Biological Behavior of Tritium after Administration of Tritiated Water in the Rat

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Distribution and retention of tritium in various tissue of a rat after administration of tritiated water (HTO) was investigated during about 100 days. Tritium incorporated into the body was rapidly and uniformly distributed in the whole body and excreted immediately with a biological half-life of 3.5 days for almost all the tissues. However, excretion of tritium incorporated into the tissue constituents was relatively slow and the retention curves were considerably different from tissue to tissue. At the end of this experiment, the highest concentration of total tritium was observed in the fat tissue, followed by brain and muscle. These results suggested that the intake of tritium from HTO into organic compounds and the excretion of tissue-bound tritium would be related to metabolic activity of the tissue. This prediction was reinforced by the experiment on age dependence.

The radiation dose to each tissue after the administration of 6.0 μCi/g body weight of HTO ranged from 2.0 to 9.5 rem, which was the highest in blood and the lowest in fat tissue. Contribution from tissue-bound tritium was within 10% of the dose from total tritium for each tissue, except for fat tissue which was 64%.

INTRODUCTION

Tritium is produced naturally by the interaction of cosmic rays, and also released from nuclear reactors and weapons testing into the environment. Tritium in the environment exists mostly in the form of tritiated water and diffuses easily into the biosphere and the organism.

We have studied the biological behavior of tritium in the organism as a first step for estimation of biological effect of tritium. The β-rays from tritium are of relatively low energy and of very short range, so that the biological effect of tritium varies considerably with the position of tritium in the organism. Tritium introduced as tritiated water into the organism will behave essentially as body water and disappear rapidly from the body. Nevertheless, it is known that tritium exchanges with hydrogen in organic compounds and is incorporated into nonexchangeable position in organic compounds through many metabolic processes. Thus, a part of tritium from tritiated water combine with various organic compounds in the tissue.
and turns into a tissue-bound tritium. Biological behavior of the tissue-bound tritium would be different from that of tritium in body water.

In the present work, distribution and retention of tritium in various tissues of mammals after the administration of tritiated water was investigated, with particular emphasis on the behavior of tissue-bound tritium. Also, the radiation dose to each tissue from the total tritium and the tissue-bound tritium was calculated. These experiments are also a part of a study on the metabolism of tritium, a radioisotope of hydrogen which exists in all organic compounds as is carbon.

MATERIALS AND METHODS

Tritiated water with a specific activity of 1 Ci/ml was obtained from The Radiochemical Centre, Amersham. This was diluted to an appropriate concentration with normal saline solution and administered to experiment animals. Male Wistar strain rats, obtained from the animal and plant supply section of National Institute of Radiological Science were used in these experiments. Throughout the experiment, the animals were kept in a room with controlled temperature and air flow.

The rats were given tritiated water in 1 ml of normal saline solution under ether anesthesia either orally by a stomach tube or intraperitoneally as a single administration and killed at various time intervals during about 100 days. At the time of the administration, the rats were about 100 days old and weighing about 380 g, but for the experiment on age dependence, the rats of about 60 and 300 days old were used. Tissue samples of liver, kidney, testis, spleen, heart, lung, intestine, muscle, brain, fat tissue, and blood and sample of urine in bladder were obtained from the sacrificed rats. A part of individual sample was immediately weighed and total tritium activity in this wet sample was determined. Some fraction of each sample was freeze-dried with liquid nitrogen in order to determine the tissue-bound tritium activity. The freeze-drying was performed up to constant weight and repeated twice after the addition of distilled water to remove water-form tritium and some easily exchangeable tritium. For a determination of tritium activity, these samples were subjected to combustion by a Packard Sample Oxidizer which automatically adds an aquatic scintillator and radioactivity in the combustion water obtained was counted by a Beckman 230 liquid scintillation system. Counting efficiencies were determined by external standard channel ratio method and checked by the addition of internal standard tritiated water to some samples.

RESULTS

Incorporation of Tritium into Various Tissues

Variations in the concentration of tritium in various wet tissues after oral ingestion and intraperitoneal injection of 0.5 μCi/g body weight of tritiated water are shown in Figure 1, as example for blood, liver, kidney and testis. Each point represents a determination on a single animal. In case of intraperitoneal injection,
the total tritium concentration reached a peak value within 1 hr. While in the case of oral ingestion, peak of the concentrations occurred at about 6-9 hr after administration. As these results show, the rate of tritiated water incorporation into the tissues following oral ingestion was slower than that after intraperitoneal injection. This fact would be explained by assuming that in the former case, tritiated water is directly absorbed by diffusion into bloodstream and circulates throughout the whole body, but in the latter case, majority of tritiated water is not absorbed into bloodstream until it reaches the intestine.

In both modes of administration, some small difference existed in the peak values of the concentrations among various tissues. The data of water content shown in Table 1, which was determined by the method of freeze-drying, indicate that the concentrations are proportional to the water content of the tissue. The peak concentration in blood was higher than that in the testis which showed the highest water content, and this seems to be due to the fact that blood is the first site of entrance into every organ of the body.

Retention of Total Tritium in Various Tissues and Urine

Excretion curves of total tritium from various tissues after ingestion of 6.0 μCi/g body weight of tritiated water are illustrated in Figure 2. Each point represents
Table 1. Water content of various tissues of rats.

| Tissues   | Water content* (%) ± SD |
|-----------|------------------------|
| Blood     | 80±3                   |
| Liver     | 69±2                   |
| Kidney    | 73±2                   |
| Testis    | 85±3                   |
| Spleen    | 74±2                   |
| Lung      | 76±3                   |
| Heart     | 73±2                   |
| Intestine | 72±3                   |
| Muscle    | 72±3                   |
| Brain     | 75±2                   |
| Fat Tissue| 15±3                   |

* Water content was determined by the method of freeze-drying, and expressed as average of ten experiments with standard deviation.

![Graph showing variations in the concentrations of total tritium in several tissues and urine after administration of tritiated water (6.0 μCi/g body wt.)](image)
the average of determinations on two rats. The concentration of total tritium did not vary so much among different tissues, except for fat tissue, until about 25 days after the administration of tritiated water, and by this time the concentration had fallen to 1-2% of the initial level. Hereafter the difference of these concentrations was obviously distinguished with time. The rate of excretion was relatively fast from the liver and intestine, and slow from the muscle and brain. At the end of this experiment, the highest concentration was observed in the fat tissue, followed by the brain and muscle, and lower in the liver and intestine. For other tissues the concentration showed intermediate values. In Figure 2, the concentration of tritium in urine is also drawn, which is considered to show the same level as tritium in body water. The concentration was obviously lower than that in other tissues at later period after the ingestion.

Retention of Tissue-bound Tritium in Various Tissues

Figure 3 shows the variation in the concentration of tissue-bound tritium as a function of time. Each point represents the mean value from two rats. For the first several days, the concentration of tissue-bound tritium was relatively low in fat tissue, muscle and brain, and high in liver and intestine. However, this situation was reversed after 20 days, and the concentration was high in the fat tissue, brain

![Graph showing variations in the concentrations of tissue-bound tritium in several tissues after administration of tritiated water (6.0 μCi/g body wt.).](image)

**Fig. 3.** Variations in the concentrations of tissue-bound tritium in several tissues after administration of tritiated water (6.0 μCi/g body wt.)
and muscle, and low in the liver and intestine.

Since the dried materials analyzed for tissue-bound tritium in this experiment were removed easily exchangeable tritium by washing with distilled water, the behavior of tissue-bound tritium must indicate that of tissue constituents themselves to which tritium has bound stably. Therefore, we would be able to interpret the retention curves in terms of metabolic turnover of the tissue constituents. On this basis, it will be concluded from our data that the tissues such as liver and intestine contain the constituents which are turning over relatively fast, while the tissues such as fat tissue, brain and muscle contain the constituents which are slowly turning over.

Quantitative Relationship between Total Tritium and Tissue-bound Tritium

Variations in the quantity of total tritium and tissue-bound tritium were plotted as a function of time. Figure 4 shows the graph obtained for kidney, as a typical example. Initially, the ratio of tissue-bound tritium to total tritium was about 3% in the kidney and 1-5% in other tissues, but it increased gradually with time, and resulted in about 65% in the kidney and 50-80% in other tissues after 40 days. It seems that the difference among these values in different tissues depends on the

![Graph showing variations in tritium activity](image-url)

**Fig. 4.** Variations in the tritium activity in the kidney after administration of tritiated water (6.0 μCi/g body wt.)
water content and/or the metabolic characterization of each tissue. After 40 days, the slope of the retention curves of total tritium was in parallel with that of tissue-bound tritium. This result shows that during the period the release of tritium from the tissue is controlled by degradation and turnover of tissue constituents in which tritium has been incorporated.

**Biological Half-life of Total Tritium and Tissue-bound Tritium**

Both of the retention curves for total tritium and tissue-bound tritium were resolved respectively into two exponential components. The biological half-lives of tritium for various tissues, as determined from the slope of the component line, are shown in Table 2. The half-life of the short components of the total tritium retention curves was about 3.5 days for all tissues studied, which is similar to those reported by Tompson, Tompson, Boxer and others. On the other hand, the half-life of the long components of the retention curves varied from tissue to tissue, ranging from 17 to 52 days, and the values were in good agreement with that obtained from the retention curves of tissue-bound tritium in the reference tissue. These results indicated that each component of the retention curves for the total tritium probably reflects, respectively, the release of tritium in the body water and the release of tissue-bound tritium.

In the retention curve of the tissue-bound tritium, the components with short half-life ranging from 5.5 to 7.5 days were also resolved for tissue samples. We cannot make clear whether this short component truly exists or not, since significant tritiated water remains in the body water at this early period after administration and additional incorporation take place in parallel with release of tissue-bound tritium. However, we are convinced of the presence of a component, at least different

| Tissues and urine | Total tritium | Tissue-bound tritium |
|------------------|--------------|---------------------|
|                  | Short comp. | Long comp. | Short comp. | Long comp. |
| Blood            | 3.4          | 17          | —           | —           |
| Liver            | 3.5          | 21          | 6.5         | 21          |
| Kidney           | 3.5          | 23          | 5.8         | 23          |
| Testis           | 3.5          | 25          | 6.9         | 27          |
| Spleen           | 3.5          | 35          | 5.8         | 35          |
| Lung             | 3.5          | 26          | 5.5         | 26          |
| Heart            | 3.5          | 27          | 7.0         | 27          |
| Intestine        | 3.5          | 17          | 6.3         | 18          |
| Muscle           | 3.4          | 35          | 6.8         | 36          |
| Brain            | 3.6          | 41          | 7.5         | 43          |
| Fat Tissue       | 3.4          | 52          | —           | 54          |
| Urine            | 3.4          | 25          | —           | —           |
from the long component with a half-life of about 20 days or longer, although the slope of the component may change slightly by the remaining tritium.

**Age Dependence of Metabolism of Tritium**

In order to investigate the effect of age of animals on the incorporation and excretion of tritium, an experiment was carried out on rats of two age groups, 60 and 300 days old, which probably have a different metabolic activity. The rats of the two groups were given orally tritiated water of 0.5 μCi/g body weight. Thereafter, the animals were sacrificed at various time intervals until 20 days, and the total tritium and tissue-bound tritium activities of various tissues were determined. The result is shown, as an example of the data obtained for the liver, in Figure 5. The rate of the excretion of total tritium by younger rats was considerably faster than that by older rats. The biological half-lives of tritium in the younger and older rats were 3.2 and 4.5 days, respectively. During the period of this experiment, the majority of tritium in the tissue existed in the form of water (HTO). Therefore, these results show that the rate of water metabolism by the younger rats is faster than that by older rats.

In the case of tissue-bound tritium, its rate of release in younger rats was also faster than that in older rats. Generally, the younger rat is metabolically more active than the older rat. Therefore, this result seems to indicate that the retention and excretion of tissue-bound tritium depend on the metabolic activity of the animal, as was indicated by the result of the experiment described in the previous section.

![Fig. 5. Comparison of the excretion rate of tritium in the liver on two age groups of rats after administration of tritiated water.](image-url)
Radiation Doses to Various Tissues

The average dose in each tissue following ingestion of tritiated water was calculated using the formula \( D = 51.2 \cdot E \cdot I \), where \( D \) is the dose in rem, \( E \) is the effective energy of tritium \( \beta \)-rays \( (5.7 \times 10^{-3} \text{ MeV}) \), and \( I \) is the integrated activity in \( \mu \text{Ci} \)-days per gram wet weight of tissue.\(^{12,13} \) It was also calculated on the assumption that either water form tritium or tissue-bound tritium is uniformly distributed and quality factor for tritium \( \beta \)-rays is 1, on basis of progress report from ICRP.\(^{14} \) Table 3 shows the doses received by various tissues in rem following the administration of tritiated water of 6.0 \( \mu \text{Ci} / \text{g} \) body weight. The average dose delivered by tritiated water is more or less uniform throughout most tissues studied, and some little difference seems to depend on their water content, especially low in fat tissue. The dose received by blood, which is the first site of tritiated water incorporation into the body, was 9.5 rem, which was the highest value among the tissues. Contribution of the tissue-bound tritium was never more than 10% of total tritium in any tissues, with the exception of fat tissue. However, the contribution was somewhat greater than was expected from the amount of tritium incorporated into tissue constituents initially.

**Table 3.** Doses to various tissues of rats during 100 days after ingestion of tritiated water.

| Tissues      | Doses* (rem) | Contribution from tissue-bound tritium (%) |
|--------------|--------------|-------------------------------------------|
| Blood        | 9.5          | --                                        |
| Liver        | 7.0          | 9.3                                       |
| Kidney       | 7.4          | 6.9                                       |
| Testis       | 7.6          | 4.2                                       |
| Spleen       | 7.0          | 8.1                                       |
| Lung         | 6.8          | 6.3                                       |
| Heart        | 6.8          | 7.2                                       |
| Intestine    | 7.0          | 7.1                                       |
| Muscle       | 7.1          | 8.0                                       |
| Brain        | 7.1          | 8.5                                       |
| Fat Tissue   | 2.0          | 64.0                                      |

* Doses from ingestion of 6.0 \( \mu \text{Ci} / \text{g} \) body weight of tritiated water.

DISCUSSION

In view of evaluation of radiation dose from tritium, the biological behavior of tritium introduced as tritiated water into rats was investigated. When rats were administered tritiated water orally, tritium activity in blood reached a maximum in about 6 hr. This result was different from the previous human data which showed that tritium in human blood reached equilibrium in about 40 min after ingestion of tritiated water.\(^{15,16} \) The difference may suggest that the pathway for gastrointestinal-
al absorption of water by the rat is somewhat different from that by man. However, it should be considered that tritiated water was ingested to the rat under ether anesthesia in our experiment and tritiated water was ingested by fasted human in previous experiment. Hence, further study is needed to convince this suggestion.

Tritium incorporated into the body was rapidly and uniformly distributed in the whole body, although some small difference existed in the tritium concentration of each tissue depending on the water content. Subsequently, tritium was promptly excreted with half-life of about 3.5 days from almost all tissues, whereas, the slope of disappearance curve was more gentle after 20-30 days and the half-life values were in agreement with those of tissue-bound tritium in reference tissues. These results show that the first component with a short half-life of 3.5 days represents the release of tritium in body water and the second component with a long half-life represents the release of tissue-bound tritium. In other words, they indicate that the excretion of tritium in body water is relatively fast, whereas tritium which has been incorporated into tissue constituents is released slowly.

In many cases, tritium concentration in animal and human body is estimated by the measurement of tritium concentration in urine and plasma, as a nondestructive means. However, as shown by our results, the tritium concentration in urine does not reflected the amount of tritium deposited in the body, particularly at later period after administration. This fact must be considered when we evaluate the radiation dose delivered by tritium in the body from the data of urine samples.

Our results also indicated that the retention of tissue-bound tritium was high in these tissues in which the initial tritium concentration was relatively low, such as fat tissue, brain and muscle. The excretion of tritium incorporated into the tissue constituents are largely determined by the metabolic fate of the constituents. Hence, it seems reasonable that the tissues, such as fat tissue, brain and muscle, which are generally considered to be metabolically inert rather than liver and intestine show slower excretion. Thus, it is suggested that there is a strong correlation between the rate of excretion of tissue-bound tritium and the metabolic activity of the tissue constituents into which tritium was incorporated. This suggestion was reinforced by the experiment on age dependence, which showed that the retention of tritium incorporated into the tissue constituents in older rats was higher than that in younger rats. As shown by these results, the study on the biological behavior of tritium incorporated into tissue constituents is essentially the study of the metabolism of the constituents which compose the tissue.

Siri and other previously reported that, with a single administration, the amount of tritium metabolically incorporated into tissue constituents of rabbits or mice may be less than 2% of tritium originally given to the whole body. Our result showed that the amount of tissue-bound tritium in the dry tissues initially ranged from 1 to 5% of total tritium in the wet tissues. Some small difference exists in the two results, but it does not seem to be an essential difference when our data are converted into that for the whole body. At any rate, the amount of tissue-bound
tritium is very small compared to that of tritium in the body water, and the radiation dose to the tissues from the tissue-bound tritium was less than 10% of the dose from the total tritium. From these results, it seems reasonable to conclude that radiation hazard from the tissue-bound tritium is not so significant compared with that from tritium in the body water. However, as stated by Oliver and Person, a tritium β-rays with a mean range of about 1 μm can be considered to deliver most of its energy within a microscopic volume, which gives considerably high dose. If this fact gives rise to a serious biological effect, we cannot ignore the fact that a small but significant fraction of tritium introduced as tritiated water is incorporated into tissue constituents and remains in a specified position for a considerably long period.

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