Biological Assessment of the Enhancement of Tritium Excretion by Administration of Diuretics and Excessive Water in Mice

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Tritiated water/ Tritium excretion/ Diuretic/ Cumulative dose/ Mice

This study was undertaken to determine whether or not the administration of diuretics and excess water after tritium exposure would have any positive reducing effect not only on the retention of tritium but also on the radiation damage of hematopoietic tissue in mice. When mice were treated with diuretics and excess water for a few days after injection of tritiated water (HTO), radioactivity within the body fluid and tissues was reduced, and the number of CFU-s, clonability of splenic T cells and proliferative activity assayed by Con-A blastogenesis were increased in comparison with those in the controls. When the mice were injected with a large dose of HTO (811 MBq/mouse) to assay survival, no mice treated with diuretic and excess water died 80 days after injection, while 80% of the controls died during the first month. The final committed dose in the mice treated early with diuretics was calculated to be 60% of that in the controls. These results suggest that treatment with diuretics and excess water is useful for practical purposes when a human is accidentally exposed to tritium.

INTRODUCTION

With the increased use of energy and the development of energy industries, the world is becoming more dependent upon nuclear energy as a source of electric power. One of the problems in the development of the fusion reactor is the assessment of biological effects of tritium released from nuclear fusion power plants during routine operation or accidents. Human exposure to tritium may occur not only from tritiated water but also from tritiated organic compounds taken as foods. Some investigators have reported that ingestion of tritiated food leads to higher tritium retention in the tissue constituents of animals than that following tritiated water administration¹⁻⁴. Tritiated water, however, is absorbed more
readily and distributed more rapidly throughout the body. Therefore, in the case of accidents, tritiated water contributes to almost all exposure to tritium. The NCRP of the U.S.A. recommends forced fluids to enhance excretion of incorporated tritiated water from the body\(^5\). A few reports have been published concerning the enhancement of excretion of tritiated water from the body\(^6\)\(^-\)\(^9\), but there is no evidence for the protective effect of enhanced tritium excretion by diuretics against tritium-induced injury.

Since the acute effect of tritium on the hematopoietic tissue in humans may be the most important factor in accidental exposure to tritium\(^10\)\(^,\)\(^11\), it is important to understand the correlation between alleviation of the acute effect on the hematopoietic tissue and reduction of the absorbed radiation-dose by the administration of diuretics and excess water. The present investigation was undertaken to examine the protective effect of treatment with diuretics and excess water on the hematopoietic tissue in mice after a single injection with tritiated water.

**MATERIALS AND METHODS**

*Animals*

B6C3F\(_1\) female mice aged 8 weeks were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan). After arrival, the mice were kept for two weeks to check the body weight and for infections. The mice at 10 weeks of age were used for experiment. They were housed, 10 per cage, on wood-chip contact bedding in a 24±2°C air-conditioned clean room and given commercial pellet chow and water ad libitum.

The mice injected with HTO were maintained in a cabinet specially made for this study, within which they were housed separately in a special small cage installed with a filter of silica gel in order to absorb tritiated water secondarily evaporated from the urine.

*Experimental Procedures*

1) **Study of hematopoietic function**

Three experiments were done to analyze the relationship between tritium retention and hematopoietic function after treatment with diuretics and excess water. The mice were divided into three groups in each of the three experiments: the control group in which mice were injected HTO alone, the treated group in which excretion was enhanced after HTO injection, and the normal group in which mice were neither injected with HTO nor treated with diuretics and excess water. Each group consisted of 5 mice (23 g~26 g body weight). HTO was diluted to an appropriate concentration with sterilized water. Mice in the first two groups were injected once intraperitoneally with 0.5 ml of HTO (3.7 GBq/g, NEN Research Products, Boston, U.S.A.). The HTO activities injected for the following Exps. a, b and c were 237, 237 and 204 MBq per mouse, respectively (Fig. 1).

Exp. a: The mice of the treated group were injected intraperitoneally with 3 ml of furosemide (8 mg/kg; Hoechst Japan Limited, Tokyo) as a diuretic twice, 2 h and 24 h after
HTO injection, and with 2 ml of glucose solution (5%) twice, 20 h and 44 h after HTO injection. The mice were then given drinking water containing acetazolamide (0.4 mg/ml, Lederle Japan Limited, Tokyo) ad libitum. The mice of the control and normal groups were given a usual water ad libitum. They were all sacrificed for analysis simultaneously 48 h after the HTO injection.

Exp. b: The protocol was the same as Exp. a except for the drinking water which did not contain acetazolamide.

Exp. c: The mice of the treated group were injected intraperitoneally with 3 ml of the furosemide (8 mg/kg) three times, 2 h, 24 h and 48 h after HTO injection, and with 2 ml of glucose solution (5%) three times, 20 h, 44 h and 68 h after HTO injection. These mice were then sacrificed for analysis 72 h after HTO injection (Fig. 1).

Determination of tritium radioactivity in body fluids and tissues
Mice were sacrificed by chloroform and heart puncture 2 or 3 days after tritium injection, and the spleen, tibiae, femurs, whole blood and plasma were sampled immediately after the sacrifice. A part of each tissue and blood sample was weighed and the radioactivity

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**Exp. a)**

\[
\begin{align*}
F_{2h} & \quad F_{24h} & \quad \text{Assay} \quad 48h \\
\text{HTO i.p.} & \quad \text{(237MBq/mouse)} & \quad \text{---(acetazolamide)} & \quad \text{---}
\end{align*}
\]

**Exp. b)**

\[
\begin{align*}
F_{2h} & \quad F_{24h} & \quad \text{Assay} \quad 48h \\
\text{HTO i.p.} & \quad \text{(237MBq/mouse)} & \quad G_{20h} & \quad G_{44h}
\end{align*}
\]

**Exp. c)**

\[
\begin{align*}
F_{2h} & \quad F_{24h} & \quad F_{48h} & \quad \text{Assay} \quad 72h \\
\text{HTO i.p.} & \quad \text{(204MBq/mouse)} & \quad G_{20h} & \quad G_{44h} & \quad G_{68h}
\end{align*}
\]

F: furosemide (8 mg/kg)/5% glucose 3 ml i.p.
G: 5% glucose 2 ml i.p.
acetazolamide: drinking water with acetazolamide (0.4 mg/ml)

**Fig. 1.** Scheme of the experimental procedure.
Evaluation of cellularity and bone marrow stem cell content

a) Preparation of cell suspension

Bone marrow cells were flushed from the femur by a syringe containing with Hanks' balanced salt solution (BSS). The spleen cells were gently dissociated and then filtered through a stainless steel mesh. The concentration of white blood cells (W.B.C.) in the peripheral blood, the number of cells within the bone marrow and spleen were counted in a hemocytometer using Turk solution.

b) Assay of Spleen Colony-Forming Units (CFU-s)

The method of determination of the CFU-s was basically the that described by Till and McCulloch12). Marrow cells obtained from the femurs were suspended in a Hanks' BSS, and the nucleated cells were counted in a hemocytometer. Suspensions containing the required number of cells, which differed among different groups, was prepared by serial dilutions. The suspensions were kcp at ice-water temperature until injected. The recipient mice were given 7 Gy of whole body X-irradiation 1 day before marrow cell injection. The X-irradiation factors were 250 kVp, 12 mA, 0.5 mm Cu+0.5 mm Al filter, 50 cm FSD and dose rate 0.45 Gy/min. They were housed individually in plastic cages and allowed food and HCI-acidified water (pH 2.2) ad libitum. A suspension containing 1×10^5 (from the normal mice) or 2×10^5 (from the treated mice) bone marrow cells in a 0.2 ml Hanks' BSS was slowly infused through the caudal vein into the recipient mice. Ten recipient mice were used for each group. After 9 or 10 days, the spleen was removed and fixed with Bouin's solution, and the number of nodules formed within the spleen was counted.

Evaluation of spleen cell activities

a) Assay of T cell colony forming activity

T-cell clonability was assayed according to the technique described in our previous report13). Spleen cells were used for assay after lysing red blood cells at 4°C for 5 min by Gey's solution in which NaCl was replaced by NH_4Cl. The culture medium was RPMI1640 medium (GIBCO Laboratories, Grand Island, N.Y. U.S.A.) supplemented with 10% fetal calf serum (FCS: the same supplier), 20% T-cell growth factor (TCGF), 20 mM Hepes, 2 mM L-glutamine, 2mM sodium pyruvate, 100 U/ml penicillin and 100 µg/ml streptomycin. The TCGF used was a supernatant from the spleen cells of Wistar rat stimulated by Concanavalin-A (Con-A, 10 µg/ml: Sigma Chemical Co., St. Louis. Mo. U.S.A.) and phorbol 12-myristate 13-acetate (PMA, 10 ng/ml: the same supplier) according to Gillis et al.14). The spleen cells for assay were seeded into each well of a 96-well microtiter plate (flat bottom, Corning, N.Y. U.S.A.) at an appropriate cell density in 0.2 ml of culture medium with lethally irradiated EL-4 cells as feeder cells. After 11–12 days of culture at 37°C in a 5% CO_2 humidified atmosphere, the number of cloned wells were counted and the cloning efficiency (CE) was calculated by the following formula for each plate assuming
a Poisson distribution:

\[
CE = \frac{-\ln \left( \frac{\text{No. of empty wells}}{\text{No. of total wells}} \right)}{\text{No. of cells seeded per well}}
\]

b) Assay of proliferation activity by Con-A blastogenesis

\(^3\text{H}\)-thymidine incorporation into replicating cells was taken as an index for the ability of cell population to proliferate. The spleen cells were seeded to each well of a 96-well, round-bottom microtiter plate at a concentration of \(2 \times 10^5\) cells per well. They were incubated with or without Con-A (1 µg/well) for 66 h at 37°C in 5% CO\(_2\). \([6-\text{\(^3\)H}]\) thymidine (\(^3\text{H}\)-TdR, 185 GBq/mmol; Amersham International plc, Buckinghamshire, England) was then added at a concentration of 3.7 kBq per well; this was followed by a 6h-incubation period. The cells were then harvested and the radioactivity bound to the fiberglass filter paper was determined by a liquid scintillation counter (Scintisol EX-H, Wako Pure Chemical Industries, Ltd., Osaka). \(^3\text{H}\)-TdR uptake was expressed as cpm in the well with Con-A minus cpm in the well without Con-A.

II) Study of acute lethality

a) Survival assay

Twenty mice were injected intraperitoneally with a single 0.5 ml dose of HTO at 811 MBq per mouse, corresponding to 37 MBq/g body weight. The mice were divided into two groups: ten were given 3 ml of the furosemide (8 mg/kg) three times at 2 h, 24 h and 48 h, and 2 ml of glucose solution (5%) three times at 20 h, 44 h and 68 h after HTO injection as in Exp. c; another ten served as non-treated controls. The survival rates of these mice were determined daily.

b) Analysis of urinary tritium concentration

Fresh urine of the mice was collected at the same time each morning. The urine was diluted to an appropriate concentration with water and the tritium activity was measured by a liquid scintillation counter.

A detailed description for the calculation procedures of the absorbed dose from beta radiation of HTO is given by Tsuchiya et al.\(^{10}\). Briefly, the calculation is as follows: the absorbed dose rate in Gy per day (d\(_\beta\)) is directly proportional to the product of tritium radioactivity per gram of wet tissue (Q µCi/g or Q' Bq/kg) and the average energy (E\(_\beta\)=5.7 keV) released per disintegration, or

\[
d\beta \ (\text{Gy/day}) = 2.92 \times 10^{-3} \times Q
\]

\[
(\approx 7.89 \times 10^{-11} \times Q')
\]

where \(2.92 \times 10^{-3}\) is the conversion factor \((3.7 \times 10^4 \text{ dis}^{-1} \cdot \text{µCi}^{-1} \cdot \text{g} \times 60 \times 60 \times 24 \text{ s} \cdot \text{day}^{-1} \times 5.7 \times 10^3 \text{ eV} \cdot \text{dis}^{-1} \times 1.602 \times 10^{-19} \text{ J} \cdot \text{eV}^{-1} \cdot 1 \text{ Gy} \cdot \text{J}^{-1} \cdot \text{kg} \times 1 \times 10^3 \text{ g} \cdot \text{kg}^{-1} = 2.92 \times 10^{-3})\).

The total dose (Dt) may be calculated by the following formula:
\[ D_t = \int_0^t d_\beta (t) \ dt \]
\[ = 2.92 \times 10^{-3} \int_0^t Q(t) \ dt \]
\[ (\approx 7.89 \times 10^{-11} \int_0^t Q'(t) \ dt) \]

where \( Q(t) \) or \( Q'(t) \) is a mean tritium concentration in the tissue at time \( t \).

**RESULTS**

1) *Tritium activity in body fluids and tissues*

To evaluate the effect of treatment with diuretics and excess water after HTO injection,

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Tritium activity (MBq/g)</th>
<th>Ratio (T/C)</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (C)</td>
<td>Treated (T)</td>
<td>(T/C)</td>
</tr>
<tr>
<td>Exp. a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>6.37±0.39*</td>
<td>3.51±0.29</td>
<td>0.55</td>
</tr>
<tr>
<td>Femur</td>
<td>5.42±0.30</td>
<td>3.36±0.15</td>
<td>0.62</td>
</tr>
<tr>
<td>Tibia</td>
<td>3.71±0.39</td>
<td>2.55±0.31</td>
<td>0.69</td>
</tr>
<tr>
<td>Blood</td>
<td>9.16±2.60</td>
<td>5.10±1.03</td>
<td>0.56</td>
</tr>
<tr>
<td>Plasma</td>
<td>6.86±0.54</td>
<td>4.42±0.36</td>
<td>0.64</td>
</tr>
<tr>
<td>Exp. b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>3.17±0.57</td>
<td>3.77±0.80</td>
<td>1.19</td>
</tr>
<tr>
<td>Femur</td>
<td>7.79±0.50</td>
<td>4.95±0.57</td>
<td>0.64</td>
</tr>
<tr>
<td>Tibia</td>
<td>4.73±0.88</td>
<td>3.78±0.48</td>
<td>0.80</td>
</tr>
<tr>
<td>Blood</td>
<td>7.22±0.80</td>
<td>4.60±0.50</td>
<td>0.64</td>
</tr>
<tr>
<td>Plasma</td>
<td>7.90±0.11</td>
<td>4.92±0.36</td>
<td>0.62</td>
</tr>
<tr>
<td>Exp. c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>2.10±0.26</td>
<td>0.77±0.20</td>
<td>0.37</td>
</tr>
<tr>
<td>Femur</td>
<td>1.74±0.32</td>
<td>0.77±0.19</td>
<td>0.44</td>
</tr>
<tr>
<td>Tibia</td>
<td>1.19±0.19</td>
<td>0.49±0.10</td>
<td>0.41</td>
</tr>
<tr>
<td>Blood</td>
<td>1.45±0.26</td>
<td>0.59±0.09</td>
<td>0.41</td>
</tr>
<tr>
<td>Plasma</td>
<td>1.61±0.30</td>
<td>0.64±0.09</td>
<td>0.40</td>
</tr>
</tbody>
</table>

* Results are expressed as mean value ± standard deviation of five mice of each group in individual experiments.

** Difference between values analyzed statistically by Student's two-tailed t test.
tritium activities of body fluid and various organs were measured at the end of each experiment. The results are shown in Table 1. The concentrations of tritium in tissues of the mice treated with diuretics and glucose solution were significantly lower than those of the control mice except for the spleen and tibia in Exp. b.

2) **Cell numbers**

The numbers of nucleated bone marrow cells, peripheral white blood cells and spleen cells were compared between the treated and control groups. The results are shown in Table 2. The reduction of these cells was apparent in both the treated and control groups injected with HTO compared with the normal group without HTO. The reduction of nucleated bone marrow cells in the treated group with diuretics and excess water was less than in the control group injected with HTO alone in Exp. b and Exp. c. On the other hand, there were no differences in the numbers of peripheral white blood cells and spleen cells between the treated and control groups, suggesting that these indices are not useful for following the effect of this treatment.

3) **CFU-s assay**

The CFU-s survival was calculated from the splenic colony forming efficiency of the

### Table 2. Effect of the treatment with diuretics and excess water on the numbers of hematopoietic cells after HTO injection.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Normal (without HTO)</th>
<th>Control (C)</th>
<th>Treated (T)</th>
<th>Ratio (T/C)</th>
<th>P value**&lt;sup&gt;***&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.M.</td>
<td>(×10&lt;sup&gt;6&lt;/sup&gt;)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>16.22±1.04</td>
<td>7.75±2.11</td>
<td>6.81±1.76</td>
<td>0.88</td>
</tr>
<tr>
<td>W.B.C.</td>
<td>(/mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>4817±1421</td>
<td>2849±756</td>
<td>2701±798</td>
<td>0.95</td>
</tr>
<tr>
<td>Spleen</td>
<td>(×10&lt;sup&gt;7&lt;/sup&gt;)&lt;sup&gt;**&lt;/sup&gt;</td>
<td>15.59±1.38</td>
<td>8.48±1.08</td>
<td>7.23±1.30</td>
<td>0.85</td>
</tr>
<tr>
<td>Spleen wt.</td>
<td>(mg)</td>
<td>71.2±6.6</td>
<td>50.2±8.4</td>
<td>46.2±4.5</td>
<td>0.92</td>
</tr>
<tr>
<td>Exp. b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.M.</td>
<td>(×10&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>16.84±2.51</td>
<td>8.53±1.47</td>
<td>11.86±1.77</td>
<td>1.39</td>
</tr>
<tr>
<td>W.B.C.</td>
<td>(/mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>4856±1029</td>
<td>2257±466</td>
<td>2027±400</td>
<td>0.90</td>
</tr>
<tr>
<td>Spleen</td>
<td>(×10&lt;sup&gt;7&lt;/sup&gt;)</td>
<td>16.13±1.69</td>
<td>7.47±0.55</td>
<td>8.92±1.67</td>
<td>1.49</td>
</tr>
<tr>
<td>Spleen wt.</td>
<td>(mg)</td>
<td>72.8±2.2</td>
<td>51.8±3.7</td>
<td>55.8±10.0</td>
<td>1.08</td>
</tr>
<tr>
<td>Exp. c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.M.</td>
<td>(×10&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>16.72±2.76</td>
<td>9.26±1.59</td>
<td>10.79±0.93</td>
<td>1.17</td>
</tr>
<tr>
<td>W.B.C.</td>
<td>(/mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>4716±552</td>
<td>1642±258</td>
<td>1399±31</td>
<td>0.85</td>
</tr>
<tr>
<td>Spleen</td>
<td>(×10&lt;sup&gt;7&lt;/sup&gt;)</td>
<td>14.76±1.46</td>
<td>6.33±0.56</td>
<td>6.89±0.36</td>
<td>1.09</td>
</tr>
<tr>
<td>Spleen wt.</td>
<td>(mg)</td>
<td>73.0±7.3</td>
<td>44.6±3.6</td>
<td>46.6±4.3</td>
<td>1.05</td>
</tr>
</tbody>
</table>

* Number of bone marrow cells from femur.
** Number of cells per spleen.
*** Difference between values analyzed statistically by Student's two-tailed t test.
**** not significant.
bone marrow cells (Table 3). The reduction of CFU-s was significantly less in the treated group with diuretics and excess water compared to that in the control group with HTO alone in Exp. a and Exp. c.

4) *The responses of splenocytes*

To determine the proliferative ability of splenocyte subpopulations, the assays for splenic T cell colony forming and Con-A blastogenesis were performed (Table 4). Both the cloning efficiency of splenic T cells and the $^3$H-TdR uptake in Con-A blastogenesis were clearly decreased by the injection of HTO. But in the treated groups with diuretics and excess water, the decreases in the cloning efficiency and in the $^3$H-TdR uptake were significantly less than those in the corresponding control groups with HTO alone.

**Table 3.** Effect of the treatment with diuretics and excess water on the spleen colony-forming units in the mouse bone marrow after HTO injection.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>CFU-s per $10^5$ bone marrow cells</th>
<th>Ratio</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (without HTO)</td>
<td>Control (C)</td>
<td>Treated (T)</td>
</tr>
<tr>
<td>Exp. a</td>
<td>17.4±3.4</td>
<td>3.3±1.6</td>
<td>6.0±0.9</td>
</tr>
<tr>
<td>Exp. b</td>
<td>-*</td>
<td>4.3±1.5</td>
<td>5.2±1.2</td>
</tr>
<tr>
<td>Exp. c</td>
<td>9.4±3.3</td>
<td>1.3±0.6</td>
<td>2.3±1.0</td>
</tr>
</tbody>
</table>

* The datum was discarded because of technical error.
** Difference between values analyzed statistically by Student’s two-tailed t test.
*** not significant.

**Table 4.** Effect of the treatment with diuretic and excess water on the clonogenicity of T cells and Con-A blastogenic response in splenocytes after HTO injection.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Normal (without HTO)</th>
<th>Control (C)</th>
<th>Treated (T)</th>
<th>Ratio</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloning efficiency of T cells (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp. a</td>
<td>14.2</td>
<td>5.5</td>
<td>8.6</td>
<td>1.56</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Exp. b</td>
<td>14.8</td>
<td>7.5</td>
<td>9.3</td>
<td>1.24</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Exp. c</td>
<td>11.8</td>
<td>5.4</td>
<td>7.6</td>
<td>1.41</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>$^3$H-TdR uptake in Con-A response (cpm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp. a</td>
<td>17860</td>
<td>2794</td>
<td>7718</td>
<td>2.76</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Exp. b</td>
<td>26974</td>
<td>4527</td>
<td>11443</td>
<td>2.53</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Exp. c</td>
<td>38254</td>
<td>14995</td>
<td>23760</td>
<td>1.59</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

* Difference between values analyzed statistically by Student’s two-tailed t test.
5) Survival study, urine monitoring and dose calculation

As shown above, it appears that the administration of diuretic and excess water is useful for accelerating the excretion of incorporated tritium. The effect of the treatment with diuretic on the surviving fraction of mice was then examined according to the procedure described in Material and Methods. In the control group without treatment after HTO injection, 8 mice out of 10 mice died during days 14–20, as shown in Fig. 2. On the contrary, no deaths were observed in the group treated with diuretic and excess glucose solution even 80 days after HTO exposure. The concentrations of tritium measured in urine samples are shown in Fig. 3. There is a considerable deviation in the figures for the control group 2 weeks after HTO injection, because of decrease in sample number due to death of mice. The exponential decline of tritium activity in urine as a function of time after HTO injection indicates the presence of at least two rate constants in the control group. The shorter half-life was about 2.76 days and the longer half-life was about 14.1 days. On the other hand, in the treated group the presence of at least three rate constants is suggested. The shortest half-life was about 1.36 days, the intermediate half-life about 2.86 days and the longest half-life about 14.4 days. In Figure 4 the cumulative doses calculated from urine measurements are plotted.

![Graph](image)

Fig. 2. Comparison of percent survival between the controls (●—●) and the treated mice (▲—▲) with diuretics and excess water after single intraperitoneal injection with HTO at a dose of 811 MBq/mouse. The body weight of mice was 22±0.5 g. The treatment scheduled was the same as that in Exp. c of Fig. 1 except for the HTO dose. Ten mice were used for each group.
The equations obtained by the least squares method in the controls (•—•) and the treated mice (▲—▲) are

$$Q(t)=63.80 \exp\left(-\frac{0.693}{2.76} t\right) + 0.086 \exp\left(-\frac{0.693}{14.1} t\right) \text{ (MBq/ml)}$$

and

$$Q(t)=46.35 \exp\left(-\frac{0.693}{1.36} t\right) + 14.82 \exp\left(-\frac{0.693}{2.86} t\right) + 0.042 \exp\left(-\frac{0.693}{14.4} t\right) \text{ (MBq/ml)}$$

respectively.

**DISCUSSION**

The studies and data on the treatment of accidental incorporation of tritium are few and insufficient, and there still remains a wide unexplored domain. The present study was made in an attempt to obtain more fundamental knowledge.

As a model for the treatment of acute incorporation of tritium, the effects of the administration of diuretics and excess water on mice injected with HTO was studied using...
the following biological endpoints: tritium activity in body fluids and tissues, the number of nucleated cells, CFU-s in bone marrow cells, colony forming ability of splenic T cells, Con-A blastogenesis of splenocytes, the survival rate of whole animals and tritium concentration in the urine.

Fig. 4. Cumulative dose calculated from the urinary data of Fig. 3 in the control group (---) and the treated group (——).

The excretion rate of tritium from the body is an important factor for the radiation dose following exposure to tritium. The biological half-life, however, is known to vary with animal species and other conditions. Many valuable reports on the metabolism of tritium in human and animals are published\(^6,15-19\). Most of them, however, described the data under ordinary conditions without treatment. The animal studies by Thompson\(^{15,16}\) and Pinson and Langham\(^6\) gave early information about tritium metabolism. Thompson\(^{15,16}\) found the biological half-life was 1.1 days in mice and 3.3 days in rats. Pinson and Langham\(^6\) reported a value of 1.9 days in mice. A typical value for the clearance half-life of tritiated water in humans is approximately 10 days according to ICRP 30\(^{20}\). This value represents clearance without any treatment such as increased drinking or medical intervention.

In cases accidentally exposed to tritium, excretion of tritium from the body should be enhanced. NCRP No. 65 stated that it was possible to increase excretion of incorporated tritium by forcing fluids orally\(^5\). This report recommended that 3000–4000 ml per day of water (including fruit juices, tea, coffee and beer) should be administered orally or 5% glucose in water or saline should be administered intravenously up to 3000 ml per day if fluids could not be given orally. However, there was no actual data in this report regarding to the use of fluid forcing. The enhancement of excretion of incorporated tritium by excess
water intake has already been reported by some investigators\(^6,9,17\). Pinson and Langham\(^6\) noted that excretion of incorporated tritium was enhanced by excess water intake in mature volunteer males. They also observed that the half-life of body water was reduced from 10 days to 2.4 days by increased water intakes (12.8 l/day). Ichimasa et al.\(^9\) showed that both free water tritium and tissue bound tritium were successfully decreased by long-term instillation of various fluids, which included some diuretics, after a single injection of tritiated water into rats. Butler and Leroy\(^17\) measured 309,000 urine samples from more than 310 cases under the routine operation of the Savannah River Plant's health physics program. They showed that half-lives were shorter when assimilations occurred in the warmer months, and this was attributed to increased water intake. However, there are relatively few reports describing the accidental incorporation of tritium treated with this method\(^7,8,21–23\). Moghissi et al.\(^21,22\) reported a highly seasonal dependence of tritium body water concentration in tritium luminous dial painters. They assumed that increased water intake during summer had diluted the tritium which was located in the tissues and constantly released into the body water. Takada et al.\(^7\) tried to enhance the excretion of tritium by excess water intake in a male worker, who inhaled tritiated water vapor while working at a heavy-water-moderated reactor, JRR-2. However, they failed in this procedure, because the normal daily water intake decreased under no special control. Lloyd et al.\(^8\) described two cases of accidental inhalation of tritiated water treated by forced diuresis or extra drinking, both of which were effective in accelerating the rate of tritium excretion.

Most of these reports, however, described only the changes of the tritium body water concentrations. There are many ambiguous statements about the biological changes in these reports. It is interesting to compare the change in excretion rate of tritium with the change in many biological endpoints.

In the present study, we observed first the reduction of the concentration of tritium in the group of mice administered diuretics and excess water (Table 1). In the first experiment (Exp. a), however, the procedure was so powerful that the mice achieved a state of dehydratation and hemoconcentration, and signs of cytotoxicity for hematopoietic cells in a cold experiment (data not shown). On the other hand, if the mice were given a glucose solution with or without the diuretic furosemide at a concentration of less than 1 mg/kg, the mice achieved a state of overhydration demonstrated by body weight change and concentration of blood cells (data not shown). Thus, we changed the procedure to use only furosemide as the diuretic at a concentration of 8 mg/kg, which was several times higher than the normal clinical dose, in Exp. b and Exp. c. In both the experiments, we were able to reduce the concentration of tritium in mice by this treatment.

No difference was observed in cell number between the control group and treated group except for bone marrow cells (Table 2). Assay of CFU-s has proven to be a useful and sensitive measure of damage and recovery of bone marrow cells after irradiation. Harriss and Fliedner\(^24\) showed both CFU-s and CFU-c were reduced, although the mature peripheral blood cells were not reduced by chronic exposure to tritium using tritiated drinking water at a concentration of 222 kBq/ml (6 µCi/ml). In the present study the number of CFU-s was apparently restored proportional to the reduction in activity of
tritium by treatment with diuretics and excess water (Table 3). Clonability of splenic T cells and \(^3\)H-thymidine uptake in Con-A blastogenesis were also restored significantly by this treatment (Table 4).

We conclude that administration of furosemide as a diuretic along with excess water is useful to reduce the concentration of tritium in the body and contributes to reduction of radiation damage and the accelerates recovery of the hematopoietic cells, especially stem or immature cells.

In our study of acute lethality, the biological half-life of 1.36 days in the treated group is shorter than 2.76 days in the control group. In the treated group, however, the half-life increased to the control level immediately after interruption of treatment with diuretic and excess water (Fig. 3). Cumulative dose in the control group achieved over 70% of the final committed dose during the first 5 days. As a result, the final committed dose in the mice treated with early diuresis was reduced almost to 60% of the dose in the control group (Fig. 4). While the mortality 30 days after injection with HTO (37 MBq/g body weight) was 80% in the control group, the mortality in the treated group was 0%. This reduction in mortality may be ascribed to the reduction of cumulative dose, as shown in Fig. 2. There are few reports describing acute lethality in mice after a single injection of HTO. Furchner\(^{25}\) studied the RBE of tritium beta radiation using 30-day mortality of mice after a single injection with HTO. He observed 30-day mortality in mice was 7 and 81% when the mice were injected with HTO at doses of 25.9 and 36.3 MBq/g body weight, respectively. Our results agree well with his data.

In conclusion, we have shown that treatment with diuretics and excess water efficiently accelerates the excretion of incorporated tritium, reduces the damage to the hematopoietic system, and protects from acute radiation death in mice. These results suggest that the administration of diuretics and excess water is useful for practical purposes when a human is accidentally exposed to tritium.

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