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Preparation of Millicurie Quantities of Oxygen-15 Labeled Water *

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SUMMARY

Due to the annihilation radiation of the positron emitting oxygen-15, $H_2{}^{15}O$ has great potential for tracer studies. The method of preparation utilized the fast exchange reaction between carbon dioxide and carbonic acid. It was found that the rate of exchange depended on the temperature, the solution used and the dynamic state of the solution. Utilizing this exchange reaction, water with a specific activity of 80 mCi/cc was prepared.

OXYGEN-15-LABELED WATER.

Water labeled with deuterium or tritium has frequently been used in bio-medical studies for the measurement of body water pools [1] and water diffusion [2]. The use of the deuterium labeled compound necessitates the mass-spectrometric analysis of the samples while the use of tritiated water is limited by quantity of the long-lived isotope that can be injected into the subject. Another disadvantage of both of these labels is that they cannot be detected *in vivo,* only tracers containing a γ-emitting isotope can be detected from outside the subject. The only potential γ-emitting label is the two minute half-life oxygen-15, which decays by positron emission so the 511 keV annihilation radiation can be detected externally. West and Dollery [3] have prepared small quantities of $H_2{}^{15}O$ by passing ^{15}OO and hydrogen over a palladium catalyst. Preliminary experiments on this method gave low yields due to the problem of trapping and transferring the labeled water in sterile pyrogen-free condition suitable for human use.

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METHOD.

The method used for the preparation of labeled water utilized the fast exchange between carbon dioxide and carbonic acid :

$$H_2O + CO_2 \rightleftharpoons H_2CO_3$$

At neutral pH, approximately 70 % of carbon dioxide is in the chemical forms dissolved carbon dioxide and carbonic acid [4], the remainder being present as bicarbonate ions.

The reactions :

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$
$$H_2CO_3 \rightarrow H_2O + CO_2$$

are very fast so that the rate of exchange of oxygen-15 between a trace of carbon dioxide and an excess of water will depend on the rate of the reaction :

$$H_2O + CO_2 \xrightarrow{k} H_2CO_3$$

There is a probability of 0.33 that the oxygen-15 will be transferred with each cycle —

$$H_2O + CO_2 \rightarrow H_2CO_3 \rightarrow H_2O + CO_2$$

so the rate of exchange of —

$$H_2O + C^{15}OO \xrightarrow{k_1} CO_2 + H_2{}^{15}O$$

should be 0.33 k.

Published values [4] of k indicate that at 38° C more than 95 % of the label initially present as $CO^{15}O$ should be $H_2{}^{15}O$ in less than two minutes.

To check the actual rate of exchange, $C^{15}OO$ was made by the following method :

Oxygen-15 labeled oxygen was produced by irradiating commercially obtained nitrogen gas with the 7 MeV deuteron beam of the Washington University Medical School Cyclotron. Oxygen-15 produced by the reaction $^{15}N(d, n)^{15}O$ reacts preferentially with the trace of oxygen present in the nitrogen [5] and the $O^{15}O$ formed is then converted into labeled carbon dioxide by passage over activated charcoal heated to 400° C.

The rate of exchange was determined by quickly passing the carbon dioxide contained in 50 cc of carrier through water and withdrawing liquid samples at various times. Each sample was added to barium hydroxide solution which precipitated the carbon dioxide and carbonate as barium carbonate. After filtering the barium carbonate and counting both the solution and the precipitate in a well scintillation counter, an assessment of the activity was made.

The decrease in $C^{15}OO$ activity versus time is shown in Figure 1. It is seen that at 38° C, in less than 3 minutes, exchange is virtually complete, while at room temperature the time is over 6 minutes.

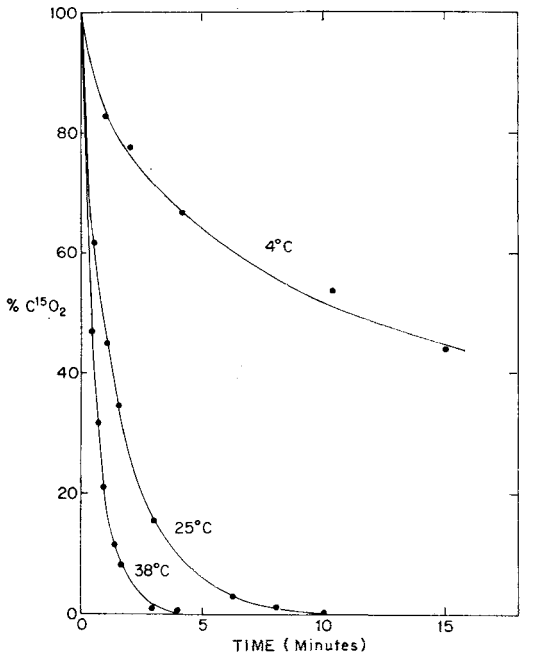

FIG. 1. Plot of the decrease in activity present as $C^{15}OO$ when labeled carbon dioxide is dissolved in distilled water.

If the carbon dioxide were dissolved in blood rather than water, and the barium hydroxide added to separated plasma, over 99.99 % of the activity was present as $H_2{}^{15}O$ in 30 seconds. The same result was obtained if whole blood was used, but in this case the minimum time studied was two minutes since it was necessary to add digitonin to hemolyze the red blood cells before filtration. It can therefore be seen that the exchange in blood is much faster than in aqueous solution due to the enzyme action of carbonic anhydrase —

$$H_2O + CO_2 \underset{\text{anhydrase}}{\overset{\text{carbonic}}{\rightleftharpoons}} H_2CO_3$$

From these preliminary studies it appears that $H_2{}^{15}O$ can be produced by dissolving $C^{15}OO$ in either blood or water. If blood is used the reaction should be very fast, while if water is used it appears necessary to wait a few minutes for the exchange to proceed to completion.

The apparatus used for labeling is shown in Figure 2, the target chamber has $\simeq$ 400 cc volume and the gas is circulated using a varistaltic pump (Manostat obtained from Fisher Scientific Company, Cat. # 13-875-100U2).

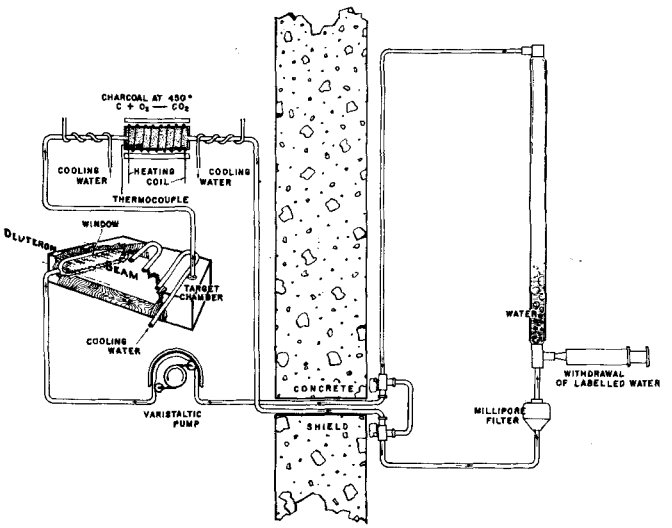

FIG. 2. Apparatus used for producing $H_2{}^{15}O$.

A deuteron beam of 20 microamperes was used for an irradiation time of 6 minutes, the gas bubbling through 5 cc of solution for the whole time. The activity induced in various solutions is given in Table 1.

TABLE 1. Activity in Various Solutions

Solution	Blood	Distilled water	Physiolgical saline
(Activity mCi/cc)	25	80	80
% $H_2{}^{15}O$ 30 sec after irradiation	99.99	99.99	99.99

When water or saline containing the activity induced by a "bubbler system" was added to barium hydroxide solution, over 99.9 % of the activity appeared as water after only 30 seconds. This is a much faster exchange than was observed in the trial experiments.

DISCUSSION.

It is observed from this work that large amounts of $H_2{}^{15}O$ can be induced into solutions that can be used on human subjects. The water can be prepared with a minimum of technical manipulations, therefore enabling large quantities to be prepared safely. By using large quantities, tracer studies remain possible up to 30 minutes post administration.

Two other features that need mention are :

Firstly, the exchange in water during the labeling process is much faster than anticipated.

The preliminary experiments were all performed in a condition where equilibrium was reached quickly, the $CO^{15}O$ being dissolved in less than five seconds. In the actual labeling process the gas was continuously bubbled through the solution, therefore the equilibrium condition was never reached. It has been suggested that the rate at which carbon dioxide dissolves in and is evolved from water depends on the dynamic state of the water [6]. It appears that when the water is continuously agitated the exchange is much faster than under equilibrium conditions.

Secondly, it is also seen that the labeling in blood is much less efficient than in water. A reasonable explanation is that blood normally contains a large carbonate pool. When the nitrogen containing only a trace of dioxide is passed through the blood, the partial pressure of carbon dioxide in the gas is much less than in the air. The equilibrium in blood —

$$CO_2 \text{ (gaseous)} \rightleftharpoons CO_2 \text{ (dissolved)} + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3$$

is therefore moved to the left and carbon dioxide is given off from the blood. This results in less chance of the labeled dioxide dissolving in blood and hence less activity in the final observation.

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REFERENCES

1. NOVAK, L. P. — *In* : Compartments. Pools and Spaces in Medical Physiology. A. E. C. Publication, Conf. 661010, p. 197 (1967).
2. JOHNSON, J. A., CALVERT, H. M. and LIFSON, N. — *Amer. J. Physiol.*, **171** : 687 (1952).
3. WEST, J. B. and DOLLERY, C. T. — *Nature*, **189** : 588 (1961).
4. KERN, D. M. — *J. Chem. Ed.*, **37** : 14 (1960).
5. WELCH, M. J. and TER-POGOSSIAN, M. M. — *Radiation Research*, **36** : 580 (1968).
6. WOLKOWSKI, Boleslaw. — *Przen, Ferment, Rolny*, **10** : 420 (1966).