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Report Written:
October 1952

LA-1467

Report distributed: DEC 8 3 1952

INCORPORATION OF TRITIUM INTO THE ORGANIC COMPONENTS OF VARIOUS TISSUES OF THE MOUSE

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HEALTH AND BIOLOGY

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ABSTRACT

Two groups of mice were given chronic and acute exposures, respectively, to tritium as HTO in body fluids. Both groups eliminated the tritium from body fluids with an initial half-time of 1.6 to 2.0 days. After most of the tritium had been eliminated, the half-time for elimination increased to about 12 days as the rate of growth of tritium out of the organic constituents of the body became the limiting factor in the elimination rate. Mice killed when the half-time for elimination had lengthened to about 12 days showed a specific activity of tritium in the organic components of tissues which was many times the specific activity of tritium in body water. However, the radiation dose to the mice from the tritium incorporated in the organic constituents was small in comparison with the radiation dose from tritium in body water at levels necessary to induce the activity into the organic components either on chronic or acute exposure. It was concluded that the overall radiation hazard due to incorporation of tritium into the organic constituents of mice is minor compared to the radiation hazard from tritium in body water. The same is probably true for man.

CONTENTS

TABLES

ILLUSTRATIONS

1. INTRODUCTION

This report supplements LA-1218.[1] These experiments were undertaken to determine the extent to which tritium might be incorporated into organic components of the various tissues of mice upon both acute and chronic exposure to high levels of tritium as HTO in body fluid and to evaluate the radiation hazard due to this incorporation. Previous research[2] shows that tritium taken into the body as HTO is distributed in all body water and in the readily labile hydrogen positions of organic components of the body. It is eliminated from these body components at a rate which is proportional to the rate of water excretion by the animal.[1,2] In addition, when the HTO activity in body water is high, tritium activity has been shown to enter slowly into the less labile hydrogen positions of organic constituents from which it is slowly eliminated.[1,3] The rate of growth and elimination of tritium in the various tissues and organic components of the body has not been determined previously except to show that the average rate of elimination from the combined organic components of mice is slower than that from body water by a factor of 3 to 30.[3]

In the experiments reported here, the level of tritium activity prevailing in the organic components of various tissues of the mouse after both acute and chronic exposures to high levels of tritium activity in body fluids has been determined. A comparison is then made between the radiation dose received by the body due to tritium activity in the organic components of the body and the radiation dose received by the body due to tritium activity in body water.

2. METHODS

Chronic Exposure

One group of three mice was subjected to a chronic exposure of tritium as HTO by ingestion. For 5 months all water which these mice drank contained 4.14 μc/ml of tritium. Periodic analysis of the water from the urine of these animals during the 5 month period was made to evaluate the concentration of HTO maintained in body fluids. The average tritium activity maintained in the urine of each of the three mice during the exposure period was 2.99, 2.70, and 2.96 μc/ml, respectively, with an over-all average of 2.88 μc/ml. The above data indicate that about 30 per cent ($\frac{4.14 - 2.88}{4.14} = 0.3$) of the water the mouse exchanges comes from sources other than the drinking water. This dilution effect results from dilution of the drinking water with water obtained from the food eaten and from water absorbed from the atmosphere due to water exchange through the skin and lungs. The water absorption through the skin and lungs is estimated to correspond to a water intake into the body of the mouse from the atmosphere of 0.4 g/day at Los Alamos where the average

water vapor pressure (yearly) is 8.05 mm Hg. This vapor pressure corresponds to a water content of about 8 mg/liter in the atmosphere. The hourly respiratory volume of a full-grown mouse is calculated to be about 1 liter from measurements of O_2 consumption and CO_2 excretion. Assuming 100 per cent exchange of inspired water with body fluids in the mouse[4] a daily absorption of 0.2 g of water into the body through the lungs is calculated (8 mg/liter x 1 liter/hr x 24 hr/day = 192 mg/day). Water absorbed through the skin of the mouse is estimated to be about equal to that absorbed through the lungs. This is true for man and the rat[5] but has not been measured in the mouse. The 0.4 g of water thus obtained by exchange through the skin and lungs corresponds to about 5 per cent of the daily water exchange of the mouse with its environment and leaves 25 per cent of the daily water exchange to come from food. By measuring the daily water and food intake, together with the dilution noted, it was possible to calculate that 45 per cent of the weight of the food eaten appeared as water in the body. (This compares to a value of 58 per cent water in the food when burned with oxygen in a combustion furnace.) The values for water exchange obtained in the manner described above were used in the subsequent part of these experiments to determine total daily water exchange by the mouse with the environment.

The beta radiation dose received by the mice during the 5 months of chronic exposure was calculated to be 5 to 7 roentgens per week as follows:

$$\frac{2.88 \times 0.75 \times 3.7 \times 10^4 \times 6 \times 10^3 \times 1.6 \times 10^{-12} \times 6.05 \times 10^5}{93} = 5 \text{ r per week}$$

where 2.88 is the activity in body fluids in μc/ml; 0.75 is the milliliters of fluid per gram of tissue (assuming no activity other than that in the body fluid); 1 $\mu c = 3.7 \times 10^4$ d/s; 6×10^3 ev is the average energy per disintegration; 1.6×10^{-12} = ergs/ev; no. sec/week = 6.05×10^5; and 1 rep = 93 ergs/g. If one assumes the activity prevailing in the fluids to be also prevailing in the organic constituents of the tissues, then a radiation dose of about 7 r/week may be calculated. Over the 5 month period a total radiation dose of about 110 to 130 rep was estimated to have been delivered to the mice during the chronic exposure.

At the end of the 5 month period the mice were given normal water to drink, and the decline in HTO activity in body fluids was followed for about 1 month. The water and food ingested were carefully measured daily, and the total water intake into the body was calculated each day by adding to the water ingested as such, an amount corresponding to 45 per cent of the weight of food ingested and 0.4 g for the water intake through the skin and lungs from the environment.

After the HTO activity in body fluids had declined to a low level, the mice were killed, dissected, and various component parts of the body separated, so that samples of bone, skin, muscle, brain, spleen, thymus, and gastrointestinal tract were obtained. These tissues were

dried, and the water evaporated from each was analyzed for HTO activity. The dried tissues were then burned separately in a combustion furnace over copper oxide at about 850°C, and the water of combustion was analyzed for HTO activity. The HTO activity was measured as described in LA-1218.[1]

Acute Exposure

A group of three mice was subjected to an acute exposure of tritium as HTO by intraperitoneal injection of 0.3 ml of water containing 2.8 mc of tritium as HTO. This resulted in an average equilibrium activity of tritium in the total body fluids of about 1.4×10^5 μc/liter. The decline in this activity was followed daily as described in the case of the chronically exposed animals. Measurement of water and food ingested was made daily and total water intake was calculated. As a result of the higher initial activity of HTO in body fluids, it was possible to follow the decline for about 2 weeks longer than in the mice chronically exposed. Fifty days after injection of the acute dose the mice were sacrificed and dissected, and analysis of tritium activity in tissue fluids and organic material was made in the manner previously described. The amount of HTO injected in the mice that received acute exposure was such as to give an integrated radiation dose approximately the same as that delivered to the animals receiving chronic exposure. In the chronic experiment the radiation dose was spread over several months, whereas in the acute experiment most of the dose was delivered in the first 2 weeks after injection.

3. RESULTS

When mice chronically exposed for 5 months to a relatively constant level of HTO in drinking water sufficient to give a radiation dose of about 20 times the tolerance of 0.3 r/week were placed on ordinary water, they showed an exponential decrease of HTO activity in body fluids with time. This decrease had a half-time of 1.6 to 2.0 days, depending on daily water intake, and was fairly constant for about 10 to 15 days. After 10 to 15 days the activity of HTO in body fluids was reduced to about 1 per cent of the activity maintained during the 5 month chronic exposure period (Fig. 1). The percentage of body weight with which the water passing in and out of the body was exchanging during this period may be calculated by the following formula:

$$\frac{\text{Average daily } H_2O \text{ intake} \times \text{half-time of decrease} \times 100}{\ln 2 \times \text{body weight}} = \text{per cent body weight.}$$

Calculated for each of the three mice, this value ranged between 70 and 80 per cent of the body weight, which suggested that exchange of body water plus rapidly exchangeable hydrogen in organic constituents were mainly involved in the decrease in tritium activity during this

period.

After the initial 10 to 15 day period the rate of loss of HTO from body fluids began to slow down and approached a biological half-time of about 11 days between 20 and 32 days after the mice were placed on ordinary water intake. The longer half-time is presumed to represent an approximation of the rate at which tritium was growing out of the organic constituents of the body as a whole during this period. The activity of HTO in urine after 32 days on ordinary water had decreased to 0.2 per cent of the activity maintained for 5 months during the exposure period (Fig. 1).

After 32 days on ordinary water the chronically exposed mice were sacrificed and dissected to obtain the various tissues and organs indicated in Fig. 1. The various samples obtained were dried in vacuo at room temperature, and the water that was evaporated from each tissue was analyzed for tritium activity. The results are given in Table I. It was expected that this water would show about the same HTO activity as that prevailing in urine at the time of the sacrifice. Such was true within the limits of the error of measurement for water from all the tissues except muscle and fat. There was a lapse of several months between sacrifice and the time of drying and analysis, during which time the tissues were kept frozen. It is presumed that during this period there was some autolysis in the muscle and fat, which resulted in an increase in tritium activity in the water of these tissues.

After evaporation of water was completed, the dried tissue was burned, and the water obtained from the combustion was analyzed for HTO activity. The results are given in Table II and Fig. 1. These data show that the highest activity was obtained in brain, skin, and muscle, and that the lowest activity was obtained in liver. The other tissues were intermediate in activity. The activities in Table II and Fig. 1 are given in μc/liter. To convert these values to μc/g of dry tissue, one may use the following formula:

$$\frac{\text{Activity in } \mu\text{c/liter} \times \%\ H_2 \text{ in dry tissue} \times \text{sp. gr. of tissue}}{1000 \times \%\ H_2 \text{ in water}} = \mu\text{c/g of dry tissue}$$

The per cent water and per cent dry weight of tissue, as well as per cent hydrogen in the dry tissue are given in Table III. The data given in this table are for both the chronically and acutely exposed mice and will be discussed subsequently.

In the fifth column of Table II is given the average activity in the water obtained from combustion of the dry tissue. If these activities are compared to the 2880 μc/liter average activity maintained in body fluids during the 5 month chronic exposure, it is observed that they range from 9.1 per cent of that value in the case of brain to 0.94 per cent in the case of liver. If the tolerance level of tritium activity in the body (140 μc/liter) had been maintained during the chronic exposure period, a proportionally lesser activity would have been

present in the organic hydrogen. Although the average level of tritium maintained for 5 months in the body fluids of these animals gave a radiation dose of about 20 times the accepted tolerance 32 days after being placed on ordinary water, the radiation dose due to tritium activity remaining in the organic component of the brain was less than one half of tolerance and for liver less than one twentieth (Fig. 1). From these results it is concluded that after chronic exposure to not more than a tolerance dose in body fluids, the radiation dose due to activity remaining in the organic components of tissues of mice after 32 days would be negligible, or about one fortieth of tolerance in the brain and about one four-hundredth of tolerance in the liver.

If one may assume that similar considerations hold for man, it may be concluded that the growth of tritium into the organic components of the tissues on chronic exposures not exceeding the tolerance level poses no serious long-range hazard from radiation exposure.

In the mice exposed to an acute dose of HTO the initial level of activity in body fluids following injection was 1.4×10^5 μc/liter. The activity decreased rapidly with an average half-time of 1.9 days during the first 20 days following injection (Fig. 2). Calculation of the percentage of body weight involved in the dilution of water passing through the body during this time showed that 66 to 76 per cent of the body weight was involved in the dilution, which is not significantly different from that found in the chronically exposed mice. Twenty days after injection, the activity of HTO in body fluids had fallen to less than 0.1 per cent of the initial level attained after injection. The integrated radiation dose received by the mice during the first 20 days was approximately 120 rep. After 20 days the rate of decay of HTO in body fluids began to slow up until it approached a half-time of about 12 days between 35 and 50 days after injection. This again is assumed to represent an approximation of the rate of decay of tritium out of the organic constituents of the body as a whole during this period. By 50 days after injection, HTO activity in body fluids had diminished to about 0.003 per cent of the initial level. At this time the mice were sacrificed, dissected, and the various tissues analyzed in a manner similar to that described for the chronically exposed mice, except that for the acutely exposed mice only a few weeks elapsed between sacrifice and analysis.

The water evaporated from all tissues showed essentially the same HTO activity as that prevailing in urine at the time of sacrifice (Table IV). The only exception appeared in water evaporated from the skin which had a significantly higher activity. This may have been due to some autolysis having occurred in the organic components of the skin during the period between sacrifice and analysis.

The various dry tissues were burned, and the water of combustion was analyzed for HTO activity. The results are shown in Table V and Fig. 2. As in the case of the mice ex-

posed chronically to HTO, the acutely exposed animals showed the highest activity of tritium in the organic constituents of brain, skin, and muscle. Activity in other tissues was in about the same order as in the chronically exposed mice, with fat and liver being lowest in tritium activity.

The level of tritium activity found in the organic constituents of any of the tissues was less than that which would be required to give a radiation dose of 0.3 rep/week to the tissues (Fig. 2), even though the acute dose was of the order of about one fifth of a lethal dose. It would appear that the radiation dose received by mice due to incorporation of tritium into the organic components of tissues is not a serious hazard following acute exposure to a level of HTO activity in body fluids which approaches a lethal dose. If one may extrapolate from mouse to man, then the same conclusion may apply.

4. SUMMARY

A group of mice was exposed for a period of 5 months to a level of HTO activity in body fluids sufficient to give a radiation dose of 5 rep/week. After this exposure the mice were placed on ordinary water for 32 days. At the end of this period the HTO activity in body water had dropped to a level (6 μc/liter) which would result in a radiation dose of about 0.01 rep/week. At this time the tritium activity in the organic components of various tissues was such as to give a radiation dose ranging from about 0.1 rep/week in the brain to 0.01 rep/week in the liver, with the dose to other tissues lying between these values.

Another group of mice was exposed to an acute high-level dose of HTO by a single intraperitoneal injection of 0.3 ml of water containing sufficient HTO to raise the activity of HTO in body fluids at equilibrium to 1.4×10^5 μc/liter. The activity in the body fluids decreased with an initial half-time of 1.9 days so that the total integrated radiation dose to the mice resulting from the single injection was about 120 rep. Seven weeks after injection the HTO activity in body fluids had declined to 4 μc/liter, which would result in a radiation dose of about 0.007 rep per week. At this time the tritium activity in the organic components of various tissues was such as to give a radiation dose ranging from about 0.2 rep/week in the brain to 0.04 rep/week in the fat and liver. The radiation dose to other tissues was intermediate between these values.

In either chronic or acute exposure of mice to HTO the radiation hazard due to incorporation of tritium into the organic constituents of the body would appear to be of relatively minor significance in comparison to the dose resulting from tritium distributed in the body water.

Following either chronic or acute exposure to HTO, mice eliminate tritium from body

water with an initial half-time of 1.6 to 2.0 days. After an initial period of 10 to 20 days this rate of elimination begins to slow down as the amount of tritium growing out of the organic constituents begins to approach a significant percentage of the tritium being eliminated from the body. When the organic components of the tissues are contributing the major fraction of tritium being eliminated from the body, the half-time of elimination is increased to about 12 days, which is assumed to approximate the average half-time of turnover of the organic body components that have fixed tritium. This half-time would presumably be longer at later times when relatively short-lived organic components had decayed and longer-lived organic components had assumed relatively greater significance in the tritium elimination picture.

5. REFERENCES

1. E. A. Pinson, LA-1218 (1951).
2. E. A. Pinson, Physiol. Revs. 32: 123 (1952).
3. R. C. Thompson, HW-20092 (1951).
4. I. G. Campbell, D. F. White, and P. R. Payne, Brit. J. Radiology 24: 682 (1951).
5. C. W. De Long, Science 114: 397 (1951).

Table I

ACTIVITY OF HTO IN TISSUE WATER OF MICE AFTER 5 MONTHS' CHRONIC EXPOSURE TO HTO IN BODY FLUIDS[a] FOLLOWED BY 32 DAYS ON NORMAL WATER INTAKE

	Activity in Tissue Water, μc/liter			
Tissue	Mouse No. 1	Mouse No. 2	Mouse No. 3	Average
Brain	---	---	---	4.3
Skin	2.1	3.9	2.1	2.7
Muscle	5.7	14.8	13.6	11.4
Heart	---	---	---	3.8
Spleen and thymus	---	---	---	5.0
Fat	8.6	20.7	22.2	17.2
Kidney	---	---	---	2.5
Liver	3.4	3.9	4.1	3.8
(Urine)	4.5	4.5	3.6	4.2

(a) Body water maintained at a level of 2880 μc/liter.

Table II

ACTIVITY OF HTO FROM COMBUSTION OF DRY TISSUES OF MICE AFTER 5 MONTHS' CHRONIC EXPOSURE TO HTO IN BODY FLUIDS[a] FOLLOWED BY 32 DAYS ON NORMAL WATER INTAKE

	Activity in Water of Combustion, μc/liter			
Tissue	Mouse No. 1	Mouse No. 2	Mouse No. 3	Average
Brain	---	---	---	263
Skin	168	205	175	183
Muscle	146	139	121	135
Heart	---	---	---	94
Bone	---	---	---	91
Spleen and thymus	---	---	---	67
G. I. tract	40	50	57	49
Fat	45	46	56	49
Kidney	---	---	---	48
Liver	25	32	23	27
(Body water) Urine	5	5	3	4

(a) Body water maintained at a level of 2880 μc/liter.

Table III

PER CENT WATER, PER CENT DRY TISSUE, AND PER CENT HYDROGEN IN DRY ORGANIC CONSTITUENTS OF VARIOUS TISSUES OF MICE

Tissue	Water, per cent		Dry Tissue, per cent	Hydrogen in Dry Tissue, per cent	
	Range	Average[a]		Range	Average[a]
Skin	46.3 - 54.7	50.7	49.3	7.0 - 9.1	8.1
Liver	68.7 - 82.8	74.2	25.8	7.0 - 8.2	7.6
Muscle	62.9 - 72.0	70.7	29.3	7.7 - 8.2	8.0
Fat	28.8 - 65.1	47.5	52.5	9.2 - 10.8	9.9
G. I. tract	81.6 - 88.9	85.2	14.8	6.9 - 7.8	7.5
Kidney	74.0 - 76.2	75.1	24.9	7.4 - 7.6	7.5
Brain	76.9 - 84.8	80.8	19.2	7.7 - 9.0	8.4
Nonweighted average		69.2	30.8	--- ---	8.1

(a) Average results from six animals.

Table IV

ACTIVITY OF HTO IN TISSUE WATER OF MICE 50 DAYS AFTER ACUTE EXPOSURE TO 1.4×10^5 μc/liter HTO IN BODY FLUIDS

	Activity in Tissue Water, μc/liter			
Tissue	Mouse No. 1a	Mouse No. 2a	Mouse No. 3a	Average
Brain	---	---	---	4.6
Skin	12.5	7.9	5.9	8.8
Muscle	5.0	5.7	5.7	5.5
Kidney	---	---	---	5.0
Liver, spleen, thymus, and lungs	4.5	4.1	4.8	4.5
Fat	3.8	3.9	4.3	4.0
(Urine)	4.0	3.8	4.2	4.0

Table V

ACTIVITY OF HTO FROM COMBUSTION OF DRY TISSUE OF MICE 50 DAYS AFTER ACUTE EXPOSURE TO 1.4×10^5 μc/liter HTO IN BODY FLUIDS

	Activity in Water of Combustion, μc/liter			
Tissue	Mouse No. 1a	Mouse No. 2a	Mouse No. 3a	Average
Brain	---	---	---	516
Skin	711	200	373	428
Muscle	265	327	338	310
Bone	---	---	---	277
Kidney	---	---	---	126
G.I. tract	89	138	103	110
Liver, spleen, thymus, and lung	92	82	106	93
Fat	69	65	80	71
(Body water) Urine	4	4	4	4

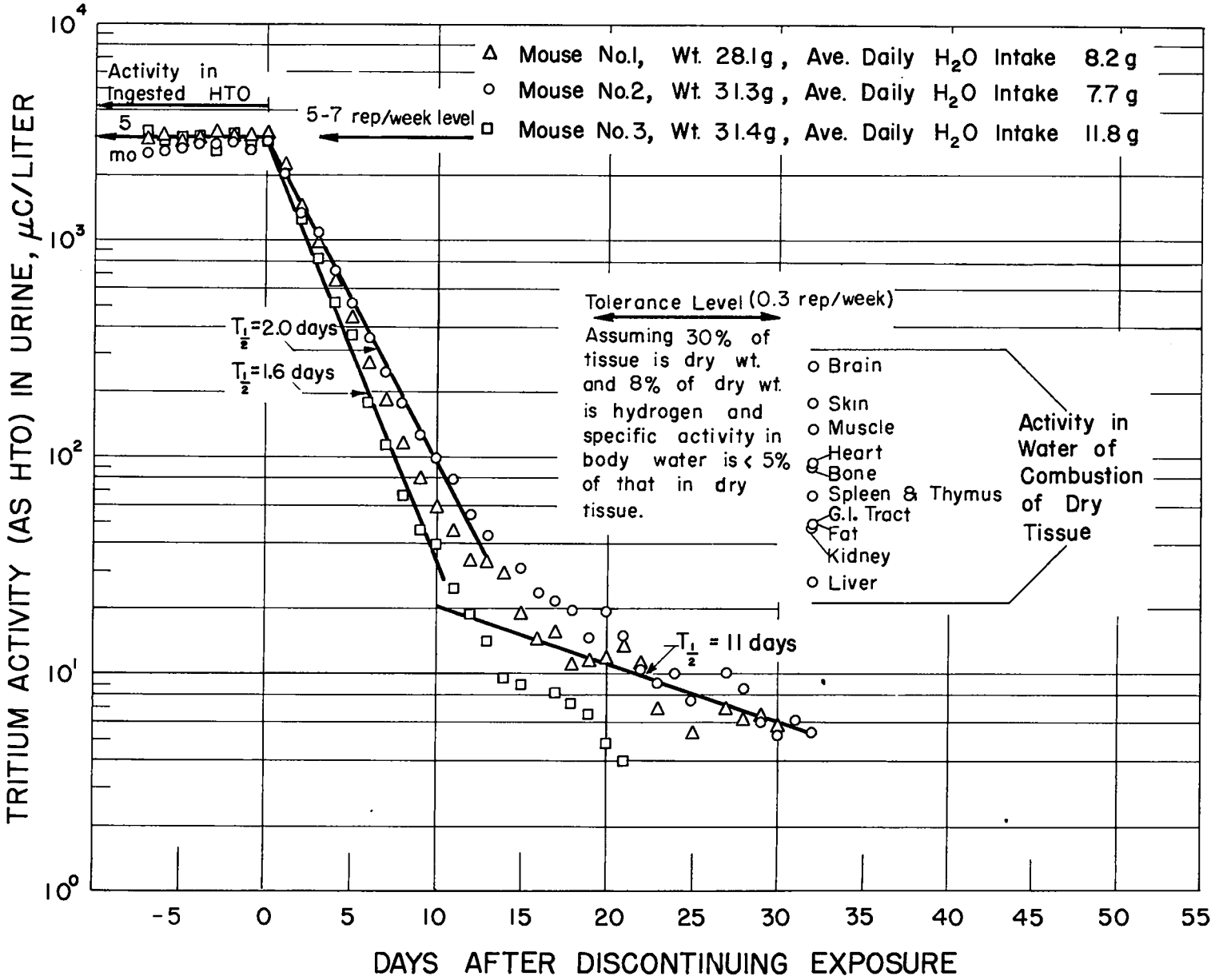

Fig. 1. Rate of elimination of tritium by mice following chronic exposure to ingested HTO.

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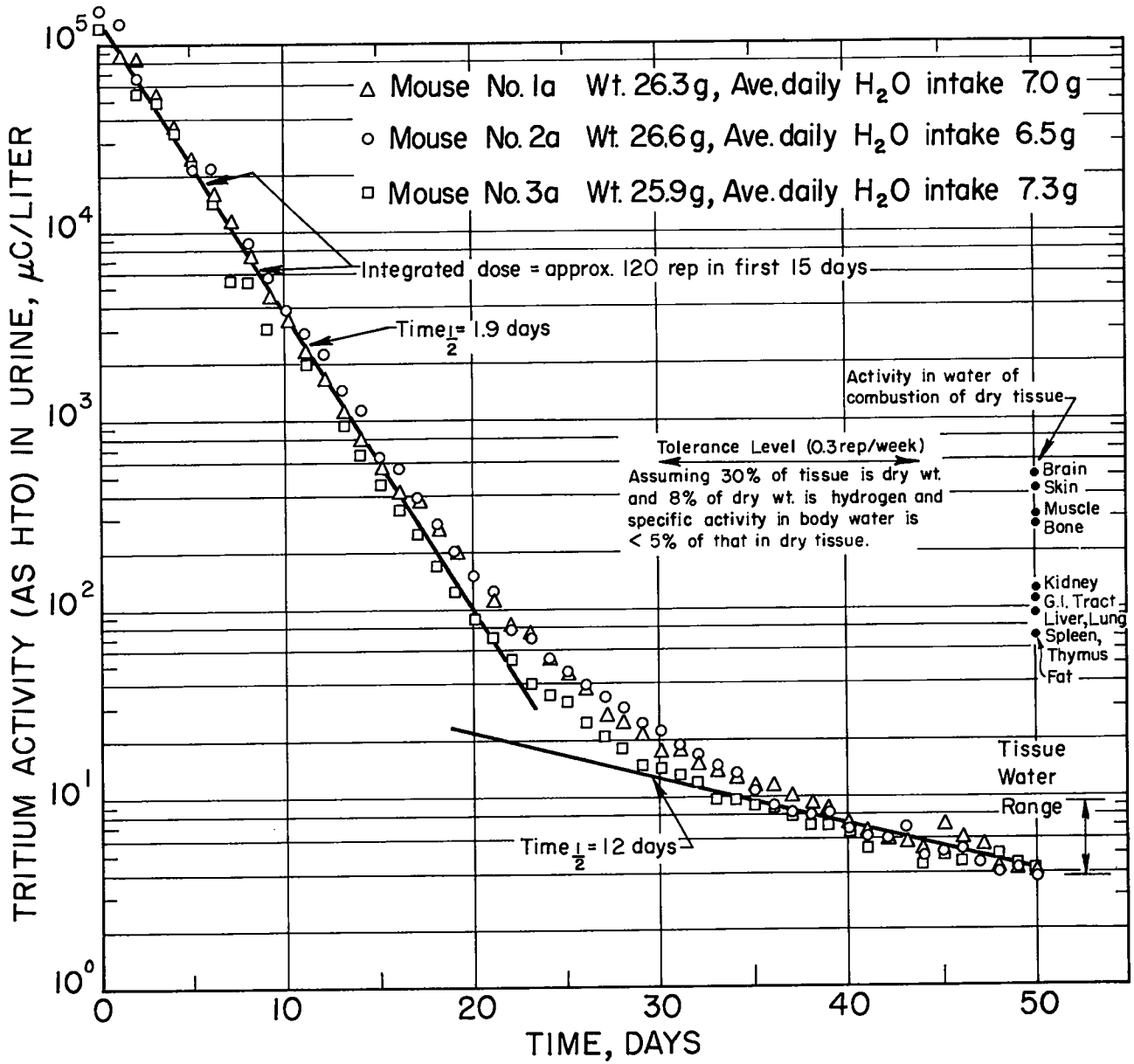

Fig. 2. Rate of elimination of tritium by mice following acute exposures to HTO.