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解　説

トリチウム水の放射線化学および放射線生物学

山本　　修

広島大学理学部生物学科
〒724　東広島市鏡山１－３

Newly Constructed Tritium Laboratory and Chemical and Biological Studies with Tritiated Water

Osamu　Yamamoto

Department of Biology, Faculty of Sciences,
Hiroshima University
1－3, Kagamiyama, Higashi-Hiroshima 724, Japan
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Abstract

A building was recently constructed which has facilities specially designed for exclusive use of HTO studies.

As chemical evidences of HTO, a specific UV spectrum, the luminescence exhibition only with luminol but without peroxydase and the epoxide formation from mesityl oxide were found, from which it was concluded that the radiolytic reaction of water with HTO is [$H_2O \rightarrow H_2O^* \rightarrow H_2 + O$] followed by [$O + M \rightarrow (MO)$, $(MO) + H_2O \rightarrow M(OH)_2$ or $\cdot MOH + \cdot OH$], [$O + O \rightarrow O_2$] and [$O + H_2O \rightarrow H_2O_2$]. This was supported by the low oxygen effects observed in thymine and adenine radiolysis with HTO.

Low-dose-rate effect (Kada effect) was reconfirmed using the transfection system of M13 phage DNA irradiated, and then it could be explained by that the ratios of [$O + M \rightarrow (MO)$, $(MO) + H_2O \rightarrow M(OH)_2$ or $\cdot MOH + \cdot OH$] to [$O + O \rightarrow O_2$] increases with the decrease of dose rate.

RBE higher than one was obtained also in the case of the killing of radioresistant *E.coli* TG 1. It was concluded that this is due to the high reactivity of the nascent O which can act recembling two OH.

$LD_{50/30}$ was estimated to be 5.6×10^8 Bq (8 Gy) for C57BL／6 N female young

mice and 9.3×10^8 Bq (13 Gy) for (C57BL／6 N×C 3 H／He) F_1 young female mice by single-injection of HTO. At doses lower than 5.6×10^8 Bq (8 Gy), mice died inducing leukemias with higher doses and other solid tumors with lower doses.

In continuous administration of HTO, (C57BL／6 N×C 3 H／He) F_1 female adult mice died of haematopoietic failure with in 50 days at dose rates more than 0.48 Gy／day, while the appearance of lymphomas changed to that of various non-lymphoma tumors with the decrease of dose rate to less than 0.24 Gy／day and surviving over 150 days.

1. Intorduction

Studies are being made on nuclear fusion throughout the world in order to obtain a new energy source. A special research project on nuclear fusion under the auspices of the Ministry of Education, Science and Culture, Japan has been initiated in April 1980. One group of this research organization is engaged in studies of the biological effects of tritium, a raw material for nuclear fusion. The author as a member of this group have constructed a building for tritium experiments, especially for tritiated water (HTO) experiments at the end of the fiscal year of 1982. There were set up two chambers (one is one cabinet type and another is four cabinets type) for animal breeding, one chamber for cell treatment, and one chamber for chemical experiment. Using these chambers, radiation chemistry and radiation biology for beta-particles from HTO were studied. Some chemical characteristics specific for HTO were found differing from those for ^{60}Co gamma-rays. Also, single or continuous administration of HTO in mice resulted in some biological interesting effects.

2. Newly Constructed Tritium Laboratory

A diagram of the building constructed for tritiated water experiment is shown in Fig. 1. A schematic diagram of the facilities in this building is also shown in Fig. 2.

Dilution of the concentrated tritiated water is done in a glove box (Sangyo Kagaku Co., Model SK - 470). For the treatment of cells, a biohazard chamber (Air Tech Co., Model BHL - 1300IIB) was set up. Small and large chambers (Chiyoda Hoan Yohin Co., Model THY - 2350 and Model THY - 2350L) for animal breeding and a draft chamber for chemical experiment (Chiyoda Hoan Yohin Co., Model THY - 1800) which were developed by the author and some members of Technology Department of Chiyoda Hoan Yohin Co. were also set up. The animal breeding chambers have an water flusher and a blender to remove the feces and urine as previously reported[1]. The chemical

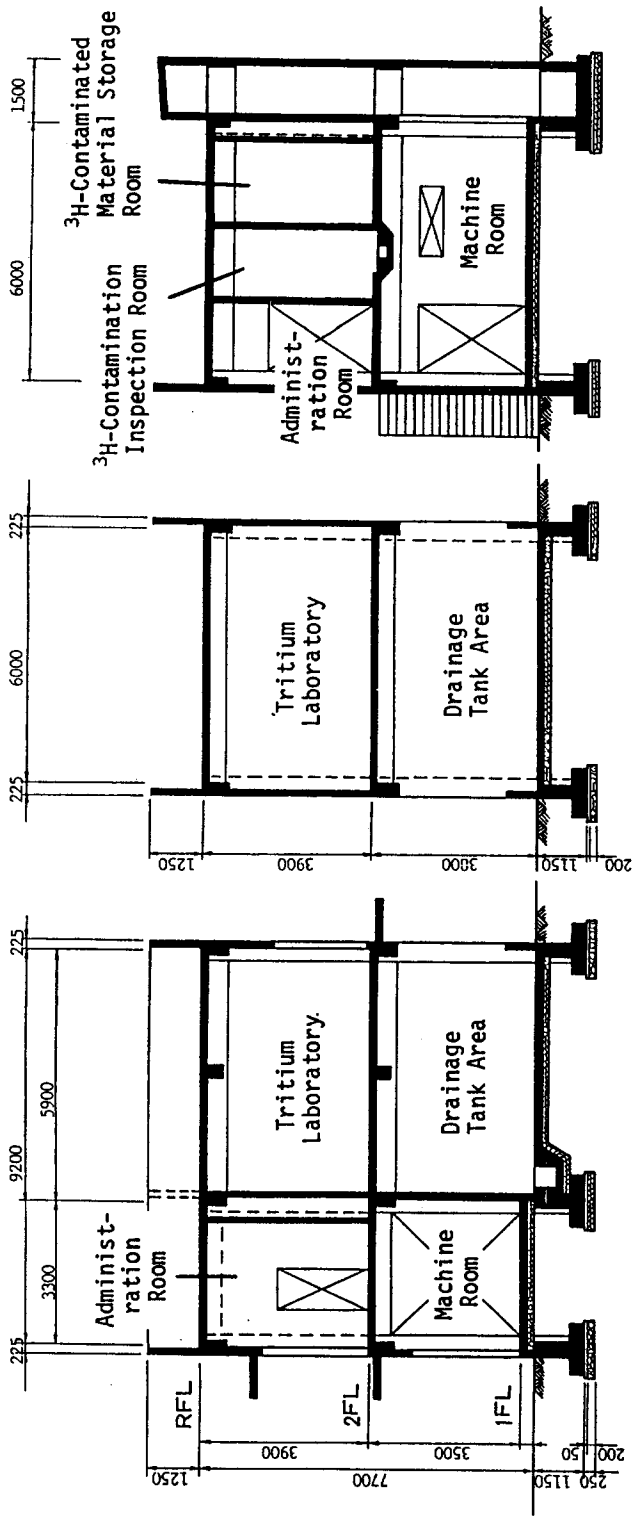


Fig. 1. Structure of the building constructed for HTO experimental studies.

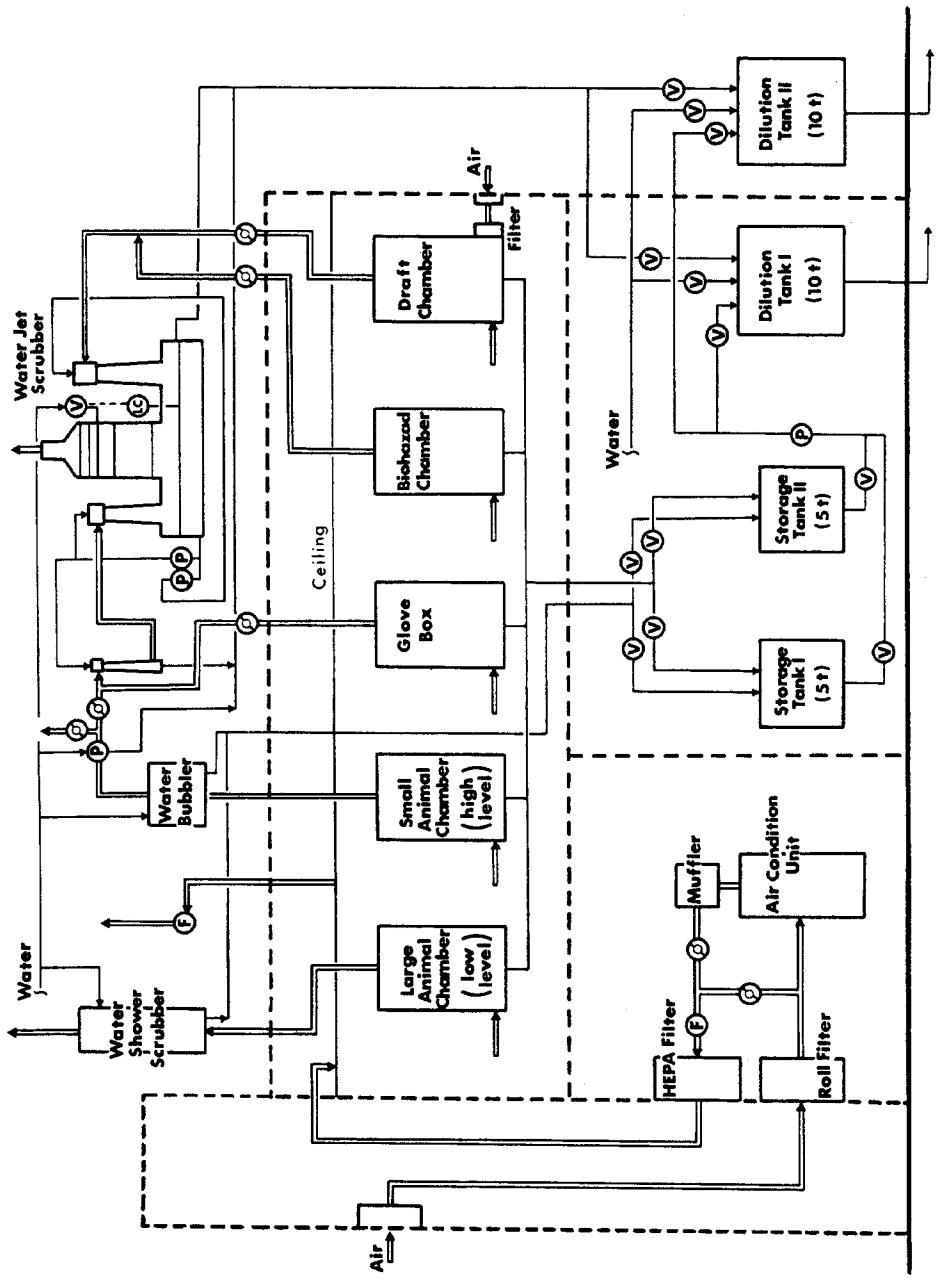


Fig. 2. Schematic diagram of the facilities for HTO experimental studies.

draft chamber has pipes to use N_2, O_2 and N_2O and a suction pump, including city-gas, electricity and water sources. These chambers were installed on the second floor.

For the collection of HTO vapor, some workers have used a molecular sieve system[2] or a cold-trapping system[3]. However, the author was faced with the problem of how to treat the bed materials contaminated with the feces and urine for breeding animals. In order to resolve this problem, a complete water dilution system was developed for removing the HTO vapor released from all chambers. To absorb the HTO vapor, three types of absorbers were set up, whichi are a water bubbler, a water shower scrubber and a water jet scrubber. These absorbers were installed on the roof.

The HTO vapor-absorbed water flows into a storage tank installed on the first floor. On the other hand, the feces and urine flow with the flushing water into a blender, where after being ground and suspended in water they flow also into a storage tank. When one of the two storage tanks ($5\,m^3$) becomes full, the flow is automatically changed to another storage tank. After measuring the ^3H activity of the slops, the slops are diluted with water to a concentration lower than the level of prescribed by law in the dilution tank ($10\,m^3$) and then released into the public sewage system.

3. Chemical Studies with Tritiated Water

Alexander and Rosen[4] have reported that alpha-rays gave rise to which were qualitatively different from those brought about by X-rays in a study of protein radiolysis in aqueous solution. They confirmed the spectral changes produced by treating tryptophan with ozone closely resemble the changes produced by alpha-irradiation, and regarded at triplet state of H_2O_2 as its active species. It was expected also in ^3H beta-irradiation that there is a similar high LET reaction as the ozone- or concentrated H_2O_2 - like oxidation reaction with some specially active species different from OH. Indeed, some evidences specific for HTO were found through the some experiments as mentioned below.

3. 1. UV Absorption[5]

Fig. 3 shows the UV spectrum of HTO, which was different from that of H_2O_2. The maximum absorbance at 195 nm did not change even after one year. The spectrum of HTO is characterized by a short trail. The maximum absorbance is also different from other active oxygen species such as OH, HO_2^-, and O_2^-.

3. 2. Luminescence[5]

Fig. 4 shows luminescence at different concentrations of HTO. Usually, when

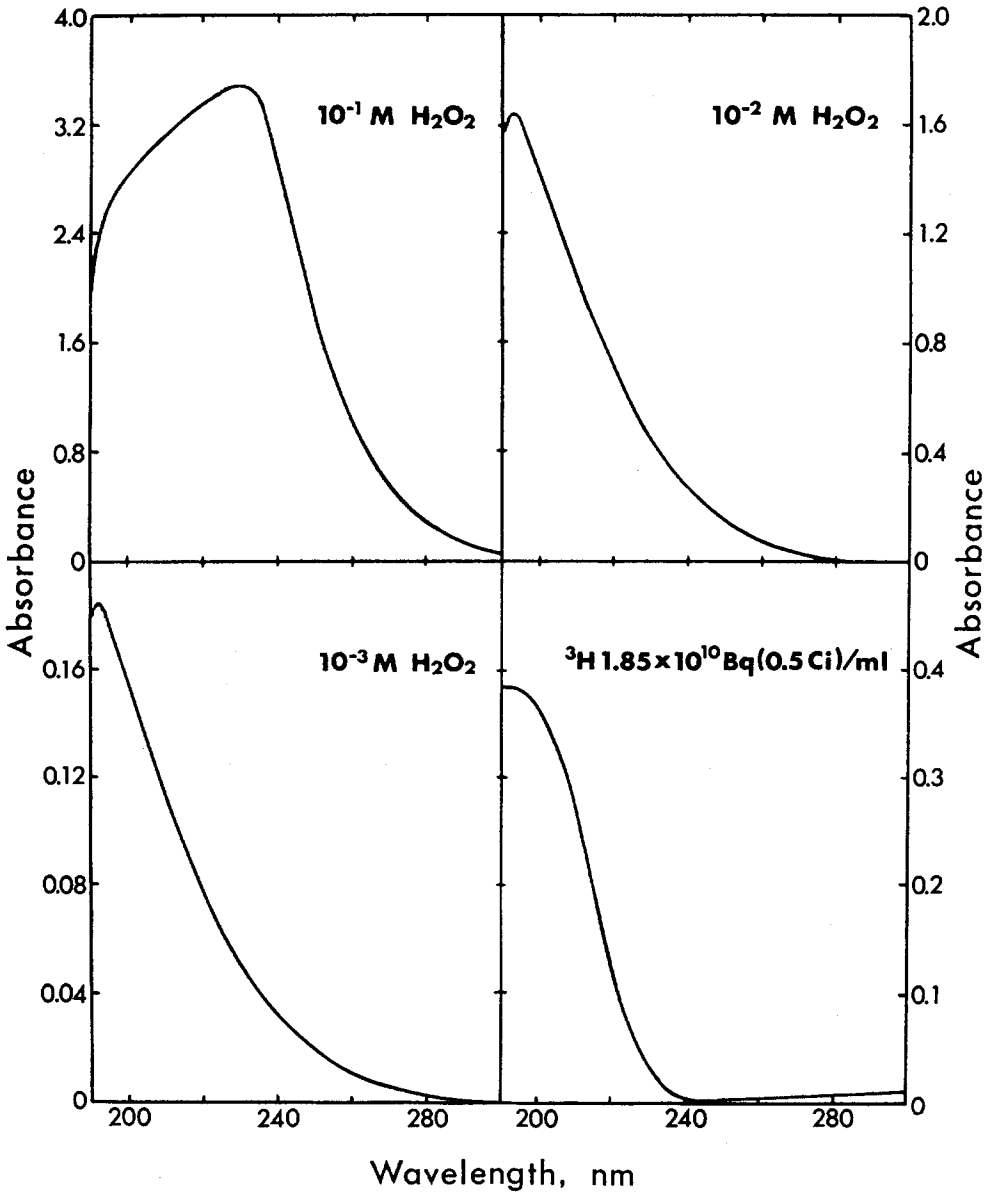


Fig. 3. UV absorption spectrum of HTO at a concentration of 1.85×10^{10} Bq/ml compared with spectra of H_2O_2 at concentration of 10^{-3}, 10^{-2}, and 10^{-1} M.

luminol and peroxidase are added to H_2O_2 solution, luminescence appears. In the case of HTO, the luminescence was observed constantly only with luminol but without peroxidase under N_2, O_2, or N_2O. The luminescence intensity decreased when *t*-BuOH, which is an OH scavenger, was added. If this luminescence is caused by OH, the intensity should increase under N_2O ($N_2O + e^-_{aq} \rightarrow N_2 + \cdot OH + OH^-$). But no difference was

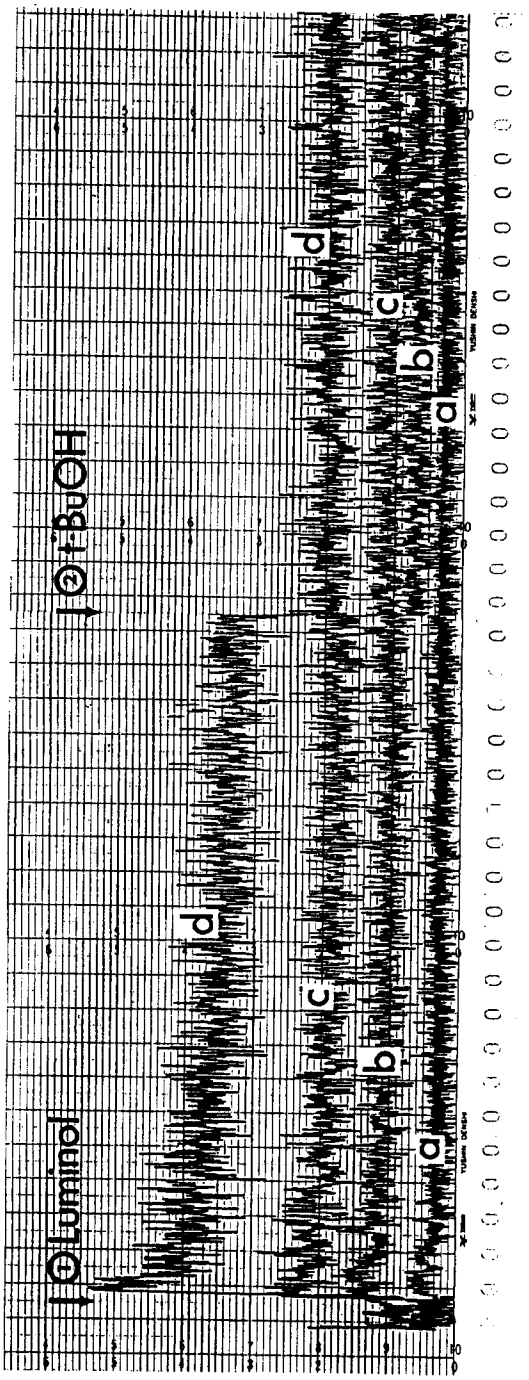
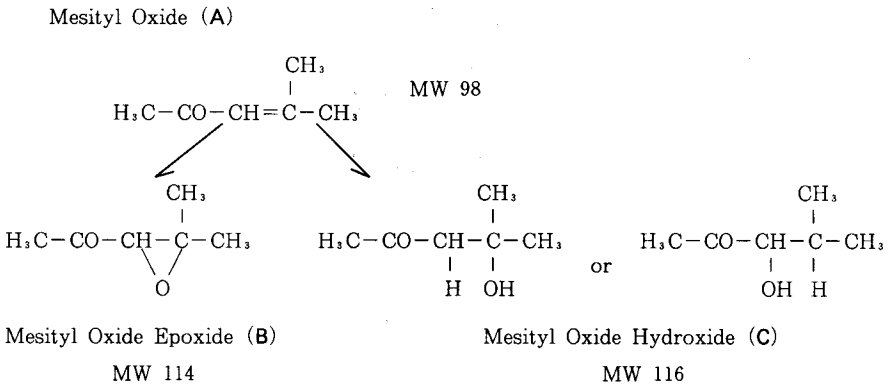


Fig. 4. Luminescence appearing in HTO mixed only with luminol but without peroxidase under O_2 (the same as under, N_2, or N_2O) as registered with a bioluminescence reader. This oxidative species was scavenged by *t*-BuOH.
a : 0 Bq/ml ; **b** : 1.48×10^9 Bq/ml ; C : 2.96×10^9 Bq/ml ; d : 5.92×10^9 Bq/ml as the concentrations of ^3H in water. Ordinate ; intenisity of luminescence ; absissa : time. ① : Addition of luminol ; ② : addition of *t*-BuOH.

found among the three atmospheric conditions (N_2, O_2, and N_2O). Therefore, there should be fouded some active oxygen species other than OH , HO_2^-, and O_2^-.

3. 3. Mesityl Oxide Radiolysis[5]

When mesityl oxide (A) suspended in water was irradiated with ^3H beta-rays or ^{60}Co gamma-rays, an epoxide (B) and a monohydroxide (C) formed as products.



Products were analyzed by a gas-chromatograph and a mass spectrometer. The yield of the epoxide was much higher with ^3H beta-rays than with ^{60}Co gamma-rays, while the yields of the hydroxide were not so different from each other as for those of the epoxide (Table 1)[6].

Brown and Hart[7] also studied the radiolytic formation of nascent O using the cyclopentene oxidation method, but the yield of such species was very low by ^{60}Co gamma-irradiation. This supports our above data.

According to this result, there should be the formation of nascent O in water with ^3H beta-rays which are one of high LET radiations. Stief[8] and Cottin *et al.*[9] presented the reaction $H_2O + h\nu \rightarrow H_2 + O$ as one of the primary processes occurring in

Table 1. Radiolytic yields of mesityl oxide degradation, epoxide formation and monohydroxide formation, and relative effectiveness of ^3H beta-rays to ^{60}Co gamma-rays. Dose : 10^4 Gy ; dose rate : 0.4 Gy/min.

Yield (%)	−Mesityl oxide	Epoxide	Monohydroxide
^{60}Co gamma-rays	21.0	4.4	11.7
^3HHO beta-rays	52.0	23.2	20.6
$^3HHO/^{60}Co$	2.5	5.2	1.8

water vapor. Burns *et al.*[10] and Buxton[11] also implicated the same reaction with high LET radiation. The author added an arrow in a figure given by Buxton (Fig. 5), which means that the yield of nascent O would be the higher when the higher the LET of radiation.

In HTO, the formation of nascent O may take place in a very limited local area because of the short range of ^{3}H beta-rays, which formation will be followed by the reaction of the solute with nascent O and also by the prouduction of O_2. The nascent O may also react with a water molecule to produce H_2O_2.

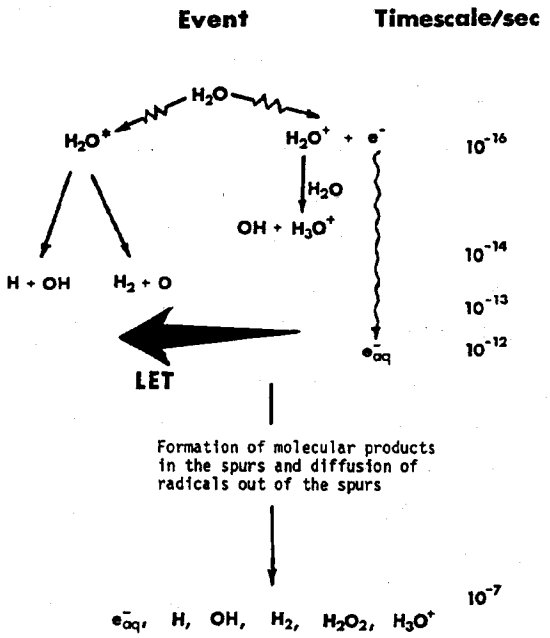


Fig. 5. Scheme for the radiolysis of water.

$H_2O \rightarrow H_2O^*$ (excited form) $\rightarrow H_2 + O$ (1)

followed by

$O + M \rightarrow (MO)$, $(MO) + H_2O \rightarrow M(OH)_2$ or $\cdot MOH + \cdot OH$ (2)

$O + O \rightarrow O_2$ (3)

$O + H_2O \rightarrow H_2O_2$ (4)

If the yield of nascent O could be the higher when the higher the LET of radiation, the yield of O_2 would be also the higher. Indeed, a high G value of 0.4 for the formation of O_2 has been esablished in neutral deaerated water after exposure to a high LET radiation, ^{222}Rn alpha-rays (134 keV/μm LET)[12, 13].

3. 4. Low Oxygen Enhancement Ratio in Deaerated Solution

a. Radiolysis of Thymine and Adenine[14, 15]

Aqueous solutions of thymine or adenine (5×10^{-4} M containing ^{14}C-compound and buffered at pH 7.0) were irradiated with ^{60}Co gamma-rays and ^{3}H beta-rays from tritiated water in the presence of N_2, O_2, N_2O or *t*-BuOH-N_2.

Thin-layer chromatography (TLC) was carried out bidimensionally for separation of the radiolytically produced products and autoradiography was performed. Considerable differences were observed in the dose-yield curves for the decomposition of the original compound and for the product formation between gamma- and beta-radioly-

ses. The low oxygen enhancement ratio (OER) was found in both experiments (Table 2).

Table 2. Oxygen enhancement ratios for nucleobase decomposition and DNA strand breaks in ^{60}Co gamma-irradiation and ^{3}H beta-irradiation.

	^{60}Co Gamma-Irradiation	^{3}H Beta-Irradiation
Thymin Decomposition	1.35	1.08
Adenine Decomposition	1.35	1.06
DNA Single Strand Breaks	1.66	1.06
DNA Double Strand Breaks	1.87	1.08

b. Strand Breaks of DNA[16]

Lambda DNA (125 μg / ml in Tris buffer, pH 7.4) was irradiated with ^{60}Co gamma-rays and ^{3}H beta-rays, respectively, and the number of strand breaks was determined by electrophoresis. Number of single-strand breaks increased linearly with radiation dose in both gamma- and beta-radiations. Number of double strand breaks increased with the square of the radiation dose in gamma-irradiation, but it increased linearly with radiation dose in beta-irradiation (Fig. 6). Oxygen effect was observed by gamma-irradiation (OER=1.66 for single strand breaks and 1.87 for double strand breaks) but was minimal after beta-irradiation (OER=1.06 for single strand breaks and 1.08 for double strand breaks).

Large OER values in gamma-irradiation and small OER values in beta-irradiation were observed not only for decomposition of nucleobases but also for DNA strand breaks. These are not due to no oxygen effect by ^{3}H beta-irradiation, but due to O_2 production in deaerated solution which is the same to oxygenated solution. In deaerated neutral water, O_2 production has been demonstrated during alpha-irradiation[4]. Burns *et al.*[10] have also measured O_2 yield in $FeSO_4$ solution irradiated with heavily charged particles. But there was no clear evidence to explain the mechanism of O_2 formation with high LET (linear energy transfer) radiation. ^{3}H beta-rays are known to be a moderately high LET type of radiation. By the above results, it is supported that there is the O_2 production also in ^{3}H beta-irradiation.

The yield of double strand breaks of DNA was much higher in ^{3}H beta-irradiation than in ^{60}Co gamma-irradiation as seen in Fig. 6. The reaction of the active oxygen species, produced locally and densely in water, with the solute molecules in the spur can only be accepted if the time scale of this reaction shorter (10^{-12} sec) than that of the diffusion of the active species from nonhomogenous spur to homogenity (10^{-7} sec). Such highly reactive species must be the nascent O which is much more reactive

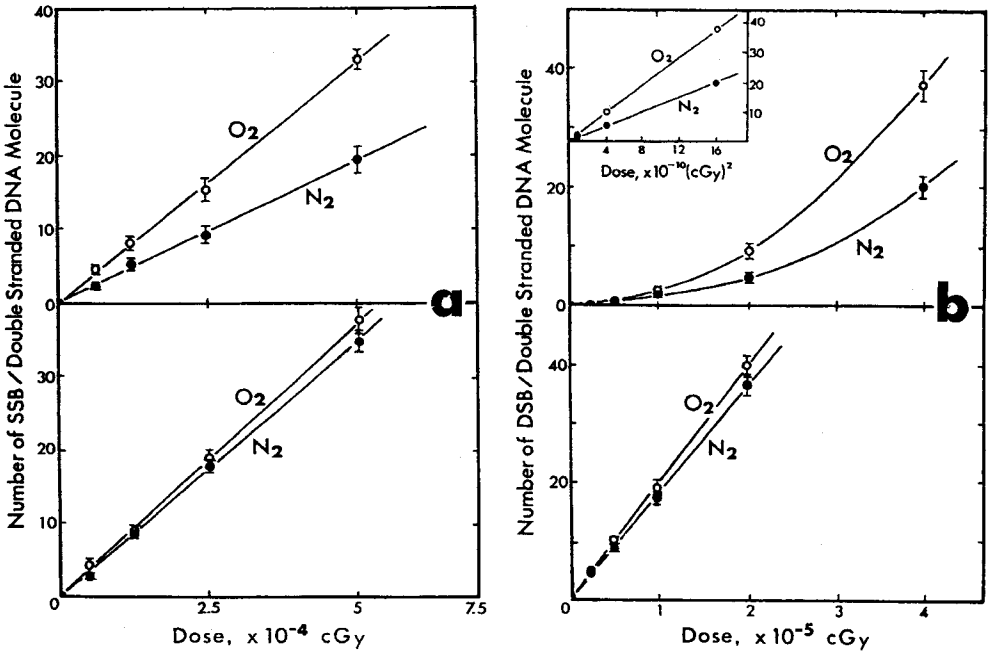


Fig. 6. Strand breaks of lambda DNA (125 μg/ml of 10 mM Tris-HCl − 5 mM NaCl − 1 mM Na_2EDTA, pH 7.4). a : ^{60}Co gamma-irradiation; b : ^{3}H beta-irradiation.

than OH, $HO_2^{\cdot}$, and $O_2^{\cdot-}$. There coluld be and similarity, at least in a qualitative sense, among ^{3}H beta-rays, soft X-rays and heavy particles. Indeed, a high relative effectiveness value (1.93) for soft X-rays to ^{137}Cs gamma-rays has been reported by Bonura *et al.*[17]

4. Biological Studies with Tritiated Water

4. 1. Low-dose-rate effect

Using HTO, Kada *et al.*[18] found a low-dose-rate effect (when the lower the dose rate, the larger the effect) of ^{3}H beta-rays on transforming DNA using *B. subtillis*, a phenomenon termed the " Kada effect". But in that study the HTO was used without eliminating the H_2O_2 contained in it. Since a similar low-dose-rate effect has been observed in H_2O_2 solution[19], it was unclear whether the ^{3}H beta-rays or the H_2O_2 contained in HTO caused the effect. In order to confirm which, we studied the transfection activity of M13 mp10 phage DNA that had been exposed to HTO from which the H_2O_2 had been eliminated. Figure 7 shows the relationship between radiation dose and transfection activity at three different dose rates. It is clear that the lower

the dose rate, the larger the effect at the same irradiation dose; that is, the transfection activity decreased more markedly in parallel with decreasing dose rate. Thus the low-dose-rate effect was reconfirmed.

The author presents a model of the low-dose-rate effect mechanism[5] as shown in Fig. 8 which explains the difference between reactions of nascent O with DNA and with another nascent O atom at high and low concentrations of nascent O.

When the concentration of the nascent O is high, the reaction yield of [$O+O \rightarrow O_2$] will become relatively high and that of [$O+M \rightarrow (MO)$, $(MO)+H_2O \rightarrow M(OH)_2$ or $\cdot MOH + \cdot OH$] will become relatively low. On the contrary, when the concentration of the nascent O is low, the former yield will become relatively low and the

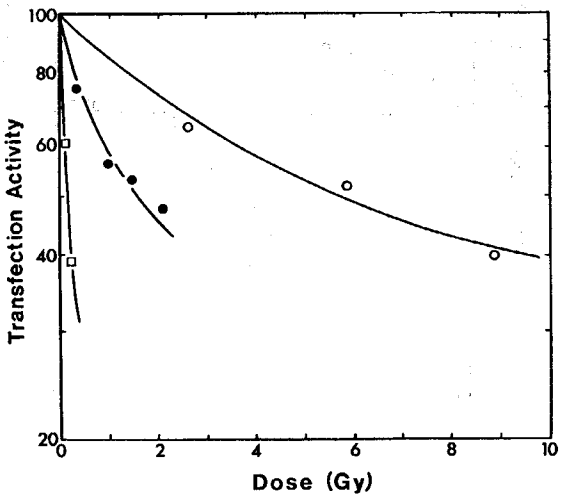


Fig. 7. Inactivation of transfection activity of phage DNA irradiated at different dose rates. ○ : 2.85 Gy/day; ● : 0.285 Gy/day; □ : 0.0285 Gy/day as the dose rates of ^3H beta-rays.

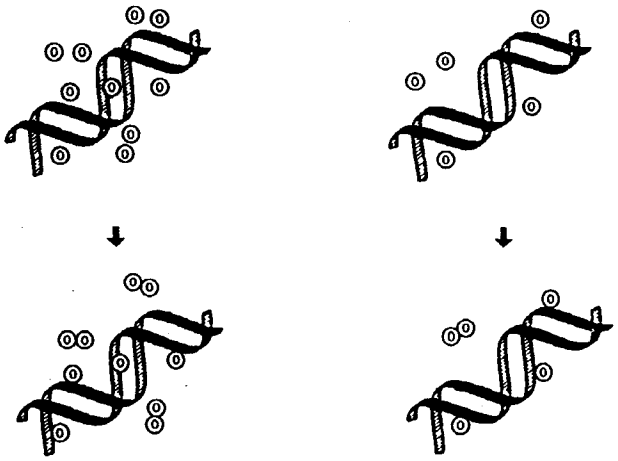


Fig. 8. Models of reactions of nascent O with DNA and with another nascent O at different concentrations. Relative yield of DNA damage higher at lower concentrations of the nascent O.

the latter will become relatively high; that is, the lower the concentration of the nascent O, the more the solute damage takes place. This low-dose-rate effect can take place not only in HTO but also in H_2O_2 solution at a constant concentration of a solute such as DNA. Because H_2O_2 may degrade in part with time as [$H_2O_2 \rightarrow H_2O+O$] with its thermodynamical instability[20]. Very recently, Takakura[21] reported the low-dose-rate effect on strand breaks in aerated solution but not in deaerate solution with ^{60}Co gamma-rays and then suggested the participation of OH. She observed, however, with ^{3}H beta-rays, the effect not only in aerated solution but also in deaerated solution. It becomes clear to produce O_2 in deaerated solution with ^{3}H beta-rays as well with other high LET radiation. Therefore, there might be included a reaction [$O_2 \rightarrow O+O$], because this reaction yield is extremly high (G value 8) as observed in oxygen-nitrogen mixture gas system[22]. Thus, three types of the formation of nascent O, [$H_2O \rightarrow H_2+O$, $H_2O_2 \rightarrow H_2O+O$, and $O_2 \rightarrow O+O$], may result in the low-dose-rate effect, and the degree of the effect may principally depend on the yields of nascent O in total. Besides, the oxygen enhancement effect which has been used as a general term in radiation biology might be due to the reaction [$O_2 \rightarrow O+O$].

4. 2. Relative Biological Effectiveness (RBE) of HTO

The RBE of ^{3}H beta-rays in comparison to ^{60}Co gamma-rays was reported in 1957 to be higher than one for $LD_{50/30}$[23], and for splenic and thymic atrophies[24], and Fe uptake[24] in mice. Later it was reported to be higher than one in a number of systems such as the killing of mammalian cells[25-31], the chromosomal aberation[32-34] in and transformation[35-37] of mammalian cells, and the mutation of mammalian[30, 38, 39], Drosophila[40], yeast[41] and bacterial[42] cells. Lunec and Cramp[43] reported an RBE higher than one for ^{3}H beta-rays to 7 MeV electrons for bacterial cell killing of radiosensitive *E. coli* B_{S-1}. These RBE values are listed in Table 3.

Iwanami and Oda[44] who made microdosimetric estimation have concluded that the RBE of ^{3}H beta-rays depends on the ability of the cell to repair DNA damage. In order to confirm it, we irradiated much radioresistant *E. coli*, TG 1 with ^{3}H beta-rays, and ^{60}Co gamma-rays respectiverly. Survival curves are shown in Fig. 9, from which RBE was caluculated to be 1.23[6]. When this value was compared to that of radiosensitive *E. coli*B_{S-1} studied by Lunec and Cramp, no difference was found between them (seeTable 3). Ito[45] estimated also microdosimetrically the RBE as 1.26, based on double strand breaks of DNA. He proposed that the double strand breaks were

Table 3. RBE of HTO obtained in various research systems

Research System	RBE	Workers	Reference Number
Mouse $LD_{50/30}$	1.7	Furchner (1957)	21
Mouse Splenic and Thymic Atrophies	1.3−1.5	Storer *et al.* (1957)	22
Rat ^{59}Fe Uptake	1.6	Storer *et al.* (1957)	22
Mammalian Cell Killing (*in vivo*)	2.3	Lambert (1969)	23
	1.6−2.9	Dobson and Kwan (1976)	24
	2.3	Dong *et al.* (1985)	25
	1.1−2.7	Satow *et al.* (1989)	26
Mammalican Cell Killing (*in vitro*)	1.7	Bedford *et al.* (1975)	27
	1.5	Ueno *et al.* (1982)	28
	1.3	LeMotte and Little (1983)	29
Mammalian Cell Chromosome Aberration (*in vitro*)	1.2	Dewey *et al.* (1965)	30
	1.7	Ikushima *et al.* (1984)	31
	2.8	Tanaka *et al.* (1989)	32
Mammalian Cell Transformation (*in vitro*)	2.2	Nikaido and Suzuki (1985)	33
	1.6−1.7	Yamaguchi *et al.* (1985)	34
	3.0	Little (1986)	35
Mammalian Cell Mutation (*in vivo*)	1−2	Russel *et al.* (1979)	36
Mammalian Cell Mutation (*in vitro*)	2.5	Ueno *et al.* (1982)	28
	2.9	Liver *et al.* (1985)	37
Drosophila Cell Mutation	2.7	Byrne and Lee (1989)	38
Yeast Cell Mutation	2	Ito and Kobayashi (1978)	39
Bacterial Cell Mutation	1.8	Tanooka and Munakata (1978)	40
Bacterial Cell Killing (sensitive cell)	1.2	Lunec and Cramp (1978)	41
Bacterial Cell Killing (resistant cell)	1.2	Yamamoto *et al.* (1991)	6

produced by two ionization (0.74% as dsb/ssb for ^{60}Co gamma-irradiation and 0.88% for ^{3}H beta-irradiation), one ionization and one OH reaction (0.80% and 1.06%, respectively), and two OH reactions (0.02% for both irradiations). If the double strand breaks do cause cell dea, his hypothesis would be adequate because his given value of 1.26 corresponds to our result of 1.23. But in his hypothesis the direct to the indirect effect are 2.71 : 1 (0.74%+0.40% : 0.40%+0.02%) and 2.56 : 1 (0.88%+0.53% : 0.53%+0.02%) for ^{60}Co gamma-irradiation and ^{3}H beta-irradiation. Such major contribution of the direct effects to cell death is inadequate in terms of the O_2 and radical-scavenger effects.

Fig. 10, based on the tables prepared by Spinks and Woods[46] and Buxton[11],

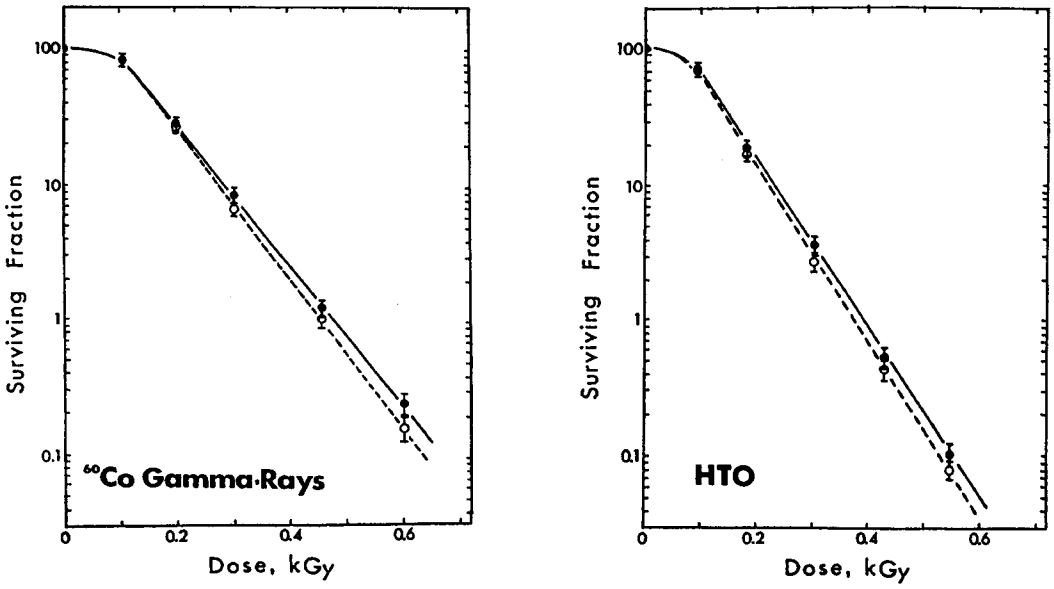


Fig. 9. Survival curves for *E. Coli* TG 1 harboring the *pUC* 18 plasmid after ^{60}Co gamma-and ^{3}H beta-irradiations. —●— : Incubation on agar plates containing LB medium without Ampicillin after irradiation, -- ○ -- : incubation on agar plates containing LB medium with Ampicillin after irradiation.

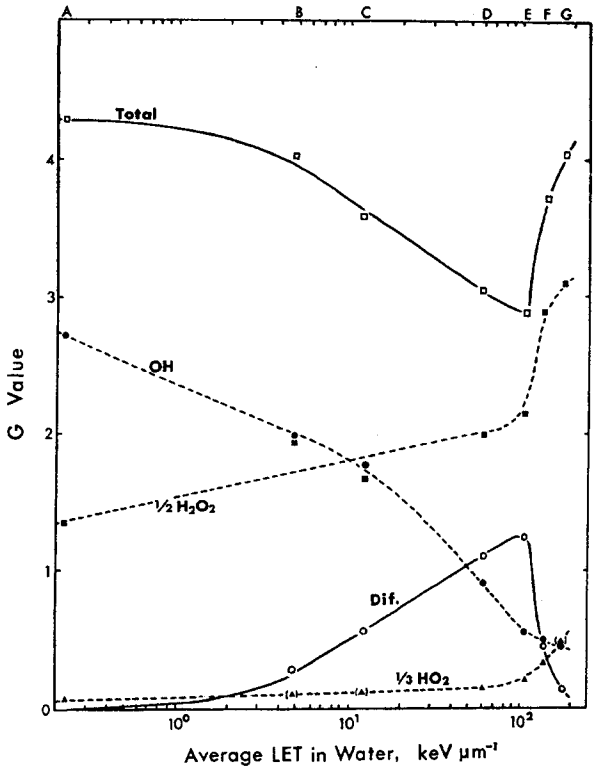


Fig. 10. Relation of the 'G values' of the three oxidative species: OH, H_2O_2, and HO_2^-, with LET. Dif. : Total 'G values' of the oxidative species ($G_{OH} + 2\,G_{H_2O_2} + 3\,G_{HO_2^-}$) for irradiation with high LET radiation was subtracted from that for ^{60}Co gamma-irradiation. ( ) : Extrapolated or interpolated. A: ^{60}Co gamma-rays, 0.23 keV/μm; B: ^{3}H beta-rays, 4.7 keV/μm; C: 18 MeV D^+, 12.3 keV/μm; D:32 MeV He^{2+}, 61 KeV/μm; E: 12 MeV He^{2+}, 108 keV/μm; F: ^{210}Po alpha-rays, 136 keV/μm; and G: ^{10}B (n,a) ^{7}Li recoil nuclei, 180 keV/μm.

shows the relations of the yields of the three oxidative species G_{OH}, $2\,G_{H_2O_2}$ and $3\,G_{HO_2^-}$ (1, 2 and 3 are shown as an OH equivalent ratio) in water with LET. The resulting yield of these three oxidative species ($G_{OH} + 2\,G_{H_2O_2} + 3\,G_{HO_2^-}$) decreases up to 100 keV/μm, and then increases; the difference in total yield for ^{60}Co gamma-irradiation and high LET particle irradiations increases up to 100 keV/μm then decreases. This difference in the resulting yield of the oxidative species for gamma-rays and other particle radiations is ascribable to some factor other than OH, H_2O_2, and HO_2^-. Therefore, the author proposes that it is due to the production of nascnet O mentioned in the preceding section. Nascent O can induce oxidation of the solute, thereby the reactions with nascent O resembling two OH [M+O→(MO), (MO)+H_2O→M $(OH)_2$ or ·MOH+·OH] may result in a higher RBE.

4. 3. Biological Effect of HTO on Mice

We have started to study a series of experiments for the acute and chronic effects of HTO on mice using a newly constructed facility designed for exclusive use as mentioned in section II.

a. Single Intraperitoneal Administration

First, the acute and sub-acute effects of HTO were studied in young female mice (7 − 8 weeks old C57BL/6N and [C57BL/6N×C3H/He] F_1). HTO was injected to mice intraperitoneally. The survival rate and changes in the body weight of irradiated mice are shown in Fig. 11. Chanages in white blood cell count, thymus weights and spleen weight also shown in Fig. 12.

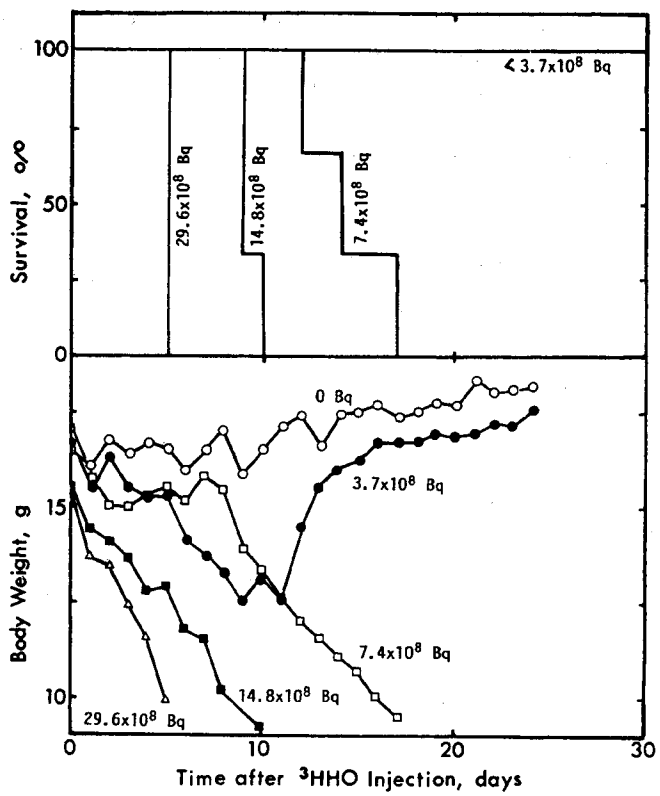


Fig. 11. Survival and body weight data for young female mice C57BL/6N after single intraperitoneal injection of HTO.

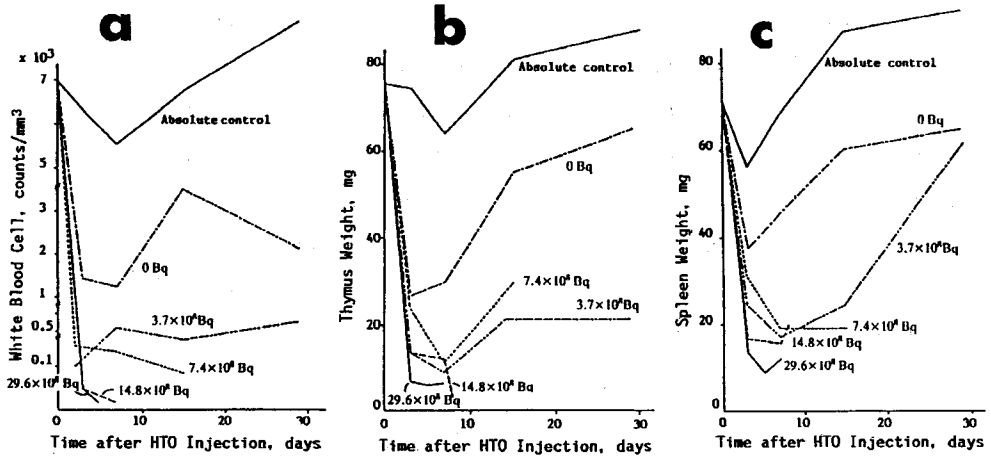


Fig. 12. Changes in white blood cell count (a) , thymus weight (b) and spleen weight (c) after single intraperitoneal injection of HTO, using young famale mice C57BL/6 N.

HTO-induced lethality in mice has been studied by Brues *et al.* in 1952[47] and Furchner in 1957[48]. Brues *et al* reported 3.7×10^{8} Bq-1.11×10^{9} Bq as le th-al HTO dose ($=5-15$Gy as absorbed radiation dose in mice organs) and Furchner reported 8.04 Gy as the $LD_{50/30}$ for CF_1 Female mice. According to our data from single injection experiments[49, 50], we estimated that the $LD_{50/30}$ was 5.6×10^{8}Bq ($=8$ Gy) for C57BL/6 N female mice and 9.3×10^{8} Bq ($=13$ Gy) for (C57BL/6 N$\times$C3H/He) F_1 female mice, becuase mice did not die with in 30 days, after doses lower than 3.7×10^{8} Bq ($=5$ Gy) for the former nor with doses lower than 7.4×10^{8} Bq ($=11$ Gy) forthe latter, but all died at doses higher than7.4×10^{8} GBq ($=11$ Gy) and 1.11×10^{9} Bq ($=15$ Gy), respectively.

When the dose of single injection de cr-eased, incidence of tumors increas-

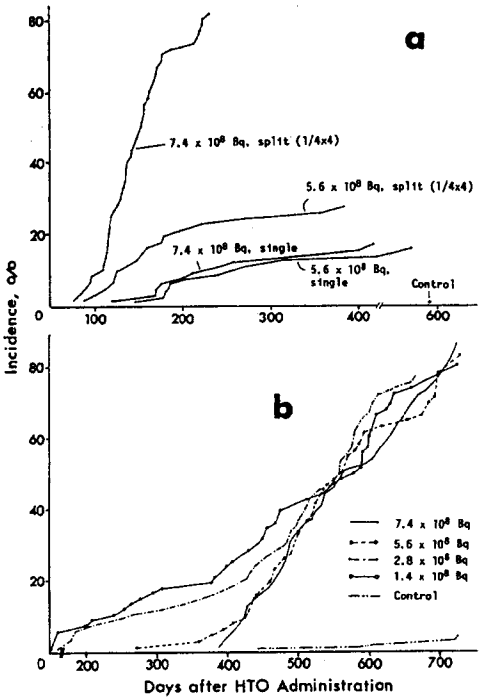


Fig. 13. Cumulative incidence of tumors. HTO were injected intraperitoneally to young female mice [C57BL/6 N $\times$ C3H/He] F_1 at various doses. a: Lymphomas; b: other tumors.

ed[49, 50]. Fig. 13 shows the relationship between the incidence and the time after injection in the various dose groups. The ear-ly occurence of lymphoma is clear in the 5.6×10^{8} and 7.4×10^{8} Bq groups, especially in the dose fractionated groups. A single injection at the lower doses seems to have a negligible effect. The development of other tumors began after the lymphoma ceasedto appear. The common sites of tumor development other than lymphoma are similarto those in mice exposed to X rays or gamma-rays.

b. Orally Continuous Administration[51, 52]

HTO in various concentrations was orally administered continuously to 10 weeks old [C57BL／6 N×C 3 H／He] F_1 female mice. Within a range of 5.92×10^{11} Bq／dm^3 to 1.48×10^{11} Bq／dm^3 as the concentration of HTO in drinking water, the time of death after initiating the administration was about 2 weeks, a typical time for haematopoietic death. A linear relationship of times of death with HTO concentrations in drinking water was observed, on a log-log scale, between 1.48×10^{11} Bq／dm^3 (about 0.96 Gy／day) and 1.85×10^{10} Bq／dm^3 (0.48 Gy／day) (Table 4 and Fig. 14.) At concenraions lower than 9.25×10^{9} Bq／dm^3 (0.24 Gy／day), mice no longer died from haematopoietic failure.

Table 4. Relevant data of continuous administration of HTO for adult female mice [C57BL／6 N×C 3 H／He] F_1.

Range of Death Type	HTO Concentration in Drinking Water (Bq／dm^3)	Dose Rate at Plateau Phase (Gy／day)	Total Volume of Drinking Water (ml／mouse)	Total Organ Dose (Gy)	Time of Death (day)	Number of Mice Used
Haemato-poietically Lethal	1. 5.92×10^{11}	_a	13	23.7	15.0±1.4	20
	2. 2.96×10^{11}	_a	14	13.1	15.4±0.5	20
	3. 1.48×10^{11}	_a	16	11.1	15.3±1.3	20
	4. 7.40×10^{10}	1.92	23	12.0	19.2±2.2	20
	5. 3.70×10^{10}	0.96	56	15.3	28.5±3.0	20
	6. 1.85×10^{10}	0.48	123	18.5	46.3±5.8	20
----------	(1.2×10^{10}) ---------	(0.3) ----------		(20) ----------		
Non-Haemato-poietically Lethal (Lymphoma)	7. 9.25×10^{9}	0.24	495	39.6	165±36	45
	8. 3.70×10^{9}	0.10	777	25.2	259±52	38
	9. 1.85×10^{9}	0.05	1242	20.0	414±66	60
----------	(1.2×10^{9}) ---------	(0.03) ----------		(13.5) ----------		
Non-Haemato-poietically Lethal (Other tumors)	10. 9.25×10^{8}	0.02	1433	11.5	481±112	60
	11. 3.70×10^{8}	0.01	1866	6.2	622±121	53

a: Not reached a plateau.

Mice receiving HTO of 9.25×10^{9} Bq／dm^{3} (0.24 Gy／day) or less survived over 150 days with high incidence of tumor development (70%－80%). A linear relationship between dose rate and time of death was observed on a log-log plot in the dose rateregion from 9.25×10^{9} Bq／dm^{3} (0.24 Gy／day) to 1.85×10^{9} Bq／dm^{3} (0.048 Gy／day). However, at dose rates less than 9.25×10^{8} Bq／dm^{3} (0.024 Gy／day), it curved downward (Fig. 15). No other tumors except lymphomas were observed in mice receiving HTO at 9.25×10^{9} Bq／dm^{3} (0.24 Gy／day). As HTO dose rate decreased, the proportion of thymic lymphomas tended to decrease, while the appearance of various non-lymphoma tumors increased (Table 5).

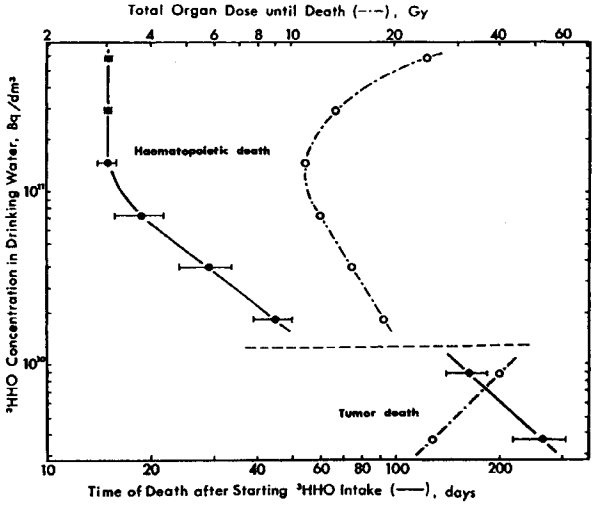


Fig. 14. Relationships of the time of death after initiating HTO intake (●) and the total organ dose until death (○) to the HTO concentration in drinking water, using adult female mice [C57BL／6N×C3H／He] F_1.

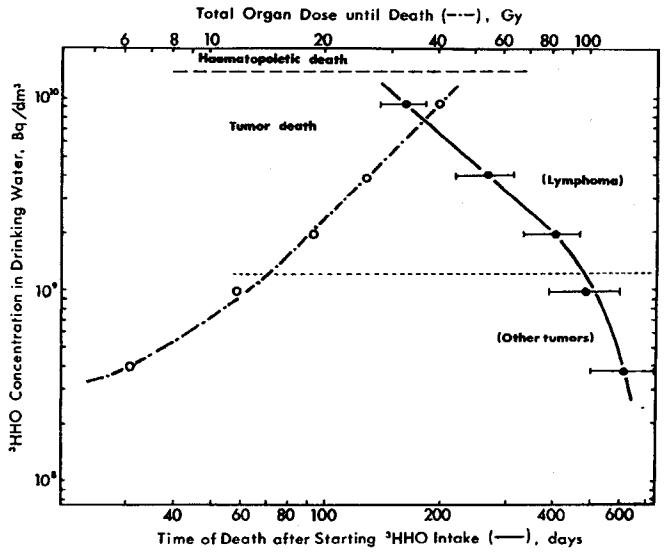


Fig. 15. Relationships of the time of death after initiating HTO intake (●) and the total organ dose until death (○) to the HTO concentration in drinking water, using adult female mice [C57BL／6N×C3H／He] F_1.

Roughly speaking, when the volume of the cell nucleus is one third the cell volume (10 μm in diameter), there is one decay per 13 min at the threshold dose rate for haematopoietic death and one decav per 130 min at the threshold dose rate for accelerated thymic lymphomatous death. In the case of haematopoietic death, the total dose and the time of death are inversery proportional to the

Table 5. Tumor formation rates at different dose-rates by continuous administration of HTO for adult female mice [C57bl/6N×C3H/He] F_1. ( ) : % ; [ ] : Latent period, days.

Dose Rate/Day	7. 0.24 Gy	8. 0.10 Gy	9. 0.05 Gy	10. 0.02 Gy	11. 0.01 Gy
Thymic Lymphoma	29 (64) [162±28]	22 (58) [273±51]	15 (25) [415±53]	4 (7) [508±202]	3 (6) [589±32]
Non-Thymic Lymphoma	5 (11) [146±27]	4 (11) [229±24]	12 (20) [433±82]	9 (15) [504±120]	11 (21) [609±70]
Lymphoma in Total	34 (76) [159±28]	26 (68) [267±50]	27 (45) [423±68]	13 (22) [505±150]	14 (26) [605±65]
Reticular Cell Sarcoma		2 (5) [179±15]	5 (8) [390±67]	12 (20) [485±144]	10 (19) [570±150]
Ovarian Tumor		2 (5) [201±18]	4 (7) [431±60]	8 (13) [511±98]	11 (21) [641±114]
Haemangiosarcoma		2 (5) [331±21]			
Fibrosarcoma			2 (3) [431±58]	4 (7) [467±97]	6 (11) [607±90]
Harderian Gland Tumor			2 (3) [423±81]	2 (3) [537±75]	
Lung Tumor			1 (2) [464]	3 (5) [460±30]	8 (15) [736±84]
Skin Tumor			1 (2) [401]		
Bladder Tumor				1 (2) [580]	
Rhabdomyosarcoma				1 (2) [298]	
Mammary Tumor					2 (4) [582±58]
Hepatic Tumor					2 (4) [685±23]
Adrenal Gland Tumor					1 (2) [623]
Double Bearing				2 (3)	10 (19)
Tumor-Formed Mice	34 (76)	32 (84)	42 (70)	42 (70)	44 (83)

dose rate. This implies that repair of damaged stem cells is or normal division of non-damaged stem cells is greater at the lower dose rate region than at the threshold dose rate (0.3 Gy or 110 decays/day in cell nucleus). Thus one decay per 13 min at the threshold dose rate may be indicative of the capacity for cellular repair in the haematopietic system. In the case of death from thymic lymphoma, the total dose is di-rectly proportinal and the time of death is inversely proportional to the dose rate, which implies that the damage is typically unreparable. The accumulaton of damage to key genes may induce stem cell mutations and result in lymphoma at dose rates higher than the threshold dose rate (0.03 Gy or 11 decays/days in the nucleus). In general, thymic lymphomas would not be expected at dose rates lower than this threshold dose rate because the amount of accumulated gene damage would not be sufficient for leukemogenesis.

At dose rates lower than 0.1 Gy or 37 decays/day, the rate of tumor incidence other than lymphoma the more increased when the lower the dose rate. But the rate of total tumor incidence is almost constant (see Table 5). In Fig. 16, the survival curves at all dose rates are shown. To know the limit of the tritium effect on the tu-

mor production and the life span and to confirm the hormesis, it is neccessary to com pare the present results with those from the further low dose rate irradiations and control which are going.

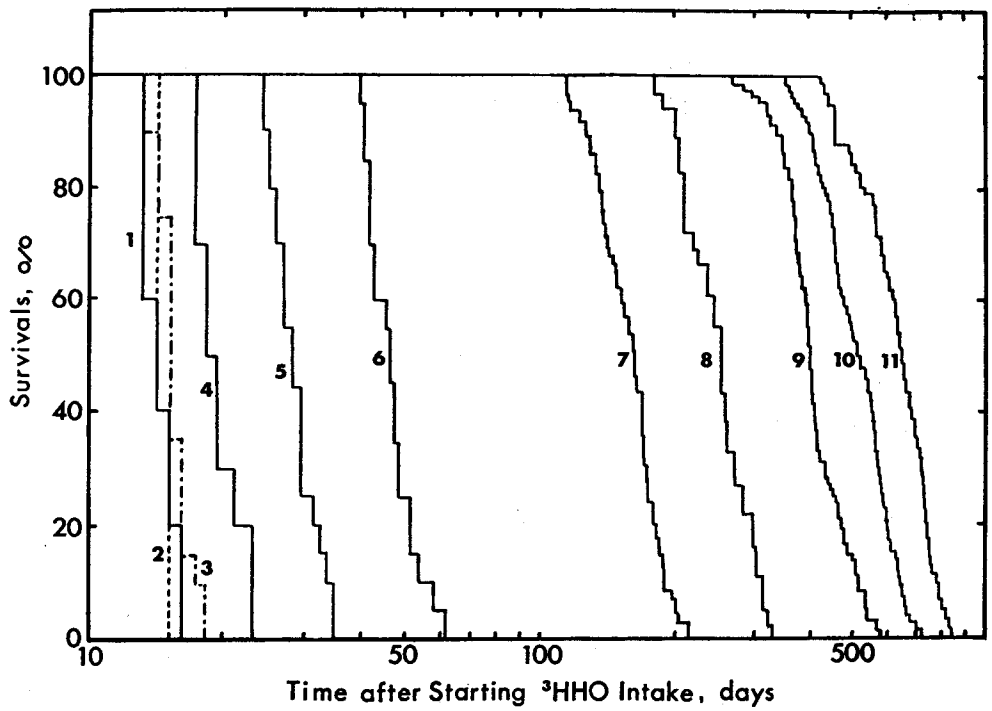


Fig. 16. Survival curves for adult female mice [C57BL/6N×C3H/He] F_1 where HTO was orally administered at different concentrations (refer the number to Table 4.)

5. Conclusions

1. A building was newly constructed, which has facilities specially designed for exclusive use in HTO studies. To use HTO for experiments, there were set up a glove box, a draft chamber, a biohazard chamber, and small and large animal chambers. The released HTO vapor can be absorbed in water by a water bubbler, an water shower scrubber and an water jet scrubber and then introduced to storage tanks. Animal feces and urine also can be introduced to the tanks. After measuring the ^3H activity of the slops, those are released into the public sewage system after dilutoin.
2. An UV spectrum specific for HTO was observed, which was different from OH, HO_2^-, O_2^- and H_2O_2. On the other hand, it was found that HTO exhibits the luminescence only with luminol but without peroxidase. Mesityl oxide rdiolysis showed

the formation of epoxide, which yield was much higher with ^{3}H beta-rays than with ^{60}Co gamma-rays. From these results, it was concluded that there was the formation of the nascen O in ^{3}H beta-irradiation [$H_2O \rightarrow H_2O^* \rightarrow H_2+O$] in aqueous systems.

3. The low oxygen effect was observed in deaerated solution with ^{3}H beta-rays for thymine and adenine radiolyses and DNA strand breaks. This supports the formation of the naccent O in ^{3}H beta-irradiation. Because the nascent O can reacts not only with substance [$O+M \rightarrow (MO)$, $(MO)+H_2O \rightarrow M(OH)_2$ or $\cdot MOH+\cdot OH$] but also with another nascent O producing O_2 [$O+O \rightarrow O_2$], that is, deaerated solution changes to aerated solution.

4. In order to confirm the low-dose-rate effect, Kada effect, that the larger the effect when the lower the dose rate, the transfection activity of M13 phage DNA was tested exposing to HTO eliminated H_2O_2. After confirmed the low-dose-rate effect, it was explained by that the reaction yield of [$O+O \rightarrow O_2$] would become relatively low and that of [$O+M \rightarrow (MO)$, $(MO)+H_2O \rightarrow M(OH)_2$ or $\cdot MOH+\cdot OH$] would become relatively high when the concentration of the nascen is low.

5. RBE of ^{3}H beta-rays to ^{60}Co gamma-rays was studied using radioresistant *E. coli* TG 1 to compare radiosensitive *E. coli* B_{S-1}. The obtained value 1.23 of the former was similar to the value 1.21 of the latter. It was concluded that the RBE value higher than one is due to the high reactivity of the nascent O which acts recembling two OH but not due to the reparability or the direct reaction with radiation.

6. According to our data from single injection of HTO, $LD_{50/30}$ was estimated to be 5.6×10^{8} Bq (8 Gy) for C57BL/6N female young mice and 9.3×10^{8} Bq (13 Gy) for [C57BL/6N×C3H/He] F_1 female young mice. Leukemias, mainly of the lymphoid type originating in the thymus, developed earliest among the neoplastic lesions observed at dose rates of 7.4×10^{8} Bq (10 Gy) and 5.6×10^{8} Bq (8 Gy) for [C57BL/6N×C3H/He] F_1. The fractionated infection resulted in a higher yield of lymphomas. At doses lower than 5.6×10^{8} Bq (8 Gy), variable solid tumors developed with a longer latency than lymphomas.

7. When HTO as drinking water was continuously administered in adult [C57BL/6N×C3H/He] F_1 female mice, the time of death was about two weeks, a typical time for haematopoietic death, within a range of 5.92×10^{11} Bq/dm^3 (about 15 Gy/day) to 1.48×10^{11} Bq/dm^3 (about 4 Gy/day). A linear relationship of time of death with HTO concentrations was observed, on log-log scale, between

1.48×10^{11} Bq／dm^3 (about 4 Gy／day) and 1.85×10^{10} Bq／dm^3 (0.48 Gy／day). At this range all mice died of haematopoietic failure within 50 days.

8. Mice receiving HTO of 9.25×10^{9} Bq／dm^3 (0.24 Gy／day) or less survived over 150 days with high incidence of tumor development (70−80%). A linear relationship between dose rate and time of death was observed on a log-log plot in the dose-rate region from 9.25×10^{9} Bq／dm^3 (0.24 Gy／day) to 1.85×10^{9} Bq／dm^3 (0.048Gy／day). No other tumors except lymphomas were observed at 9.25×10^{9} Bq／dm^3 (0.24 Gy／day). As HTO dose rate decreased, the proportion of thymic lymphomas tended to decrease, while the appearance of various non-lymphoma tumors increased.

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