Water Content in Cultured Mammalian Cells for Dosimetry of Beta-rays from Tritiated Water

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One of the critical factors for dosimetry of beta-rays from tritiated water is the water content within the cell.
We estimated the cellular water concentration in cultured mammalian cells by measuring accurately the fraction of the extracellular water in the cell sample with $[^{14}\text{C}]$inulin.

The net water content (ml·g$^{-1}$) after correcting for the extra-cellular water fraction was 0.858 for HeLa (human), 0.833 for JTC12P3 (monkey), 0.829 for NRK (rat), 0.843 for C3H10T1/2 (mouse) and 0.846 for L5178Y (mouse) cells. The mean water content ($\pm$ S.E.M.) in these 5 lines of cultured mammalian cells was 0.842±0.005.

INTRODUCTION

The importance of tritium in radiation protection has been increasing in recent years, since the use of much greater quantities of tritium is expected in future nuclear fusion reactor engineering. The most frequently occurring chemical form of tritium in submerged living cells is tritiated water. The essential factor in calculating the radiation dose from tritiated water is the concentration of water within the cells.

The water content of the cell, however, is difficult to measure as pointed out by the recommendations of the National Council on Radiation Protection and Measurements$^1$), and has often been assumed to be 0.60–0.75$^{1,2}$ or 0.80$^{3,4}$ based upon the water content of soft tissues, not upon the data on the concentration of water within the cell of interest. The main difficulty in measuring the water content of cells is the correction for the extracellular water in the wet mass of cell pallet. Inulin has been used in the determination of the extracellular water of tissue$^5$, since this polysaccharide is not toxic to the living cell and can freely diffuse into the extracellular water space, without being taken up or metabolized by
mammalian cells.

Therefore we used $^{14}$C]inulin for this purpose in several lines of cultured mammalian cells. A preliminary report was published previously$^6$.

MATERIALS AND METHODS

Cell cultures:

Five lines of cultured mammalian cells were used in this assay: HeLa cells derived from a human cervical carcinoma, JTC12P3 cells from a crab-eating monkey kidney epithelium in a spontaneously transformed state (kindly provided by Dr. Ken-ichi Manaka, Research Center of Tissue Culture, Dokkyo University Medical School, Tochigi, Japan), NRK from normal rat kidney, C3H10T1/2 normal fibroblasts from mouse embryo (kindly provided by Dr. Toyozo Terasima, the former Director-General of the National Institute of Radiological Sciences, Chiba, Japan) and L5178Y-leukemic cells derived from a mouse lymphoma.

The culture media used were Ham's F10 medium (Flow Laboratories, Irvine, Scotland) supplemented with 10% fetal calf serum (FCS) for HeLa cells, DM170 medium (Kyokuto Pharmaceutical, Tokyo, Japan) with 5% FCS for JTC12P3 cells, MEM (Flow Labs.) with 10% FCS for NRK cells, BME (Flow Labs.) with 10% FCS for C3H10T1/2 cells, and Fischer's medium (Grand Island Biological Co., New York, U.S.A.) with 10% horse serum for L5178Y cells. Penicillin (100 U ml$^{-1}$; Meiji Seika, Tokyo, Japan) was added to all the culture media. The cultures were kept at 37°C in humidified air with 5% CO₂.

The HeLa, JTC12P3, NRK and C3H10T1/2 cells were grown in plastic flasks of 175 cm$^2$ growth area (Falcon No. 3028, Becton Dickinson Labware, Oxnard, U.S.A.) and the cells in confluent state were used for the measurements. The L5178Y cells were cultured in suspension within a glass flask containing 500 ml of the culture medium and the cells at population density of about $3 \times 10^8$ cells per 1 were used for the measurement. Each assay sample consisted of about $2 \times 10^7$ to $2 \times 10^8$ cells and the data on the amount (wet weight) of the cell pellet sample actually used are shown in Table 1.

Procedures for measurement:

The cells, except L5178Y cells in suspension, were dispersed by gentle trypsinization and pipetting, and suspended in the conditioned medium derived from each cell sample at an ice-cold temperature (0–4°C). To keep the cells alive at a packed state, the following procedures were carried out under ice-cold condition unless otherwise stated. The cells, especially of a fastidious cell line such as HeLa or C3H10T1/2, died in a packed state at room temperature. If a part of the cell population within the sample were to die, the measurement will produce a larger numerical value for extracellular water, and the calculation will yield a smaller value for intracellular water, because $^{14}$C]inulin can permeate into the dead cells.

The cells in suspension thus obtained were spun down by centrifugation at 1,000 rpm for 5 min and resuspended in 4.5 ml of conditioned medium. A 0.5 ml sample of the culture
medium containing 5 mg of inulin was added to the cell suspension. After being stirred gently at 20°C to allow the cells to adsorb the carrier inulin on their surface, [carboxyl-14C]inulin having a nominal radioactivity of 18.5 kBq (0.5 μCi) (sterile; DuPont/NEC Research Product) was added to the suspension. If radioactive inulin was added to the cell suspension without prior treatment with carrier inulin, the radioactive polysaccharide might be adsorbed by the cell surface giving rise to a falsely high radioactivity within the cell pellet. After standing for 10 min at 20°C for equilibration, the cell suspension was centrifuged at 1,000 rpm for 5 min, and immediately after centrifugation the supernatant was transferred into another test tube. As much of the resulting cell pellet as possible was transferred through a plastic micropipette into a glass tube with its tightly fitted cap, both of which had been carefully dried and weighed. The tube was always handled with a pair of clean gloves. The tube containing the cell pellet was weighed and the wet weight of the cell pellet was referred to as M mg. The tube containing the cells was then uncapped and dried together with its cap in an electric drying-oven kept at 100–110°C. Every 2 h during drying, the sample tube was tightly capped and transferred into a dessicator containing silica gel and weighed after cooling down. Then the sample was again transferred into the oven to continue drying. By repeating these procedures of drying and weighing, the dry weight reached a minimum value after 6 h of successive drying periods. A further 2 h-drying caused a slight (ca. 1% of the minimum value) increase in the weight owing to the oxidation of organic materials. The minimum value thus attained was adopted as the dry weight of the cell pellet and referred to as D mg. After the drying was completed, [14C]inulin within the dried cell pellet was extracted with 2.0 ml of warm distilled water. Duplicate samples of a 0.5 ml extracted solution, each of which corresponded to one fourth of the total radioactivity within the cell pellet, were transferred to vials containing a scintillation cocktail (1 l of the cocktail contained 670 ml toluene, 330 ml Triton X-300, 6 g PPO and 0.1 g POPOP) and the radioactivity arising from 14C was counted by a liquid scintillation counter (referred to as P dpm per one forth of sample).

A 1 ml aliquot of the supernatant of the cell suspension was saved for the determination of its dry mass, which was referred to as D mg·ml⁻¹. The other two 50-μl aliquots of the supernatant were transferred into liquid scintillation vials and the radioactivity of 14C was counted (referred to as S dpm per 50-μl supernatant solution).

**Calculation of the intracellular water content:**

From the radioactivities of the cell pellet (P dpm per one forth of sample) and the supernatant (S dpm per 50-μl sample), the volume of the extracellular water, M', is calculated according to the equation:

\[
M' = P \times (1/4)^{-1} \times (S \times 50^{-1})^{-1} = 200 P \times S^{-1} \text{ (μl)}
\]

(1)

The dry mass of the culture medium within the extracellular water space, D'', is:

\[
D'' = M' \times D'' \times 10^{-3} \text{ (mg)}
\]

(2)
Since \( M' \) \( \mu l \) of the culture medium can be taken as \( M'' \) mg in wet weight, the net wet weight of the cells within the pellet, \( M \), is:

\[
M = M' - M'' \text{ (mg)}
\]  
(3)

The net dry weight of the cells within the pellet, \( D \), is:

\[
D = D' - D'' \text{ (mg)}
\]  
(4)

Consequently the water concentration, \( W \), after the correction for the extracellular water can be calculated according to the equation:

\[
W = (M - D) \times M^{-1} \\
= \{M' - 200 P \times S^{-1} - (D' - 0.2 P \times S^{-1} \times D'')\} \\
\times (M' - 200 P \times S^{-1})^{-1} \text{ (mg\cdotmg}^{-1} \text{ or ml\cdotg}^{-1})
\]  
(5)

RESULTS

The experimental data, summarized in Table 1, indicate that the resultant values of the water content were very close to each other among these 5 cultured mammalian cell lines. The mean water content (± S.E.M.) was 0.842±0.005 ml\cdot g^{-1}.

DISCUSSION

Whenever we studied the biological effect of tritiated water, we met a difficulty in estimating the beta-ray dose from tritium. The difficulty mainly came from uncertainty about the intracellular water concentration. If the water content of the cell is determined as \( W \) (ml \cdot g^{-1}), the dose-rate, \( D \) (Gy \cdot h^{-1}), absorbed by the cell equilibrated with tritiated water at a specific radioactivity of \( C \) (MBq \cdot ml^{-1}) is calculated according to the equation:

\[
D = 3.29 \times 10^{-3} \cdot C \cdot W
\]  
(6)

where \( 3.29 \times 10^{-3} \) is the conversion factor \( (1 \times 10^6 \text{ decay} \cdot s^{-1} \cdot \text{MBq}^{-1} \times 3.6 \times 10^3 \text{ s} \cdot \text{h}^{-1} \times 5.7 \times 10^3 \text{ eV} \cdot \text{decay}^{-1} \times 1.602 \times 10^{-19} \text{ J} \cdot \text{eV}^{-1} \times 1 \text{ Gy} \cdot \text{J}^{-1} \cdot \text{kg} \times 1 \times 10^3 \text{ g} \cdot \text{kg}^{-1}) \).

The value of 0-80 has often been used for \( W \) in cultured mammalian cells\(^3\). This value was derived from the water content of soft tissues\(^3\), which, however, actually varied from 0.68 to 0.92 (ml \cdot g^{-1}) as shown in Table 2. Moreover, the value was not the intracellular water content, but the water content of tissue including the extracellular fluid and matrices. These circumstances prompted us to measure the water content of cultured mammalian cells.

The concentration of water in the nucleus may be more important than that in the whole cell, since radiation damage results principally from damage to the cell nucleus. As for the distribution of water between the nucleus and cytoplasm, X-ray\(^9\) or \( \beta \)-ray\(^10\)
## Table 1. Experimental data for the water content in cultured mammalian cells.

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Cell line</th>
<th>Exptl series*</th>
<th>Measured values**</th>
<th>Calculated water content: W (ml · g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>HeLa</td>
<td>4</td>
<td>362.7, 44.5, 16</td>
<td>2136, 9746, 0.863</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>158.4, 13.9</td>
<td>2956, 8145, 0.852</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td></td>
<td></td>
<td>0.858±0.006***</td>
</tr>
<tr>
<td>Monkey</td>
<td>JTC12P3</td>
<td>1</td>
<td>613.3, 75.6, 13</td>
<td>9019, 10676, 0.835</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>481.8, 54.7</td>
<td>8395, 9104, 0.824</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>203.5, 26.9</td>
<td>2863, 9522, 0.818</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>377.3, 48.7</td>
<td>4654, 9778, 0.832</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>625.6, 86.0</td>
<td>5287, 10463, 0.839</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>515.0, 71.8</td>
<td>4624, 10300, 0.834</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>431.1, 52.8</td>
<td>4759, 9912, 0.846</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td></td>
<td></td>
<td>0.833±0.003</td>
</tr>
<tr>
<td>Rat</td>
<td>NRK</td>
<td>1</td>
<td>310.6, 36.3, 16</td>
<td>6189, 9722, 0.812</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>140.1, 16.5</td>
<td>2254, 9520, 0.831</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>206.3, 20.7</td>
<td>3957, 9826, 0.846</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td></td>
<td></td>
<td>0.829±0.010</td>
</tr>
<tr>
<td>Mouse</td>
<td>10T1/2</td>
<td>3</td>
<td>70.6, 8.0, 16</td>
<td>1347, 9409, 0.821</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>484.3, 38.0</td>
<td>10535, 9374, 0.867</td>
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<tr>
<td></td>
<td></td>
<td>5</td>
<td>135.8, 14.2</td>
<td>2279, 8975, 0.842</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td></td>
<td></td>
<td>0.843±0.013</td>
</tr>
<tr>
<td>L5178Y</td>
<td></td>
<td>1</td>
<td>305.3, 25.9, 16</td>
<td>7410, 10000, 0.850</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>227.8, 23.2</td>
<td>3961, 8569, 0.841</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td></td>
<td></td>
<td>0.846±0.005</td>
</tr>
</tbody>
</table>

* The experimental series numbered with the same figure represent the measurements performed simultaneously in parallel.

** Symbols used for the measured values signify: M', wet weight of cell pellet (mg); D', dry weight of cell pellet (mg); D'', dry weight of supernatant (mg · ml⁻¹), which was 16 for the media containing 10% serum and 13 for the medium containing 5% serum; P, radioactivity in one fourth of cell pellet (dpm); S, radioactivity in 50-μl supernatant (dpm) which was computed to be 185×60 = 11,000 according to the nominal radioactivity of the [¹⁴C] inulin preparation used.

*** The standard error of the mean.

Absorbance studies showed no significant difference in dry mass per volume⁹ or even a slightly lesser dry mass per volume¹⁰ in the nucleus than in the cytoplasm.

Thus the value of 0.84 for W is regarded to be reasonable for the dosimetry of beta-rays from tritiated water in cultured mammalian cells. Using this figure, we have obtained a reliable value (1.6—1.8) for the relative biological effectiveness (RBE) of tritium beta-rays for malignant transformation in mouse C3H10T1/2 cells¹¹.
Table 2. Water content (ml · g⁻¹) of soft tissues.
The reference of each value is found in the literatureᵃ. Values are for adult animals unless otherwise specified. The figures with asterisk (*) for mouse are by Drs. Ishida and Saito of the Research Reactor Institute, Kyoto University (personal communication).

<table>
<thead>
<tr>
<th>Tissue or Organ</th>
<th>Man</th>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain: Newborn</td>
<td>0.920</td>
<td>0.895</td>
<td>0.868–0.887</td>
</tr>
<tr>
<td>Adult</td>
<td>0.77</td>
<td>0.780–0.832</td>
<td>0.762–0.804; 0.779*</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.710</td>
<td>0.806</td>
<td>0.778*</td>
</tr>
<tr>
<td>Large intestine</td>
<td>0.727</td>
<td>0.771</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.750</td>
<td>0.700–0.760</td>
<td>0.695*</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.748</td>
<td></td>
<td>0.709*</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.787</td>
<td>0.780</td>
<td>0.775*</td>
</tr>
<tr>
<td>Lung</td>
<td>0.813</td>
<td>0.787–0.820</td>
<td>0.785*</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.784</td>
<td>0.740–0.786</td>
<td>0.752*</td>
</tr>
<tr>
<td>Testis</td>
<td>0.840</td>
<td>0.680–0.850</td>
<td>0.840*</td>
</tr>
<tr>
<td>Ovary</td>
<td>0.805</td>
<td>0.720–0.770</td>
<td></td>
</tr>
</tbody>
</table>

ACKNOWLEDGEMENTS

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REFERENCES

WATER CONTENT IN TRITIUM DOSIMETRY

