1 representative patient (C. P.) with large cell anaplastic carcinoma of the lung are presented here to permit estimates of the incorporation of labeled DNA precursors into rapidly proliferating cell populations.

The LI of samples obtained by aspiration at 1, 4, 54, and 150 hr after injection ranged between 6.9 and 11.3%, with a mean of approximately 10%. The mean grain count was the highest at 1 hr postinjection (36.9 grains/cell). The PLM curve permitted calculation of an S-phase duration of 20 hr, and a \(T_c\) of 80 to 90 hr. These kinetic parameters are typical of those observed in vivo for human solid tumors (2, 5, 15).

The efficacy of our radioautographic technique was determined by comparison of the specific activity of extracted cell DNA to mean grain counts in experiments with Sarcoma 180 ascites tumors in BALB/c \(\times\) DBA\(_2\) F\(_1\) mice (6). The observed efficiency varied from 24 to 28%. Assuming a 25% efficiency for similarly prepared radioautographs of the human biopsy material, estimates of cellular incorporation of labeled thymidine can be made. For the 1-hr sample (Patient C. P.), a mean of 36.9 grains/cell were counted following an 8-day exposure, \(i.e., 1.3 \times 10^{-2} \text{ dpm/cell. Estimating the total burden in Patient C. P. to be 0.5 kg (38), an LI of 10%}^6 \text{ and an assumed mean cell weight of 10^{-3} g, roughly 5 \times 10^{10} tumor cells are labeled.}

The estimations of the uptake of \(^{3}H\)thymidine into the bone marrow and intestinal cells require several assumptions. The number of nucleated bone marrow cells in humans has been determined to be \(2 \times 10^{10}\) cells/kg body weight (11). The LI of erythroid and myeloid cells determined by in vitro incubation in \(^{3}H\)thymidine is 20 to 25% (39). For a 70-kg man, approximately \(3.0 \times 10^{11}\) bone marrow cells would be labeled. It has been estimated that there are approximately \(6.8 \times 10^{19}\) proliferating intestinal crypt cells in a 70-kg man (R. Hagemann, personal communication). Assuming an LI of 10% (25) for these cells, a total of \(3.8 \times 10^{19}\) cells in the tumor, bone marrow, and intestinal crypt cells are labeled. Therefore, 2.2 mCi are estimated to be incorporated into the DNA of these rapidly proliferating populations.

Therefore, 7.3% of the initial dose is excreted within the 1st 60 days, essentially infinite time in terms of the biological half-life of water. Up to 19.8% of the activity is initially incorporated into cell DNA, but a substantial fraction of this is lost within the 1st several days because of continued division of the most rapidly proliferating cells. During the following weeks, gradual cell turnover in the more slowly dividing cells results in the excretion of an additional small component of activity.

Radiation Delivered to the Body. The radiation dose received by patients administered i.v. \(^{3}H\)thymidine is calculated in 2 parts, the dose from total body water and the dose from uptake in cell DNA. The dose to the total body during the 1st day is estimated rather conservatively, assuming that all injected activity not incorporated into DNA irradiates the body for the entire day. For a 70-kg man given 14 mCi, the presence in the body water of 80.2% or 11.23 mCi of tritium with a mean energy of 5.7 keV deposits 0.047 rad in 1 day. After the 1st day, activity is lost from the total body water with a half-life of 10.8 days. Integrating Equation A from Day 1, at which time the mean plasma concentration of tritium was 0.164 mCi/ml (Table 2), to infinite time, calculates the dose of the total body water to be 0.84 rad. The total dose for all days is at most 0.69 rad.

The dose to the cells is more difficult to determine and requires additional assumptions. The mean nuclear volume accounts for the excretion of a significant fraction of the 2.2 mCi estimated to be incorporated into the DNA of these rapidly proliferating populations.

It is suggested that accountable losses of 87.5%, which were in excess of the 80.2% available for loss from the body water (7.3% difference), are due to the breakdown of labeled DNA by cell turnover, particularly in the rapidly proliferating populations. The bone marrow and crypt cell pools do not change significantly in mass. With each crypt cell division, 1 daughter cell must either be sloughed off to be lost in the stool or degraded with reuptake of activity into the body water where it may be excreted or reutilized through salvage pathways (7, 37). Similarly, division of labeled bone marrow cells may result in release of differentiated cells into the bloodstream where they may remain prior to degradation for hours or months. There may also be cell destruction with reutilization or excretion of incorporated activity (13). Similar processes may coexist in tumors despite the gradual accrual of tumor mass (15). Transit of the cell cycle often implies death for a significant fraction of the proliferating cells. In general, the greater the growth fraction, \(i.e.,\) the percentage of cells in the proliferative compartment, the greater the death rate.

Of the cells in the proliferative cycle, the \(T_c\) ordinarily ranges from 1 to 5 days. The \(T_c\) of granulopoietic and erythropoietic precursor cells is 1 to 2.5 days (9). The \(T_c\) of the tumor in our Patient C. P. was less than 4 days. On the basis of this kinetic parameter, many of the labeled cells would be expected to replicate within 12 days. Hence, this accounts for the excretion of a significant fraction of the 2.2 mCi estimated to be incorporated into the DNA of these rapidly proliferating populations.

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cells. The dose to this mass depends upon the rate at which cell doubling causes the dilution of incorporated activity. Estimates can be made for some of the proliferating tissues. Assuming a $T_d$ of the proliferative cells of 1 to 5 days, as above, with each division the loss of one-half of the daughter cells and, hence, the loss of one-half of the activity occurs to retain constant mass so that between 8.2 and 41.0 rads are ultimately deposited. The tumor mass of Patient C. P. with a $T_d$ of, at most, 90 hr increases in mass with each division. Assuming no cell loss, a total of 20.5 rads would be deposited within the tumor mass.

The DNA synthetic time of bone marrow precursor cells is approximately 13 hr (28), and the $T_d$ of 1 to 2.5 days (9) indicates that the radiation hazard to these cells is less. Furthermore, most of these cells go through only a few divisions, mature, and are removed from the population. The issue of radiation to the hematopoietic stem cell cannot be resolved, since these cells have not been identified.

**Radiation Exposure to Personnel.** Estimates of radiation exposure to other patients and health care personnel have also been made. No activity was detected in the blood or urine of medical, nursing, and technical personnel who regularly associated with study group patients. Patients sharing rooms with the study group patients were continuously exposed to an atmosphere containing tritiated water (HTO). Pinson and Langham (32) showed that, during such exposure, activity is effectively taken up by inhalation and by direct absorption through the skin in equal amounts. Patient J. B., occupying the bed adjacent to Patient W. S., was oliguric. The room door was kept closed over 99% of the time. Plasma samples obtained from Patient J. B. during the 1st 2 days following the injection of 14 mCi of [3H]thymidine into Patient W. S. were found to contain a maximum of $1 \times 10^{-4}$ μCi/ml, or a total body water content of approximately 3.9 μCi. This amount is equivalent to roughly 0.5% of the total HTO activity lost in sweat and in expired air by Patient W. S. Twenty-four-hr urine collections from Patient J. B. contained up to $2.4 \times 10^{-3}$ μCi. Such estimates are necessarily crude because of the inaccuracy of extrapolating few counts above background to total body water content.

Total uptake of tritium by other patients and health care personnel was never above background counts and therefore did not approach the permissible levels for an unrestricted area as outlined in Title 10, Code of Federal Regulations, Part 20 (10.CFR.20) (40). Insensible losses of HTO were considerable, and an atmospheric activity exceeding permissible levels ($5 \times 10^{-8}$ μCi/ml of air, 10.CFR.20, Appendix B) would have been generated had there been no ventilation of the room. Since the hospital ventilation system was operative, the exposure to HTO was minimal, namely, the plasma and urine activity of Patient J. B.

**DISCUSSION**

The i.v. administration of a single dose of [3H]thymidine results in the rapid equilibration of activity with the total body water. Rubini et al. have reported 2 components with half-lives of 0.2 and 1.0 min which were attributed to the rapid clearance of plasma activity into other body water compartments (33). Our results indicate an initial half-life of 3 to 4 min, but sampling at very early points was inadequate for a more definitive appraisal of the initial events following dose administration.

Tritiated thymidine is metabolized rapidly. The availability of activity for incorporation into DNA is limited to the 1st hr after i.v. administration (33, 36). Rubini et al. (33) have determined that, after 10 min, most body water activity is present as volatile (HTO) and nonvolatile (other than thymidine) components (33). Nonvolatile activity observed after the 1st hr is largely in organic metabolites of thymidine including thymine and β-aminoisobutyric acid (14). Following the 1st day, total body water activity decayed with a half-life of 10.8 days, indicating that the predominant form of activity was in HTO. It is evident, however, that nonvolatile activity continues to comprise a significant portion of total activity. HTO is excreted at the same concentration as it exists in the body water (3, 32, 35). The clearance of activity in urine in a higher concentration and with a more rapid half-life than plasma activity is, therefore, attributed to a nonvolatile component. Urine tritium activity of up to 115 times plasma activity indicates that the renal handling of labeled nonvolatile metabolites of thymidine includes a glomerular filtration and/or secretion but without extensive reabsorption.

Activity in expired air (HTO) was less than that of plasma for the 1st 2 days. A delayed equilibration of tritium activity in saliva samples with that of plasma activity was similarly noted. Plasma and total body water compartments equilibrate rapidly. If the tritium activity in plasma were solely in the form of HTO, the expired water vapor and saliva concentration would have been equivalent to plasma activity in a much shorter period of time (3). It is suggested, therefore, that a substantial fraction of plasma activity is initially nonvolatile but that the proportion of this component diminishes during the 1st 2 days until activity is primarily in the form of HTO.

Most of the administered dose was excreted. Urinary and insensible excretion during the 1st 12 days accounted for 64.8% of the administered activity. The eventual loss was projected to be 87.5% of the initial dose. Cumulative urinary losses for 21 and 24 days were examined by Lisco et al. (27) for 2 patients given 0.1 mCi/kg. A total of 65 and 47.5% of the injected doses were excreted, in general agreement with our observations.

As indicated, the excretion data also include the activity from the metabolic turnover of [3H]thymidine initially incorporated into DNA. Approximately 20% of the injected dose of [3H]thymidine is incorporated into cell DNA within the 1st hr after i.v. administration, and the remaining 80% of the dose is excreted as HTO and as nonvolatile metabolites of thymidine. Of this 20%, it is suggested that 7.3% of the initial dose activity incorporated into DNA was metabolized and excreted during the study period. This would explain the observation that the cumulative tritium excretion exceeded the loss of activity from the total body water.

Our result of the percentage incorporation of [3H]thymidine into DNA is substantially different from that of Rubini et al. (33) wherein 2 terminal patients were administered i.v. [3H]thymidine (0.14 to 0.31 mCi/kg). Plasma,
urine, and stool activities were followed primarily during the 1st 2 hr after dose administration. Their estimates of incorporation of [3H]thymidine into cell DNA were based upon an analysis of volatile and nonvolatile components, rather than upon detailed balance studies. It was observed that less than 50% of the injected activity was converted to HTO and nonvolatile metabolites of thymidine. They concluded that at least 50% of the administered dose was retained in vivo and was presumably incorporated into cell DNA.

These results are in accord with studies of [3H]thymidine incorporation in rodents. Wade and Shaw (43) observed that, 24 hr after the p.o. ingestion of [3H]thymidine by mice, 51% of the initial dose remained in the body, with 8% present as nonvolatile activity. Kiseleski (22) found 6% of [3H]thymidine activity incorporated into DNA at 1 hr after i.p. injection. Lambert and Clifton (24) reported 14% of activity incorporated into DNA following i.p. injections of [3H]thymidine into rats. The incorporation into DNA as exemplified by these reports is somewhat less than the amount observed here for humans. The route of administration could relate significantly to this difference.

The detailed balance studies reported here permit a more accurate appraisal of the biological hazard of [3H]thymidine in humans than was previously possible. The radiation dose to the total body water of patients receiving i.v. [3H]thymidine, 0.2 mCi/kg, is roughly 0.69 rad. The calculated dose to the cell nucleus is a maximum of 4.1 rads/day. The total energy deposited in any given cell depends upon the cell cycle time and the time to cell death. Rapidly proliferating cells dilute the incorporated activity with each cell division by distribution of equal parts of the parental genome to the daughter cells. Cells with prolonged cell cycle times are far less likely to incorporate [3H]thymidine, but energy deposition will continue as long as tritium activity is present in those few cells that have incorporated label into their DNA. Pelc (31), however, observed the significant turnover of label, even when incorporated into the so-called "nondividing" tissues of the adult mouse, including brain and muscle, thereby limiting the potential dose. The mechanism of this was not apparent and could involve DNA repair mechanisms.

Similar estimates of the dose to the cell nucleus following the incorporation of [3H]thymidine have been reported previously. Van de Aiet and Shaw (41) calculated that 8.3 μCi administered p.o. to mice cause the average cumulative dose delivery of 4 to 10 rads in 8 days. Wade and Shaw (43) reported a 12-rad cumulative dose to the cell nucleus in mice that were fed [3H]thymidine, 0.4 mCi/kg.

The relative biological effectiveness of tritium β decay is taken as 1.0, indicating that radiation from intranuclear [3H]thymidine is no more efficient than X-irradiation in producing biological effect (4). At the cellular level, irradiation by β decay of tritium causes strand breaks in DNA. As with X-irradiation, most of these breaks are repaired rapidly, so that genetic lesions are inefficiently produced (8). Johnson and Cronkite (19) evaluated the effect of [3H]thymidine on mouse spermatocyte count and concluded that doses of up to 1 mCi/kg body weight were comparable to 2 rads/day of γ irradiation. Vennart (42) estimated that, in rodents, 1 to 5 mCi of [3H]thymidine per kg are equivalent to 30 rads whole-body X-irradiation. In tissue culture cells, killing is observed (dose that effectively destroys 37% of the cells) at an X-irradiation dose of 200 to 750 rads when grown in the presence of 0.07 to 0.21 μCi of [3H]thymidine per ml, which yielded a mean grain count of over 100 times that observed in Patient C. P. (assuming radiographic exposure of 8 days) (4). In our studies in Sarcoma 180 ascites tumor, 0.8 mCi of [3H]thymidine per kg had no effect on tumor cell count, compared with controls.9

It is unlikely that toxic effects of 0.2 mCi/kg, as used here, would be observable at the organism level. Baserga et al. (1) and Lisco et al. (26) observed a significant increase in tumor rate in both young and old mice receiving [3H]thymidine, 1 mCi/kg. Johnson and Cronkite (20), however, have observed no significant increase in either tumor incidence or in mortality rate in mice given 1 or 5 mCi of [3H]thymidine per kg. The radiobiological effects of incorporated [3H]thymidine have been extensively reviewed for in vitro and animal models (4, 29). Few reports, however, have dealt with human studies. Lisco et al. (27) failed to detect chromosomal abnormalities in the lymphocytes of 4 patients who received [3H]thymidine, 0.1 mCi/kg.

The radiation dose to individuals exposed to the patient study group was determined by urine and plasma collections from other patients and personnel. Activity was barely detectable in only 1 oliguric patient sharing a room with a patient given tritium. In all other cases, there were no measurable levels of activity. Unpermissible levels of exposure could not have occurred by any manner of contact with labeled patients except by accidental ingestion or injection of labeled material.

We have demonstrated that several factors limit the radiation dose in a person given injections of [3H]thymidine to levels only a few times greater than those commonly used in diagnostic radiology. First, most of the dose is rapidly excreted. Second, only 20% of i.v. [3H]thymidine is incorporated into DNA. Third, most of the activity resides in rapidly proliferating tissues which, because of cell division and death, results in limited energy deposition. Fourth, prolonged retention of activity would be expected for only a rare, mature, slowly dividing cell, thus allowing only a minimal chance of long-term somatic effects.

In our opinion, the potential importance of delineating cell kinetic parameters in patients with cancers justifies the use of the doses of [3H]thymidine we have administered. The hazard of the [3H]thymidine may be marginal at most. These kinetic data may provide insight into more effective and rational uses of therapeutic modalities which are usually highly toxic. It is not our thesis, however, that the present data necessarily justify the use of these doses of [3H]thymidine in other experimental situations. It is hoped that these data can provide a more reasonable foundation upon which appropriate determinations can be made in each given experimental situation.

REFERENCES


# The Uptake, Excretion, and Radiation Hazards of Tritiated Thymidine in Humans


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