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RETENTION BY MICE OF TRITIUM ADMINISTERED AS THE OXIDE

June 19, 1951

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by

R. C. Thompson

June 19, 1951

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SUMMARY

Tritium oxide was administered to mice by intraperitoneal injection. The retention of tritium activity in the body water and in the dry carcass was determined. The biological half-life of body water in the mouse is approximately 1.1 days. The curve of tritium loss from the tissue-bound state can be fairly well resolved into components of 9 day and 90 day half-lives. These components are thought to represent, respectively, groups of relatively dynamic body constituents, and of relatively stable body constituents. They are of comparable tritium binding capacity and under chronic exposure to tritium would accumulate about 17 per cent as much activity as would be present in the body water.

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RETENTION BY MICE OF TRITIUM ADMINISTERED AS THE OXIDE

INTRODUCTION

The results of a preliminary experiment on the retention by mice of tritium administered as the oxide have been reported in an earlier document (1). The present report describes an essentially similar investigation using higher levels of tritium and continuing over a much longer time period. For the sake of completeness and to facilitate comparison, the more pertinent data of the earlier experiment are repeated here, together with the results of the long-term experiment.

METHODS

The data reported were obtained from two experiments in which tritium was administered as the oxide to mice and the radioactivity followed in the body water and in the dried carcass. The animals used were mature CF-1 mice. They were housed in individual cages and provided with water and Purina Dog Chow ad. lib. Coprophagy was prevented by supporting the animals on wire screen floors and excluding all bedding material.

Tritium oxide was administered in 0.5 ml of physiological saline solution injected intraperitoneally. Animals were sacrificed by decapitation. Each entire carcass was ground with 100 ml of benzene in a Waring Blendor. Body water was obtained by distilling the benzene suspension into an overflow receiver which returned the supernatant benzene to the distilling flask and collected the water which separated as the bottom layer. Water recovered by this procedure averaged 64 per cent of the total weight of the animal.

Benzene was removed from the carcass by distillation and the

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dry residue mixed with copper oxide and burned in a combustion tube at 750°C. Water formed in the combustion was collected in a double trap cooled with solid CO_2. Control experiments indicated complete removal of tritium activity from the sample by this procedure.

Body water and combustion water were analyzed for tritium by a method previously described (2). It involved conversion of the water sample to acetylene by treatment with CaC_2 and measurement of the radioactivity in the acetylene with a vibrating reed electrometer. Values obtained are reproducible within ± 10 per cent.

RESULTS

In the first experiment 12 male and 8 female mice were injected daily over a 13 day period, each animal receiving a total of 1.2 mc of tritium as the oxide. Starting 24 hours after the final injection, animals were sacrificed at intervals over a 30 day period and body water and tissue-bound tritium determined. The data obtained are presented graphically in Figure 1. Each point represents a determination on a single animal, and the considerable scatter of points is thought to reflect primarily the individual differences in the animals rather than experimental error. This may not be true for the last few points on the curves. Activities below 0.5 per cent of the activity present at the end of the injection period are near the limit of detection of the analytical method and subject to greater error than the ± 10 per cent previously quoted.

For more precise results over a longer time period, a second experiment was performed at higher tritium levels. Eighteen animals were injected over a two week period with a total of 5.5 mc of tritium as the oxide. Starting 24 hours after the final injection, animals were sacrificed at intervals over a period of 160 days. The data obtained for tissue-bound tritium are presented graphically in Figure 2. The data on body-water tritium were not significantly different from those obtained in the previous experiment and fell below detectable limits before the end of the experimental period.

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In neither experiment were any consistent differences noted between male and female animals. The points in Figures 1 and 2 are, therefore, plotted without distinction as to sex.

DISCUSSION

The initial slope of the body-water tritium curve in Figure 1 indicates a biological half-life for body water in the mouse of about 1.1 days. The fact that only about 10 per cent of the total tritium administered is present in the body water at the end of the two week injection period is consistent with a biological half-life of this magnitude.

About 10 per cent of the tritium activity remaining in the animal at the end of the injection period in both experiments was not removed with the body water and must, therefore, be considered as being incorporated into the tissue compounds. This "bound" tritium amounts to 0.5 to 1.0 per cent of the total tritium administered. That most of this tritium is carbon-bound and not in equilibrium with body water, is indicated by the initially much smaller value for the slope of the bound tritium curve as compared to that of the body-water tritium curve. The decreasing slope of the body- water tritium curve after about 10 days is further evidence of the presence of bound tritium with a biological half-life much longer than that of the body water. After a sufficiently long period of time the body-water tritium curve should exhibit the same slope as the bound tritium curve. In neither experiment was it possible, however, to follow the body-water curve accurately to this point, because of the low level of activity remaining.

Conclusions as to the biological half-life of the tissue-bound tritium can best be drawn from the data of the long term experiment. As shown in Figure 2, the bound tritium curve can be resolved fairly well into two major components with half-lives of about 9 days and 90 days. Such a resolution can only be considered as a rough grouping of the tritium binding entities into two classes. There are as many actual components to the curve as there are compounds in the animal capable

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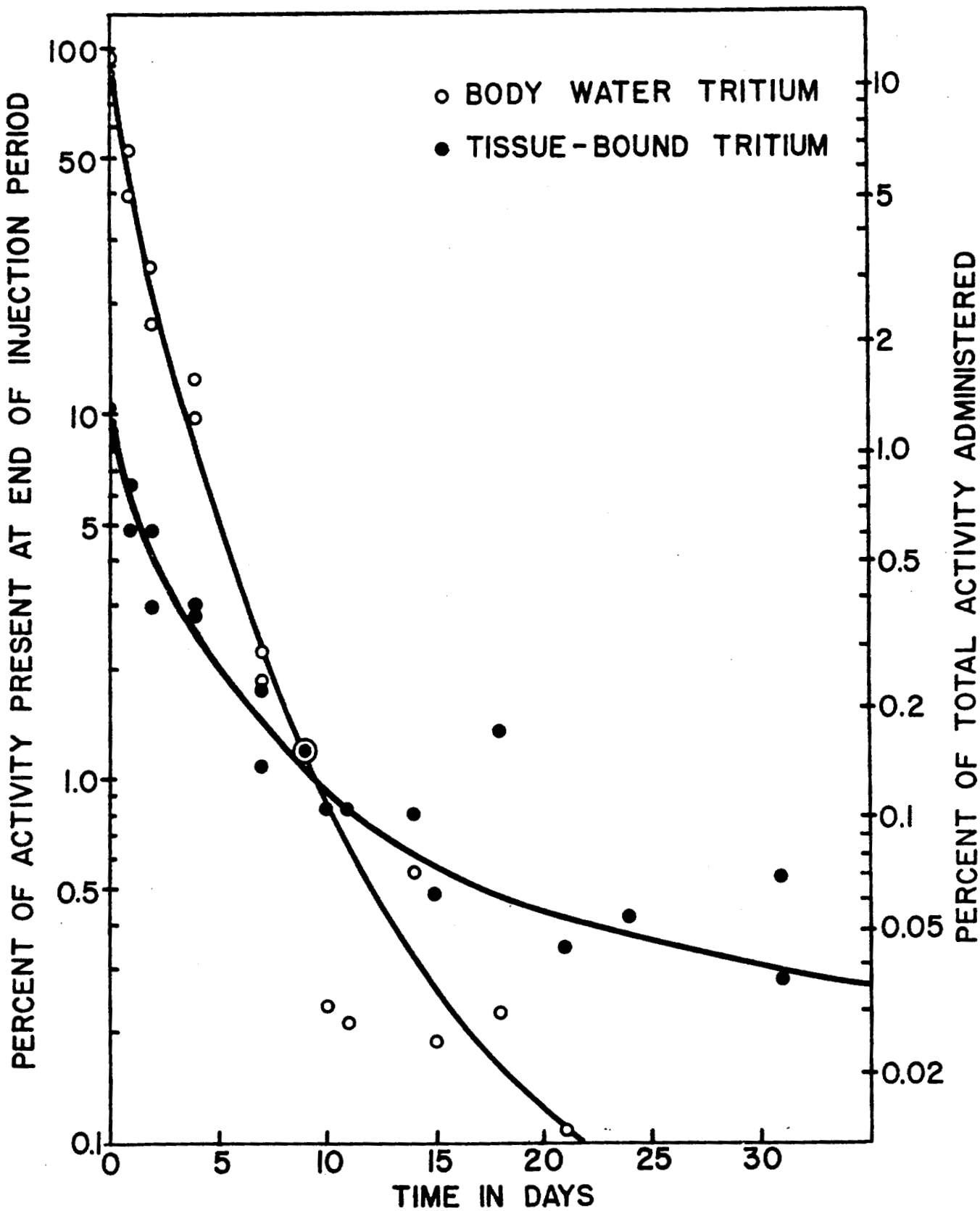

FIGURE I. RETENTION OF TRITIUM IN SHORT-TERM EXPERIMENT

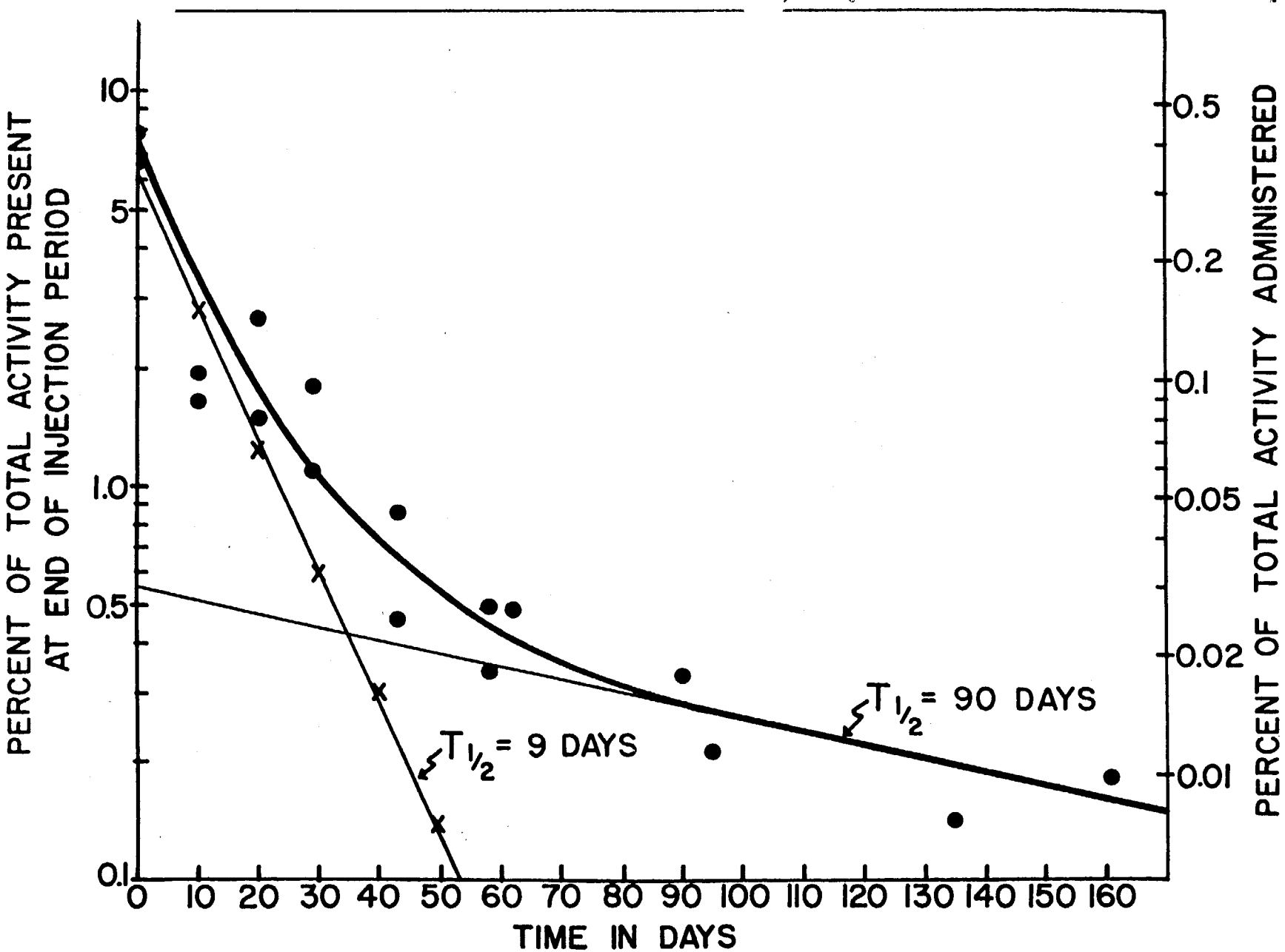

FIGURE 2. RETENTION OF TISSUE-BOUND TRITIUM IN LONG-TERM EXPERIMENT

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of binding tritium. Further uncertainty attaches to the half-life of the longer lived component because of the considerable scatter of points and the lack of information as to the course of the curve beyond 160 days. For this reason the value of 90 days for the half-life can be considered only an approximation. With these limitations in mind, it is still possible to draw certain significant conclusions from the data.

The "90 day component" constitutes, at the end of the injection period, about 0.55 per cent of the total activity remaining in the animal. The "9 day component" constitutes about 6 per cent of the activity remaining. This bound activity has accumulated over the 13 day injection period. One can calculate the equilibrium activity which would eventually be attained in these two components if tritium injection were continued indefinitely by application of the equation:

$$N_o = \frac{N}{1 - e^{-0.693\, t/T}}$$

where N_o is the equilibrium activity, N is the activity at time t, and T is the half-life. Expressing activities in the arbitrary units of per cent of activity remaining at the end of the injection period, we have:

for the "9 day component", $N_o = \dfrac{6\%}{1 - e^{-0.693 \times 13/9}} = 10\%,$

and for the "90 day component", $N_o = \dfrac{1\%}{1 - e^{-0.693 \times 13/90}} = 6\%.$

The validity of these calculations depends upon the assumption that the rate of incorporation of tritium into the tissue components is the same as its rate of loss from the tissue, a reasonable assumption for the case of mature animals in approximate weight equilibrium.

It is apparent that the potential tritium binding capacity of the two tissue components is of comparable magnitude. If we make the assumption that the compounds which make up the two components contain about the same percentage of firmly bound hydrogen we may con-

clude that the mass of these two components is comparable. This general conclusion would remain unaltered if the long-lived component of Figure 2 were drawn with a half-life as short as 50 days or as long as 150 days.

This finding is in essential agreement with that of other workers who have estimated the size of the nitrogen pool involved in the "dynamic state" by the mathematical treatment of data on the rate of excretion of N^{15}. Hoberman (3) calculates that 51 per cent of the total proteins of rats are in the "dynamic state". Sprinson and Rittenberg (4) conclude that 28 per cent of the carcass proteins of rats are in the "dynamic state", and only 6 per cent of the carcass proteins of man.

The fact that tritium administered as the oxide may be retained in the body with half-lives much longer than that of body water is of some significance in the evaluation of the radiation hazard which would result from ingestion of tritium oxide. Methods for measuring the internal contamination of humans with tritium would be limited, for practical purposes to the analysis for tritium activity in a sample in some way derived from the body water. To the extent to which tritium may exist in the tissue-bound state, such body water analyses would prove to be misleading. In the case of chronic exposure to tritium oxide the maximum possible increase in the ratio of tritium to hydrogen in tissue compounds is limited, assuming no isotopic fractionation, by the ratio of tritium to hydrogen in the ingested water. The total quantity of bound tritium could never be more than a fraction of the quantity of tritium in body water. For the case of the chronic exposure of mice to tritium oxide, the increased retention of tritium occasioned by tissue binding may be calculated from the results of the present investigation. We have already calculated the equilibrium activities of the two bound components shown in Figure 2. They total 16 per cent of the activity present at the end of a 13 day injection period. If we now calculate the equilibrium body water activity on the same basis, we have:

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$$N_o = \frac{93\%}{1 - e^{-0.693 \times 13/1.1}} = 93\%.$$

Therefore, the equilibrium bound activity resulting from chronic exposure to tritium oxide would amount to about 17 per cent of the equilibrium activity in the body water. Consequently, the radiation to the animal is 17 per cent greater than if no tissue binding occurred.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the technical assistance of Audrey Pixley, John E. Ballou, and the Analysis Group of the Biology Division.

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