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Hydrogen gas is not oxidized by mammalian tissues under hyperbaric conditions

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Kayar SR, Axley MJ, Homer LD, Harabin AL. Hydrogen gas is not oxidized by mammalian tissues under hyperbaric conditions. Undersea Hyperbaric Med 1994; 21(3):265–275.—Mammalian tissues, including heart, lung, liver, kidney, spleen, and skeletal muscle of guinea pig, rat, or pig, were exposed to tritium (T_2) and high pressures of H_2. Incorporation of the tritium label was measured to test for a latent capacity by mammalian tissues to oxidize H_2 under conditions such as those experienced by deep divers breathing H_2. Tissues were removed aseptically, and either minced, homogenized, or prepared as live cell cultures. The tissues were placed in a chamber to which 8 mCi T_2, 1 MPa He, and either 1 or 5 MPa H_2 were added. After 1 h the chamber was decompressed. The tissues were spun briefly in a vortex mixer to facilitate elimination of T_2 in the gas phase. Samples were analyzed by scintillation counting for tritium incorporation in the liquid phase or in the tissues. Saline and distilled water were used as negative controls. Palladium (Pd) beads immersed in water, and cultures of the H_2-metabolizing bacterium *Alcaligenes eutrophus* were used as positive controls. The tissues incorporated on the order of 10 nCi $T_2 \cdot ml^{-1}$, which implied a H_2 incorporation of 10–50 nmol $H_2 \cdot g^{-1} \cdot min^{-1}$. However this incorporation was not different from that found in the water controls and was attributed to radioisotope effects. The Pd and bacterial samples incorporated over 1,000-fold more T_2 than the mammalian tissues. We concluded that the mammalian tissues did not oxidize H_2 under hyperbaric conditions, with a limit of detection of 100 nmol $H_2 \cdot g^{-1} \cdot min^{-1}$.

diving, tritium, hydrogen metabolism, hydrogenase

Hydrogen is currently being evaluated as a major component of a breathing gas for deep diving. This raises the question of whether mammalian tissues have the capacity to oxidize H_2 under conditions of high H_2 pressure and concentration. It has long been thought that H_2 is biologically inert in mammals under normal atmospheric conditions. When Séguin and Lavoisier (1) placed a guinea pig under a bell jar filled with an oxygen–hydrogen gas mixture, they found that "the hydrogen did not experience any diminution, and left the lungs in approximately the same state in which it entered." More recent experiments using a hydrogen radioisotope (2) found no evidence for H_2 reaction with mammalian tissues at 1 atm.

Gaseous hydrogen is an extremely stable molecule that does not spontaneously dissociate into hydrogen ions at atmospheric temperature and pressure (3). However, numerous bacteria have enzyme systems that catalyze this reaction as part of a major metabolic pathway (4). Hydrogen metabolism has also been reported in some algae, flagellate protozoans, and plants (5). In many of these organisms the direction of the biochemical reactions is reversible, either forming or consuming H_2, depending on the concentration of H_2 (6). Hydrogen has been used in diving experiments with humans and other animal species (7–10). At pressures over 2 MPa H_2, narcotic and even psychotic effects (9, 10) have been observed. Speculations have been offered that hyperbaric hydrogen may exert biological effects such as scavenging hydroxyl free radicals, interfering with cytochrome oxidase (11) or hydrating unsaturated lipids in biological membranes (12). It is thus essential to test for oxidation of H_2 under hyperbaric conditions to determine if mammalian tissues have a latent capacity to oxidize H_2 that is only expressed under hyperbaric conditions.

In the present study, sterile tissue homogenates, minced tissues, and live tissue cultures from guinea pigs, rats, and pigs were exposed to tritium (T_2) diluted in H_2 at pressures of 1 and 5 MPa H_2. After decompression, the H_2 and T_2 gases were flushed out of the samples. The tissues and the water in which they were immersed were then analyzed for incorporation of the tritium label, as a demonstration of the capacity of the tissues to oxidize H_2.

MATERIALS AND METHODS

Tissue preparations

Guinea pigs and rats were placed under halothane anesthesia. Using aseptic technique, two organs were removed from an animal which was then euthanized. Organs used in this study included kidney, liver, spleen, heart, lung, and skeletal muscle from the quadriceps. The organs were weighed and either homogenized in sterile phosphate buffered saline, pH 7.4, or diced with a sterile scalpel blade and immersed in phosphate-buffered saline to concentrations of 0.05–0.1 $g \cdot ml^{-1}$. These preparations were used within 1 h of their removal from the animal. Samples of each organ preparation were plated on both chocolate and blood agar; the plates were incubated at 37°C for 48 h under both aerobic and anaerobic conditions to check that sterility had been maintained.

Myocytes from rat heart were prepared according to the protocol of Sakai et al. (13) by perfusing the heart with collagenase. The myocytes were used within 1 h of isolation, and formed a subconfluent culture at a concentration of 6–8 $\cdot 10^4$ cells $\cdot$ ml^{-1}.

A frozen stock of Pheochromocytoma 12 cells (PC12; cells cloned from a transplantable adrenal medulla tumor of a rat) were obtained from the American Type Culture Collection (Rockville, MD). Monolayer cultures of cells were propagated and maintained on standard growth medium (14) and used as a subconfluent culture at a concentration of 1.3–1.5 $\cdot 10^6$ cells $\cdot$ ml^{-1}.

Capillary endothelial cells were isolated from porcine cerebral cortex. The cells were propagated and maintained on standard growth medium (15) and used as a subconfluent culture at a concentration of 10^6 cells $\cdot$ ml^{-1}.

The tissue samples and cultures (3 ml each) were placed in sterile petri dishes which had been prepared with small notches in their rims to ensure gas entry into the dishes when covered with a lid and stacked. In each experiment, one dish of sterile saline was included in the center of the stack of six dishes containing tissues.

Control experiments

To determine the sensitivity of our method we included two positive control experiments, one using an organic and one using an inorganic substance, in which oxidation of T_2 was certain.

Alcaligenes eutrophus is a Gram-negative bacterium, found commonly in soil, that is known to metabolize H_2 (16). A culture of *A. eutrophus* was grown heterotrophically (17). The culture was centrifuged to concentrate the bacteria. The concentrate was frozen in liquid N_2 and stored at $-70°C$. For an experiment, a pellet of the frozen bacteria was thawed, suspended in tris-buffered saline, pH 7.4, and used within 0.5 h. Concentration of bacterial cells was estimated to be $4 \cdot 10^9$ cells $\cdot$ ml^{-1}. The suspension was pipetted into sterile petri dishes and subsequently handled like the mammalian tissues. Samples of the bacterial suspension were plated on chocolate and blood agar and incubated at 37°C for 48 h. Heavy bacterial growth verified that some bacteria were viable after freezing and thawing.

Palladium (Pd) catalyzes the reaction: $H_2 + \frac{1}{2}O_2 \rightarrow H_2O$. Beads of Pd weighing 0.1–0.7 g per sample were immersed in 3 ml sterile, Millipore-filtered water in sterile petri dishes. These dishes were treated like those containing tissues. We also performed negative control experiments in which all seven dishes contained sterile, Millipore-filtered water.

Apparatus for tritium exposure

A detailed description of the apparatus and its operation appears elsewhere (18). Briefly, the T_2 (Dupont-New England Nuclear, Boston, MA) was purchased as 1 Ci (0.38 ml) in a glass ampule. The ampule was placed inside a stainless steel pressure chamber (330 ml internal volume) which was flushed and then pressurized with helium to 2 MPa (Fig. 1). The chamber was vigorously shaken to break the ampule

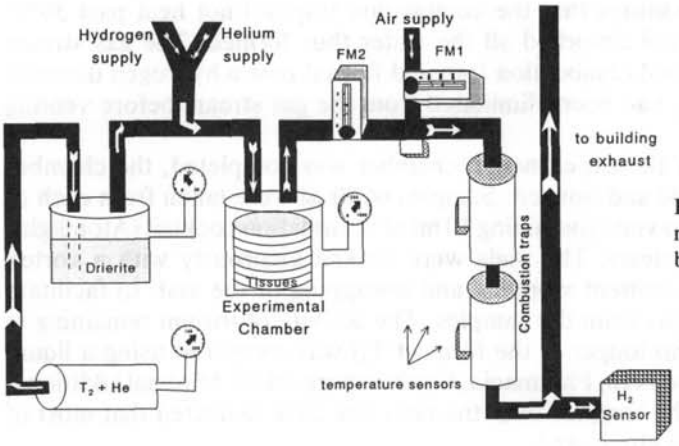

FIG. 1—Apparatus for exposing mammalian tissues to T_2 in hyperbaric H_2.

and release the T_2 into the carrier He. When a small volume of T_2 was drawn for an experiment, the gas passed from this storage chamber through stainless steel piping to a brass chamber (250 ml internal volume) containing a water vapor absorbent (anhydrous $CaSO_4$, Drierite, WA Hammond Drierite Co., Xenia, OH). The gas percolated through the desiccant to remove contaminating tritiated water vapor. The gas then passed to a second brass pressure chamber (250 ml internal volume). The petri dishes for each experiment were stacked inside this chamber, along with two additional dishes of desiccant for further removal of tritiated water vapor contamination. Free gas volume in this chamber was 210 ml when loaded for an experiment.

The activity of T_2 delivered per experiment (7.6–8.0 mCi) was computed from the dilution of T_2 in He and the pressure increase in the experimental chamber. To deliver 8 mCi, the volume of T_2 needed was 3.04 μl, which generated a partial pressure of T_2 of 1.45 Pa in the experimental chamber. Such a low pressure would have been extremely difficult to measure precisely. Diluting the T_2 with He made it possible to deliver this small volume of T_2 while generating a chamber pressure of 26–27 kPa. This pressure was easily measurable with a standard 0–200 kPa gauge (Pacific Scientific, Newport Beach, CA). The T_2–He mixture was allowed to mix with the air in the experimental chamber for 1–2 min. Helium was then added to the experimental chamber at the rate of 0.2 MPa · min^{-1}, to a gauge pressure of 1 MPa. The He diluted the O_2 in the air that had been initially sealed inside the experimental chamber to a concentration of 2%. At this reduced O_2 concentration, it was then possible to continue to pressurize the experimental chamber with either 1 or 5 MPa H_2 (0.2 MPa · min^{-1}) without danger of combustion. Final absolute pressure in the experimental chamber was thus either 2.1 or 6.1 MPa. The compression rate of 0.2 MPa · min^{-1} was chosen from standard human dive chamber protocol, and ensured viability of the cells in the minced organs and cultures. No heating of the chamber was noted during compression.

The tissue preparations remained under pressure for 1 h. The chamber was decompressed at a rate of 0.04–0.05 MPa · min^{-1}, which was dictated by safety requirements for venting H_2 and T_2. The gases from the experimental chamber were vented into a stream of air at a volume ratio of 1:100, again to avoid a combustible mixture of H_2 and O_2. The combined gas stream passed through a combustion trap consisting of a glass vessel filled with Pd-coated molecular sieve material (Science Glass Inc., Miami, FL). The Pd catalyzed the production of water from H_2 and O_2 (19) under sufficiently controlled conditions that the combustion trap did not heat past 50°C. The molecular sieve material absorbed all the water thus formed. The gas stream then passed through a second combustion trap and flowed past a hydrogen detector to verify that all T_2 and H_2 had been eliminated from the gas stream before venting to the atmosphere.

When decompression of the experimental chamber was completed, the chamber was flushed 3 times with He and opened. Samples of 20 μl were taken from each of the petri dishes and added to vials containing 10 ml of scintillation cocktail (Atomlight, Dupont–New England Nuclear). The vials were shaken vigorously with a vortex mixer for 2 min, with intermittent stopping and uncapping of the vial, to facilitate elimination of residual T_2 gas from the samples. The activity of tritium remaining in the samples (presumably no longer in the form of T_2) was measured using a liquid scintillation counter (Wallac 1410, Pharmacia, Gaithersburg, MD). Minimal additional loss of tritium activity in the samples over the next few days indicated that most of the T_2 gas had indeed been eliminated.

Postdive cell viability was confirmed in the cultures by microscopic examination. For the PC12 and endothelial cells we used the stain trypan blue (0.4% in phosphate-buffered saline, pH 7.4) as a test of cell membrane integrity. For the cardiac myocytes we checked for spontaneous contractile activity of the cells. Postdive survival was comparable to that under lab bench conditions over the same time period; over 95% for the PC12 and endothelial cells, and 10–30% for the cardiac myocytes.

Statistical analysis

Six dishes containing tissues or positive controls and one dish containing sterile saline were run together for each experiment. For the experiments with cell cultures, Pd and bacterial suspensions, all six dishes had the same contents; tritium activity was computed from the mean and standard deviation of values from all six dishes. For the experiments with tissue homogenates and minced tissues, three dishes were from one organ and three were from another organ; T_2 activity for each organ was computed from the mean and standard deviation of values from these three dishes.

Analysis of variance (ANOVA) was used to test for significant differences among the dishes in each experiment, and between the various tissues. ANOVA was also used to test for differences between the tissues and the saline control dish in each experiment, and between the tissues and the distilled water negative control dishes. For each analysis, we have included the *P* value, the *F* test value, and the number of degrees of freedom (df).

RESULTS

The mammalian tissue homogenates, minced tissues, and cell cultures incorporated nanocurie activities of the tritium label per milliliter of saline during their 1-h exposure to either 1 or 5 MPa H_2 (Figs. 2 and 3). This activity was equivalent to picomole quantities of T_2 per gram of tissues, or per 10^6 cells in culture. At the relative dilutions of T_2 in H_2 we used (1 T_2 per $6.90 \cdot 10^5$ H_2 at 1 MPa, and 1 T_2 per $3.44 \cdot 10^6$ H_2 at 5 MPa), this would imply an uptake of 10–50 nmol $H_2 \cdot g^{-1}$ tissues $\cdot$ min^{-1} (Table 1). To compute the time periods for these rates, we added the decompression times of 0.5 h from 1 MPa H_2 and 2 h from 5 MPa H_2 to the 1-h exposure at constant pressure because these time periods added significantly to the length of the hydrogen exposure.

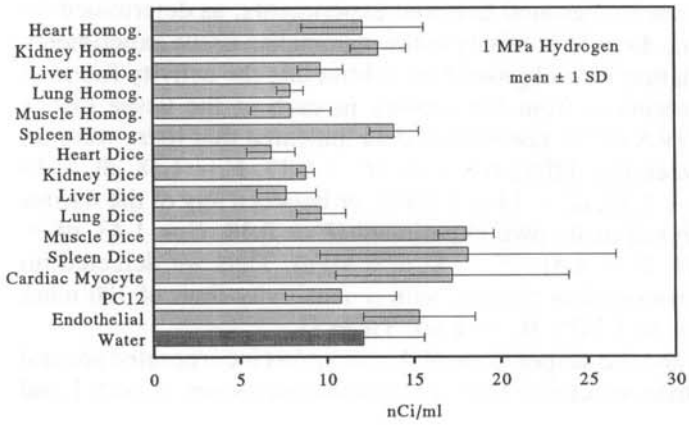

FIG. 2—Total tritium activity in samples of tissue homogenates (means of three samples from one organ ± 1 SD), dice (means of three samples from one organ ± 1 SD), or live cell cultures (means of six samples from one culture ± 1 SD) following 1-h exposure to 8 mCi T_2 and 1 MPa H_2. Also included is the total tritium activity in sterile, distilled water (mean of seven samples ± 1 SD) exposed to T_2 and H_2 under the same conditions.

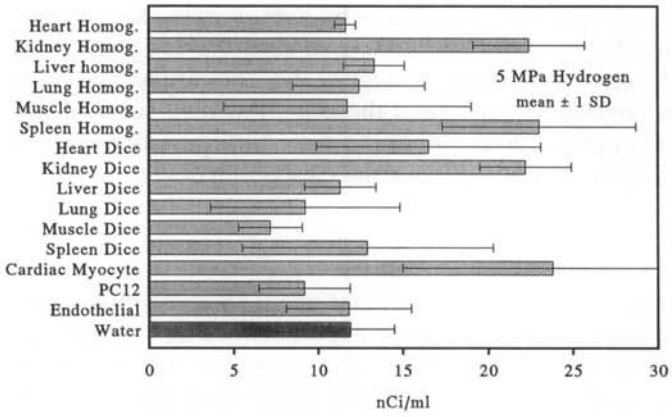

FIG. 3—Total tritium activity in samples of tissue homogenates (means of three samples from one organ ± 1 SD), dice (means of three samples from one organ ± 1 SD), or live cell cultures (means of six samples from one culture ± 1 SD) after 1-h exposure to 8 mCi T_2 and 5 MPa H_2. Also included is the total tritium activity in sterile, distilled water (mean of seven samples ± 1 SD) exposed to T_2 and H_2 under the same conditions.

Table 1: H_2 Oxidation Rate (mean ± 1 SD) Estimated From T_2 Incorporation Rate for Mammalian Tissue Homogenates and Dice (mean of 12 tissues), Pd Beads Immersed in Water (six samples), and Suspensions of the Bacterium *Alcaligenes eutrophus* (six samples) Following 1 h Exposure to 8 mCi T_2 and Either 1 or 5 MPa H_2

	1 MPa H_2 (mol $H_2 \cdot g^{-1} \cdot min^{-1}$)	5 MPa H_2 (mol $H_2 \cdot g^{-1} \cdot min^{-1}$)
Mammalian tissues	1.1×10^{-8} ($\pm$ 0.98×10^{-8})	4.8×10^{-8} ($\pm$ 4.4×10^{-8})
Palladium	1.3×10^{-5} ($\pm$ 0.41×10^{-5})	3.1×10^{-5} ($\pm$ 1.9×10^{-5})
A. eutrophus	9.5×10^{-4} ($\pm$ 1.5×10^{-4})	1.9×10^{-3} ($\pm$ 0.45×10^{-3})

A similar tritium activity was present in the saline and distilled water control dishes. ANOVA indicated that there were no significant differences between the various tissue dishes and the saline control dish in each experiment ($P = 0.11$, $F = 1.86$, df = 6 at 1 MPa, and $P = 0.29$, $F = 1.26$, df = 6 at 5 MPa). There was over 2-fold variability in the tritium background between experiments, as determined by the activity found in the saline dish. The activity in the mammalian tissue experiments was normalized for the variation in background by subtracting the activity found in the saline dish in each experiment from the activity in each of the tissue dishes from that experiment. ANOVA of the normalized data indicated that there were no significant differences between the different tissues ($P = 0.13$, $F = 1.58$, df = 14 at 1 MPa, and $P = 0.24$, $F = 1.32$, df = 14 at 5 MPa), or between any of the tissues and the distilled water analyzed as its own experiment ($P = 0.18$, $F = 1.43$, df = 15 at 1 MPa, and $P = 0.14$, $F = 1.51$, df = 15 at 5 MPa). Thus we detected no hydrogen oxidation in the mammalian tissues, with a sensitivity limit of 100 nmol $H_2 \cdot g^{-1} \cdot min^{-1}$ (mean value at 5 MPa H_2 + 1 SD; Table 1).

In contrast, the Pd beads and the suspensions of *A. eutrophus* incorporated several orders of magnitude more tritium activity than the mammalian tissues at both 1 and

5 MPa (Figs. 4 and 5). This activity was in the range of 10 $\mu Ci \cdot ml^{-1}$ for both positive controls at 1 and 5 MPa H_2. Corresponding values in mass-specific units were 100 nmol $T_2 \cdot g^{-1}$ bacteria and 1 nmol $\cdot T_2 \cdot g^{-1}$ Pd. At the dilutions of T_2 in H_2 we used, the oxidation of H_2 was computed to be 1–2 mmol $H_2 \cdot g^{-1}$ bacteria $\cdot min^{-1}$ and 10–30 $\mu mol\ H_2 \cdot g^{-1}$ Pd $\cdot min^{-1}$ for the 1-h period at constant pressure plus decompression time (Table 1).

DISCUSSION

None of the tissues of guinea pigs, rats, and pigs studied, at either 1 or 5 MPa H_2, incorporated any more T_2 than the trace quantities that were also incorporated in the various water control dishes. Thus we found no detectable capacity to oxidize H_2 in a variety of mammalian tissues exposed to high concentrations and high pressures of H_2.

Much greater tritium activity was found in our positive controls of Pd immersed in water and suspensions of *A. eutrophus*. These positive controls indicated that the apparatus and experimental protocol were adequate to demonstrate H_2 oxidation. Our estimated rates for H_2 oxidation by the bacterial suspensions (Table 1) are very

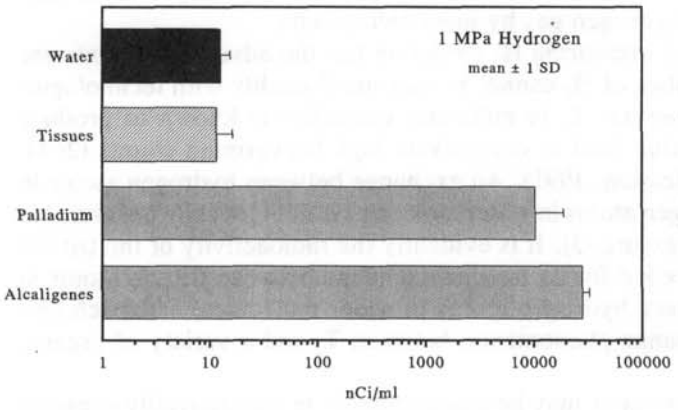

FIG. 4—Total tritium activity in all mammalian tissues studied (mean of 15 tissues ± 1 SD), in sterile, distilled water controls (mean of seven samples ± 1 SD), and in two positive controls of Pd beads immersed in water (mean of six samples ± 1 SD) and cultures of the hydrogen-metabolizing bacterium *A. eutrophus* (mean of six samples ± 1 SD), after 1-h exposure to 8 mCi T_2 and 1 MPa H_2.

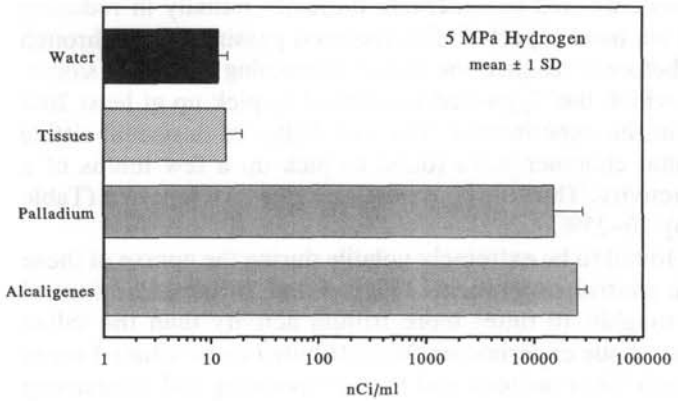

FIG. 5—Total tritium activity in all mammalian tissues studied (mean of 15 tissues ± 1 SD), in sterile, distilled water controls (mean of seven samples ± 1 SD), and in two positive controls of Pd beads immersed in water (mean of six samples ± 1 SD) and cultures of the hydrogen-metabolizing bacterium *A. eutrophus* (mean of six samples ± 1 SD), after 1-h exposure to 8 mCi T_2 and 5 MPa H_2.

close to the rate of 1.6 mmol $H_2 \cdot g^{-1} \cdot min^{-1}$ calculated by spectrophotometry in suspensions of *A. eutrophus* bubbled with H_2 under 1 atm conditions (unpublished data).

The 8 mCi of T_2 to which we exposed all the tissues and controls had a solubility in water of 11.3 pmol $\cdot$ ml^{-1} (685 nCi $\cdot$ ml^{-1}). The high activity of tritium in the positive controls demonstrated that T_2 was diffusing into these samples and being oxidized. In the case of Pd, T_2 was oxidized to water; in the bacterial suspensions, the tritium may have become incorporated into water or some organic molecule. The low activity of tritium that remained behind in the mammalian tissues represented less than the quantity of T_2 gas that was in physical solution while the chamber was compressed. Background activity for tritium in this scintillation counter and with this cocktail was determined to be approximately 50 disintegrations per minute, or 0.23 pCi per vial of scintillation cocktail. Thus the quantity of tritium label found in the mammalian tissue samples and water controls was very low relative to the quantity of T_2 used, but higher than the background in the absence of exposure to T_2.

Smith et al. (2) exposed tissues of rats to T_2 under low concentration and 1 atm conditions. They found a tritium incorporation of the same order of magnitude as that found in the mammalian tissues in this study, after correcting for differences in the amount of T_2 used. Smith et al. (2) attributed this trace incorporation to the formation of tritiated water by what they termed "physical exchange reactions," and not to an oxidation of hydrogen gas by mammalian cells.

The use of T_2 as a tool for measuring H_2 oxidation has the advantage of extreme sensitivity; picomole quantities of H_2 cannot be measured readily with technologies such as H_2 electrodes. However, T_2 in millicurie quantities is known to produce radioactive isotope effects that lead to deceptively high background counts (2; G. Östlund, personal communication, 1991). An exchange between hydrogen atoms in H_2 gas molecules and hydrogen atoms in water molecules is energetically unfavorable at room temperature and pressure (3). It is evidently the radioactivity of the tritium that provides the energy needed for an isotope exchange between tritium atoms in T_2 gas molecules and ordinary hydrogen atoms in water molecules. Wilzbach (20) described this isotope exchange phenomenon between T_2 and a variety of organic molecules.

In addition, tritiated water vapor may be a contaminant in commercially supplied T_2, despite manufacturers' analyses to the contrary (21; P. Lamburger, personal communication, 1992). Indeed, we had considerable difficulty initially in reducing the background to the level we have reported; this required passing the T_2 through or over a desiccant 3 times before it reached the dishes containing tissues or saline. The desiccant trap through which the T_2 passed was found to pick up at least 20% of the tritium activity used in the experiments. The two dishes of desiccant sitting in the top of the experimental chamber were found to pick up a few tenths of a percent more of the tritium activity. Thus the H_2 oxidation values we reported (Table 1) may be underestimated by 20–25%.

Tritiated water vapor was found to be extremely volatile during the course of these experiments. In the positive control experiments (Figs. 4 and 5) the saline control dish was found to contain roughly 10 times more tritium activity than the saline control dish in the mammalian tissue experiments. We attribute this to tritiated water being formed in the dishes with Pd or bacteria and then evaporating and condensing

into the saline dish. When such an experiment was left sealed for several days, all the dishes came into equilibrium with each other in their tritium activity.

There are several limitations to the technique we have used. The first limitation is that we had to choose whether to use the same radiation dose in the experiments at 1 and 5 MPa, or whether to use the same relative concentration of T_2 in H_2, i.e., use 5 times more T_2 in the 5 MPa experiments. We chose the former strategy, following the rationale that the undesirable but unavoidable radioisotope effects could be held constant only if the T_2 dose were constant. The actual dilution of T_2 in H_2 used in any experiment is largely a matter of convenience, and is easily dealt with mathematically when computing H_2 oxidation values from radioactivity (Table 1).

We had no method of stirring the gases in the chamber and cannot guarantee that the T_2 and H_2 were perfectly mixed throughout the chamber. However there was no effect of dish position on T_2 content in any of the experiments. This suggested that the T_2 was uniformly mixed throughout the chamber. If any stratification of gases were present, then averaging data from multiple dishes in the chamber should have eliminated bias. When two different tissues were tested together, their dishes were stacked in alternating positions, with the saline control dish in the center of the stack, to minimize this potential problem.

Another limitation is that safe operation of our apparatus required us to add the T_2 to the experimental chamber first, followed by H_2 some minutes later. For the mammalian tissues, which are known not to metabolize H_2 under 1 atm conditions, this should have posed no problem. However the Pd and bacterial suspensions may have begun to oxidize the T_2 when it was the only form of hydrogen gas present, assuming that there was no threshold of hydrogen partial pressure needed to initiate oxidation. During the compression phase, the T_2 and H_2 were not in their final relative concentrations. Any oxidation of T_2 during that time would not accurately reflect the rate of H_2 oxidation if their relative concentrations were continuously changing. Thus the computations of H_2 oxidation for these positive controls (Table 1) may be overestimates by an unknown magnitude. Our intention with these positive controls was not to quantify their activity with accuracy, but to demonstrate that hydrogen oxidation could be detected at least qualitatively with our apparatus.

It was also problematic for us to decide how to deal with the unequal decompression times of 0.5 h from 1 MPa and 2 h from 5 MPa. During decompression the tissues and controls were exposed to the same relative dilutions of T_2 in H_2 as they had at constant pressure, but they were also exposed to continuously decreasing partial pressures of both T_2 and H_2. For the positive controls in which hydrogen oxidation was occurring, we would not be able to distinguish pressure effects on oxidation rate from simple exposure time at any pressure, without further experiments. Our values for H_2 oxidation (Table 1) may thus be in error by up to 3-fold in the 5 MPa experiments if we were incorrect to have added the 2-h decompression times to the rate computations. Since the mammalian tissues never differed in their T_2 content from their water controls at either pressure, this problem does not affect our conclusions regarding the absence of H_2 oxidation in the mammalian tissues, with a limit of detection still in the range of nanomoles of $H_2 \cdot g^{-1} \cdot min^{-1}$.

We conclude that when a variety of tissues of guinea pigs, rats, and pigs were exposed to T_2 at high concentrations and pressures of H_2, the slight amount of tritium incorporated in the tissues was no greater than the amount incorporated in sterile

distilled water or saline under the same conditions. This tritium incorporation is attributable to radioisotope phenomena which set the limit of sensitivity for measuring H_2 oxidation of 100 nmol $H_2 \cdot g^{-1} \cdot min^{-1}$. We therefore did not detect a latent capacity of mammalian tissues to oxidize H_2 under hyperbaric conditions.

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