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ABSTRACT

Experiments, conducted to assess the fate of molecular tritium gas in the environment, showed that molecular tritium is metabolized by soil microorganisms and converted to tritiated water. Experiments carried out with laboratory systems containing both plants and soil indicated that molecular tritium may also be metabolized in plants.

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THE CONVERSION OF GASEOUS MOLECULAR TRITIUM TO TRITIATED WATER IN BIOLOGICAL SYSTEMS

INTRODUCTION

There is very little in the scientific literature about the incorporation or metabolism of molecular tritium gas in living organisms. Some information can be inferred from studies of molecular hydrogen metabolism. Molecular hydrogen can be reduced by bacteria containing the enzyme hydrogenase (see Reference 1 for list of hydrogen-reducing bacteria and related references). Hydrogen metabolism, as evidenced by the evolution of molecular hydrogen, has also been demonstrated in photosynthetic bacteria, green algae, and blue-green algae.[2-4] Some organisms will evolve hydrogen under certain conditions and take up hydrogen under other circumstances.[5] There is much less evidence of hydrogen metabolism in higher plants. Hydrogen uptake has been demonstrated in mosses.[5] Leaves of bean plants were exposed to molecular tritium and tritium was found in the water removed from the leaves by freeze drying.[6] While tritium uptake by plants does indicate that some form of hydrogen metabolism probably exists, it does not mean that there is necessarily a net uptake of hydrogen, because the tritium may only be exchanged with other hydrogen isotopes present in the plants.

A series of experiments are described in this report which investigated (I) the conversion of molecular tritium to tritiated water in soils, and (II) the conversion of molecular tritium to tritiated water in systems containing both soil and plants.

SUMMARY

The exposure of field soils to molecular tritium under laboratory conditions showed that molecular tritium was converted to tritiated water within the soil column. Soil conditions which promoted active metabolism of soil microorganisms also increased the amount of tritium found as tritiated water. Soil sterilization greatly decreased the conversion of molecular tritium, while reinnoculation with soil microorganisms restored the conversion ability of the soil. On the basis of these results, we conclude that molecular tritium is converted to tritiated water in soil through the metabolic action of soil microorganisms. A series of experiments was also carried out in which closed plant-soil systems were exposed to molecular tritium. Tritiated water was

found in the soil and plant water after exposure. In some cases, the concentration of tritiated water in the plants was higher than the concentration in the soil. This observation suggests that there may be conversion of molecular tritium to tritiated water associated with the metabolism of the plants.

CONVERSION OF MOLECULAR TRITIUM TO TRITIATED WATER IN SOILS

Experiments were designed to determine whether conversion of molecular tritium to tritiated water occurred in soils and to test the effect of temperature on the tritiated water content of the soil.

In these experiments, the exposures were made in closed vessels containing water, soil, or bacterial culture medium. Tritium gas produced by flowing tritiated water vapor over heated magnesium turnings was passed through soil columns or over the surface of the water or culture medium. The gas undoubtedly contained a small amount of tritiated water vapor because of incomplete conversion by the magnesium turnings.

To determine whether tritiated water was produced during the experiments, the soil or culture medium was heated and the water collected by condensation. The tritium content of the water sample was obtained using liquid scintillation counting.

Effect of Temperature on the Concentration of Tritiated Water in the Soil

As a preliminary test, soil columns were exposed to a range of temperatures to determine whether temperature could cause a concentrating of tritiated water in the soil water. One column was kept in laboratory conditions at 25°C. The second column was kept outside in the prevailing weather of the area. The third column was maintained between -1.0 and 3.0°C to determine whether the different freezing points for tritiated water and nontritiated water could cause freezing and concentration of the tritiated water. There was no evidence of concentration of tritiated water in soils from any of these treatments.

Conversion of Tritium Gas to Tritiated Water by Soil Systems

Four different soils collected in the area of the Savannah River Plant (SRP) were exposed to tritium gas for a period of three days. The tritiated water content of the soil varied by as much as a factor of 18 (Table 1). The soil having the highest tritiated water concentration (a warm, moist loam collected under a steam line) possessed all of the characteristics necessary for

rapid biological metabolism; and hence, the data would suggest a role for microorganisms in converting tritium gas to tritiated water. However, the higher moisture content of this soil would also facilitate direct exchange between tritium gas or residual tritiated water (HTO) in the gas stream and the water in the soil. A series of experiments was carried out to determine the magnitude of direct exchange (Table 2). These data show that direct exchange did take place, and this exchange was a function of the surface area available for exchange.

Because there was evidence of direct exchange, it became necessary to evaluate the effect of water content on the tritium uptake and to demonstrate that this effect would take place independently of conditions that alter the soil biological activity. Table 3 shows the results of exposing six soil samples at identical soil moisture, but different pretreatments to molecular tritium gas. The first two soil samples were maintained at the moisture content found in the field. The other four soil samples were oven-dried and brought back to their original moisture content with distilled water or rainwater. In these latter soils, some decrease in the number of soil microorganisms was assumed with a subsequent decrease in biological activity. The results show the concentration of tritiated water is less in dried soils that had water re-added. The differences observed indicate that the rates of molecular tritium conversion are at least partially dependent on biological activity.

As a further test of the hypothesis that soil microorganisms were involved in incorporating tritium in soil moisture, formaldehyde was added to sterilize the soil and the experiment repeated. The results of these tests (Table 4) show that conversion of molecular tritium to tritiated water is decreased considerably by the addition of formaldehyde. A test was also carried out to determine whether formaldehyde itself would exchange tritium for the hydrogen in the formaldehyde structure. The results of this experiment (Table 4) show that no additional exchange took place during the period of exposure.

The effect of increased activity of soil microorganisms on the tritiated water concentration was evaluated by adding an organic culture medium to soil. The results show that the conversion of molecular tritium increases when conditions are more favorable for the functioning of microorganisms (Table 5).

Soil bacteria were isolated and grown on four different culture media. In a plain agar, a beef extract in agar culture, and a peptone medium, no increases of tritiated water concentration were found when soils were exposed to tritium gas. In the fourth, Burk's medium,[7] there was a definite increase in the tritiated water content of the medium. The results of this and other

experiments illustrate that the presence of soil bacteria enhances the tritiated water content of soil (Table 6). The tritium gas used in these exposures was both the laboratory-generated gas as in all of the past experiments, and a tritium gas mixture similar to that from a tritium production facility. The increases in uptake when soil microorganisms were present were significant in both cases.

TABLE 1

Tritium Gas Exchange with Soil Water

Type of Soil	*Soil-Water Activity, cts/(min-ml)*
1. Warm, moist, loam	7978
2. Sandy loam	1492
3. Sand	640
4. Clay	443

TABLE 2

Evidence of the Direct Exchange between HT or T_2 and H_2O

A. Effect of Water Volume on the Exchange[a]

Vol. of H_2O, ml	*Activity (corrected), cts/ml*	*Activity, total counts*
10	162[b]	1620
25	65[b]	1625
50	38[b]	1900
10	175[c]	1750
25	65[c]	1625
50	36[c]	1800

B. Effect of Water Surface Area on the Exchange

Vol. of H_2O, ml	*Surface Area, cm^2*	*Activity, cts/(min-3 ml)*
15	14.4	57
15	62.2	109

a. Surface area held constant.

b. Samples were refluxed to remove any dissolved tritium gas.

c. Samples were refluxed.

TABLE 3

Effect of Soil Water Content on the Tritium Gas Exchange Mechanism

Type of Soil[a]	*Soil-Water Activity, Relative cts/(min-mℓ)*	*Water Content, mℓ*	*Remarks*
Warm, moist loam[b]	2465	55	
Warm, moist loam[b]	2587	55	
Warm, moist loam[b] (oven-dried at 85°C)	191	55	Distilled water added
Sandy loam (oven-dried at 85°C)	541	55	Rain water added
Sandy loam (oven-dried at 85°C)	359	55	Rain water added
Sandy loam (oven-dried at 85°C)	190	55	Rain water added

a. All containers were identical in size and shape, and each contained 100 g of soil.

b. Soil samples were collected in the field near a steam-trap release point.

TABLE 4

Effect of Time and Formaldehyde Additions on the Tritium Content of Soil Water

Type of Sample[a,b]	*Soil Water Activity,*[c] *Relative cts/(min-mℓ)*
Test I, 3-day exposure	
1. Warm, moist loam 1	1744
2. Warm, moist loam 2	1819
3. Warm, moist loam 3 10 mℓ 18% formaldehyde	633
4. Warm, moist loam 4 10 mℓ 18% formaldehyde	656
Test II, 16-hour exposure	
1. Warm, moist loam 1	412
2. Warm, moist loam 2	440
3. Warm, moist loam 3 10 mℓ 18% formaldehyde	52
Test III, effect of formaldehyde on content of tritiated water	
1. 50 mℓ rain water	67
2. 25 mℓ rain water 25 mℓ formaldehyde	65

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a. All samples had equal surface areas, weights, and quantities of water.

b. Soil samples were collected in the field near a steam-trap release point.

c. Formaldehyde does not quench the liquid scintillator solution.

TABLE 5

Effect of Organic Culture Media on the Tritium Gas Exchange with Soil Water

No.	*Type of Sample*[a]	*Soil Water Activity, Relative cts/(min-mℓ)*
1	Warm, moist loam	741
2	Warm, moist loam	988
3	Warm, moist loam (culture media added)	1348
4	Warm, moist loam (culture media added)	1646

a. All samples had equal weights, water contents, and surface areas. All were collected in the field near a steam-trap release point.

TABLE 6

Soil Bacteria Cultures Exposed to Tritium Gas

Type of Culture	*Water Activity, mCi/ℓ*
Laboratory Generated Gas	
Soil bacteria + Burk's medium	0.349
Soil bacteria + Burk's medium	0.200
Blank (Burk's medium)	0.095
Stack Gas	
Soil bacteria + Burk's medium	17.782
Soil bacteria + Burk's medium	14.250
Blank (Burk's medium)	10.275
Blank (Burk's medium)	8.727
Soil bacteria + sterile soil	1.535
Soil bacteria + sterile soil	1.080
Blank sterile soil medium	0.855
Blank sterile soil medium	0.850

a. All cultures had equal water contents and surface areas.

CONVERSION OF MOLECULAR TRITIUM TO TRITIATED WATER BY CLOSED PLANT-SOIL SYSTEMS

To evaluate the role of different plant-soil systems in converting tritium gas to tritiated water, plant-soil systems enclosed in glass jars were exposed to molecular tritium gas. In each experiment, seedlings were grown in soil within the glass jars. The 2-liter jars were approximately one-quarter filled with soil (∿1000 gm of dry soil).

Three species of plants were exposed: loblolly pine *(Pinus taeda)*, soybean *(Glycine max)*, and rye grass *(Lolium sp.)*. The pine seedlings were transplanted from under a mature pine stand while the soybean and rye grass were grown from seed. The plants were allowed to grow in a greenhouse for at least one month before exposure to tritium gas.

Immediately preceding the exposure of the plant-soil system to tritium gas, the glass jars were sealed with a glass cover. Preliminary trials indicated that the jars would maintain a negative pressure of 5 inches of water for more than two days when the covers were closed and sealed with a rubber gasket and stopcock grease. The exposures were made by removing a volume of gas with a gas syringe (either 1 or 10 cm^3) through a septum in the jar cover. Then a volume of commercially purchased tritium gas in argon (10 mCi/ℓ, MG Scientific, Kearny, New Jersey) equal to the volume of air removed from the jar was introduced through the septum with a gas syringe.

If the period of exposure was to be longer than one day, the plants were placed in growth chambers prior to the exposure. The growth chambers were maintained at an air temperature of 25°C during the day, an air temperature of 19°C during the night, a light intensity of 2000 foot candles, and a photoperiod of 12 hours. If the period of exposure was less than one day, the plants were placed in a laboratory hood. The air temperature was 23°C. The light intensity in the hood was not monitored but was certainly below 2000 foot candles.

At the end of the exposure period, the jars were opened and purged with air to stop tritium uptake. The plants and a soil sample were removed and separately sealed in glass containers. The samples were frozen immediately in dry ice to prevent further metabolism. The plant and soil samples were freeze-dried, and the water of freeze drying was counted by a liquid scintillation technique.[8]

The results obtained when 100 μCi of tritium gas were introduced into jars containing a pine seedling are summarized

in Figure 1. The concentration of tritiated water increased rapidly in the system. Somewhat surprisingly, the concentration peaked most rapidly and remained highest in the seedlings. This can be interpreted as evidence that the plants are active in converting the molecular tritium to tritiated water. However, this evidence by itself is not sufficient to prove that molecular tritium is converted in the aerial organs of the seedling. An alternative explanation would be that the most active microorganisms are associated with the plant roots, and the seedings are absorbing tritiated water converted from molecular tritium in this location.

The humus layer of the soil, made up of partially decomposed organic matter, also has a higher concentration of tritiated water than the soil. Similar observations were obtained in earlier experiments when carbon and mineral nutrients were made available to the microorganisms.

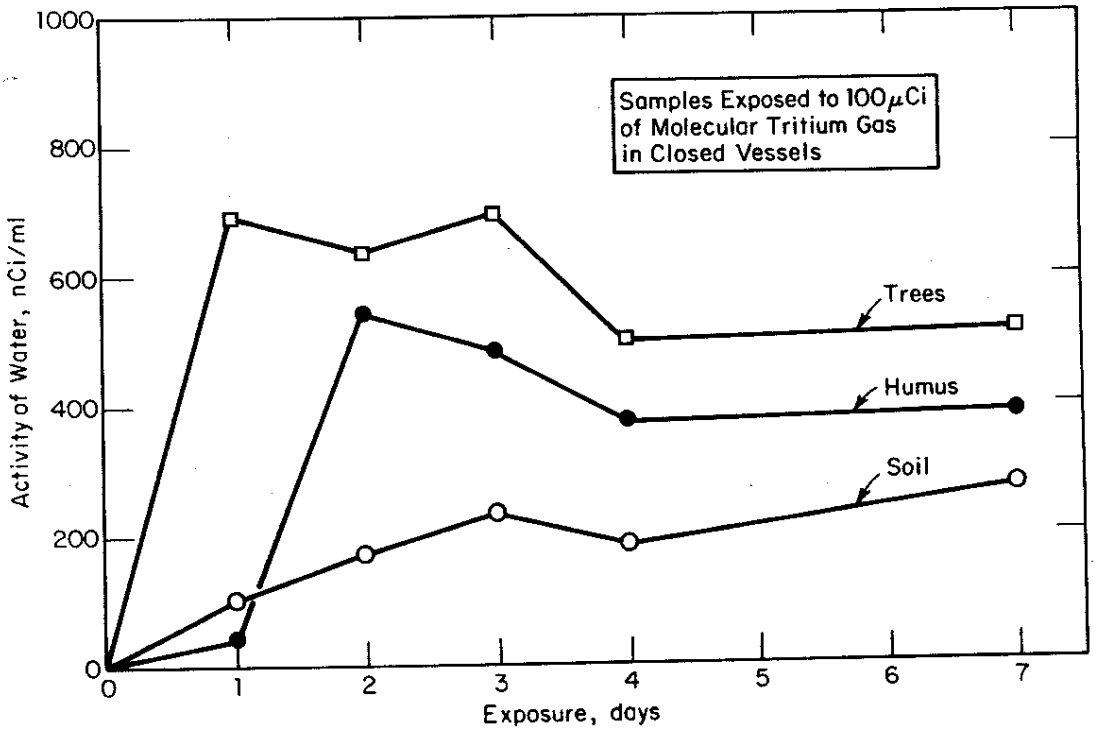

FIGURE 1. Activity of Water from Freeze-Dried Materials in Loblolly Pine Experiments

Because of the high tritium activity and rapid rate of accumulation of tritiated water associated with the pine seedlings, the exposures of the soybean and rye grass plants were conducted at lower tritium concentrations and over shorter time periods. The activity of gas added to each jar was 10 μCi for the soybean and rye grass experiments. The maximum exposure time was reduced to four hours for the rye grass and soybean study. The results are illustrated in Figure 2. Each datum point represents the value obtained from a single jar.

The high concentrations of tritiated water found in the pine seedling exposures were not present in the soybean and rye grass exposures. In these latter exposures, only one jar had a plant concentration of tritiated water higher than the soil concentration. The concentration difference between the soil and the plants was not great, and the elevated plant values occurred in jars which were exposed for short periods of time.

Patterns are apparent which are common to the uptake and conversion in the pine and the soybean experiments and to a lesser extent in the rye grass experiment. Tritium uptake by plants and/or microorganisms and conversion to tritiated water occur preferentially in the different compartments of the system; in some cases, more rapidly in plants; and in other cases, more rapidly in the soil or humus. The tritiated water may be re-distributed in the system fairly rapidly until the concentrations in all of the compartments approach equilibrium.

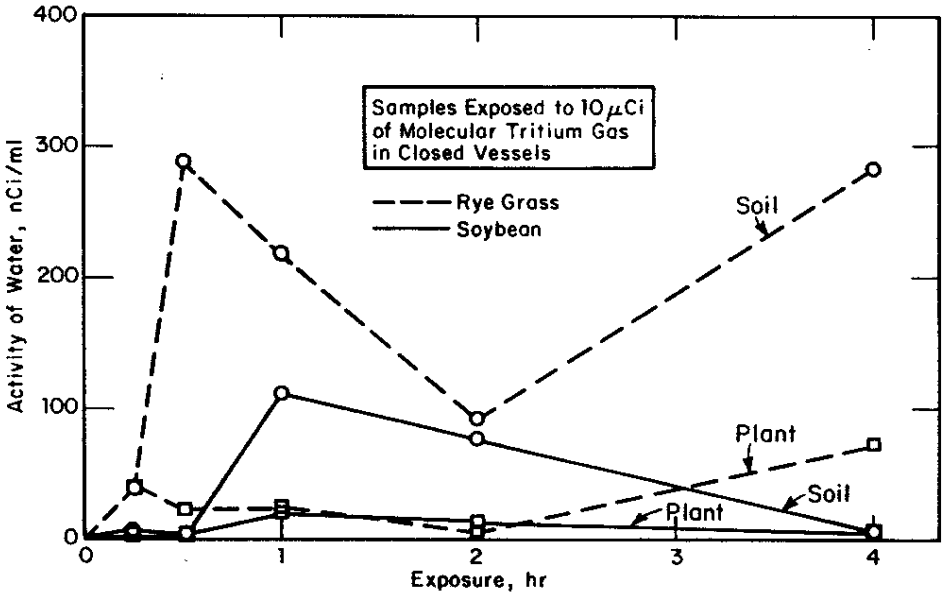

FIGURE 2. Activity of Water from Freeze-Dried Materials in Rye Grass and Soybean Experiments

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