STUDIES IN GLYCINE-2-C^{14} METABOLISM IN MAN. I. THE PULMONARY EXCRETION OF $C^{14}O_2$[1]

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INTRODUCTION

There has been considerable discussion regarding the use of C^{14} in clinical investigation, particularly from the standpoint of retention in the body of a long lived (5600 years) radioactive isotope. There is evidence from animal investigations that following the intravenous injection of simple C^{14} labeled compounds occurring in normal biochemical processes most of the radioactivity will be excreted as $C^{14}O_2$ within a few days (1–5). The rate of excretion in mice via the lungs and the rate of loss of C^{14} from the soft tissues and bone when this isotope is administered as glycine-2-C^{14} has been determined; most of the radioactivity was eliminated in the breath in the first two days and the slowest tissue component was found to have a half time of approximately 10 days (6). There is no method available for extrapolation of these animal data to man. The present study was designed to measure the rate of pulmonary excretion of C^{14} as $C^{14}O_2$ when administered as glycine-2-C^{14}.

METHODS

Methyl labeled glycine was prepared by chlorination and amination of acetic acid-2-C^{14} and had a specific activity of 12.2 microcuries/mg. of glycine (7). A series of four patients was given approximately 100 microcuries of glycine-2-C^{14} (8 mg.) intravenously in 10 ml. distilled water for the purpose of the determination of the red blood cell life (8), since previous investigations with radio-iron had indicated that many patients with polycythemia and chronic leukemia might have red blood cells with a shorter than normal life span (9). Frequent breath samples were taken by a device (see Figure 1) designed to collect the expired air in a rubber balloon for a measured period of time. The balloon was transferred to a bubbler system (see Figure 2) and the expired air drawn through a 1N sodium hydroxide solution. The carbon dioxide thus absorbed was precipitated as $BaCO_3$ by NH_4NO_3 and $BaCl_2$. The barium carbonate was filtered, dried at 110° C and weighed to determine the amount of CO_2 excreted per minute. The samples were counted with a proportional counter[2] where the specific activity was greater than 10 dis./min./mg. $BaCO_3$; those with a lower activity were measured in an ionization chamber. These instruments were calibrated with the current Oak Ridge C^{14} standard millicurie by specially prepared samples.

Inasmuch as the rate of pulmonary excretion of C^{14} when administered in this form was not known at the start of these studies, the patients were placed in an oxygen tent although no oxygen was supplied, so that the expired air would pass through the soda-lime filter and thus absorb the carbon dioxide. In addition a vacuum pump continually exhausted the air from inside the tent to the outside of the building. These precautions were maintained for approximately 36 hours.

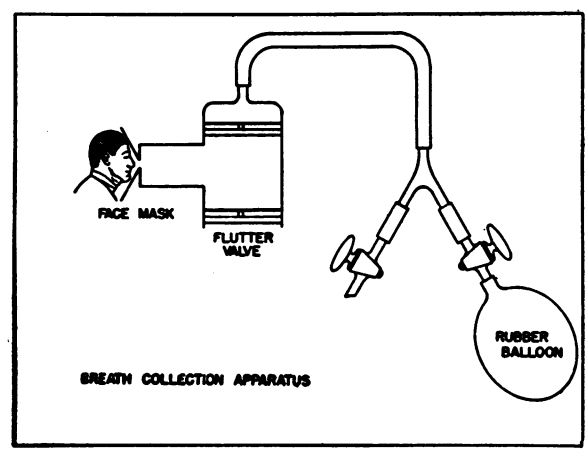

FIG. 1.
BREATH COLLECTION APPARATUS
(Note: not drawn to scale.)

RESULTS

Figure 3 shows the specific activity as a function of time of the expired carbon dioxide as $BaCO_3$. The maximum specific activity of the expired breath occurred within the first hour following the injection. A measurable amount of radio-

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[2] Nucleometer—Manufactured by Radiation Counter Laboratory, Chicago, Illinois.

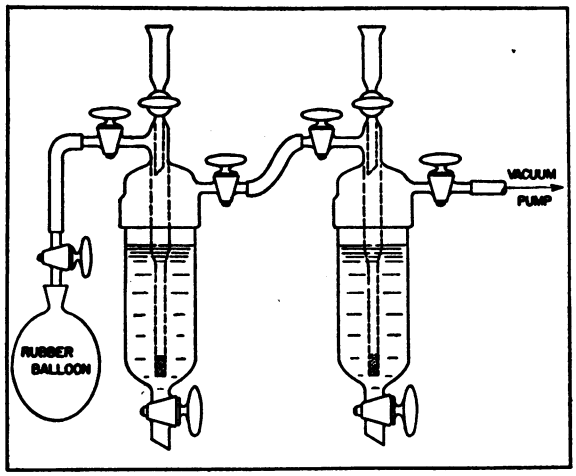

FIG. 2.
APPARATUS FOR CARBON DIOXIDE ABSORPTION

activity was present within the first 10 minutes following the injection. Figure 4 shows the calculated cumulative C^{14} excretion as determined from the amount of CO_2 excreted per minute and the rate constants obtained from Figure 1.

The rate of excretion of C^{14} when administered in this manner may be described in terms of three rate processes: the first having a half time of two

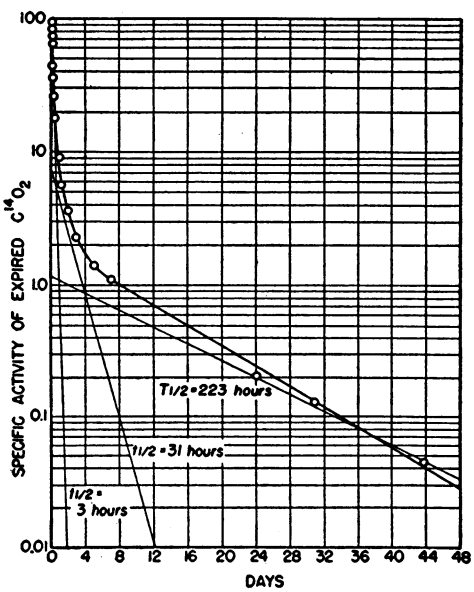

FIG. 3.
THE SPECIFIC ACTIVITY OF THE EXPIRED BREATH AS A FUNCTION OF TIME, IN PATIENT 1, IN DISINTEGRATIONS/MIN./MG. OF $BaCO_3$

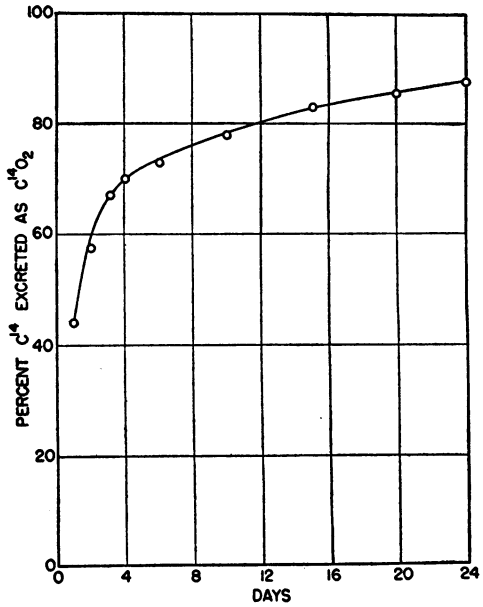

FIG. 4.
THE CUMULATIVE C^{14} EXCRETION AS $C^{14}O_2$ IN PATIENT 1
The cumulative excretion after the 24th day is not shown, but by the 45th day it will amount to approximately 92 per cent of the injected dose.

to four hours; the second, 16 to 40 hours; and the third, seven to 14 days. Table I shows the half times and the percentages of C^{14} excreted through each of the three rate processes for the four patients.

DISCUSSION

The cumulative excretion of C^{14} is so rapid that in Patient 1 at the end of one day approximately 40 per cent has been eliminated by this route, and by the end of five days 60 per cent; by 20 days, approximately 83 per cent (see Figure 4). Furthermore, in all four patients by the end of 40 to 75 days there was no significant measurable activity in the breath; *i.e.*, less than 0.04 dis./

TABLE I

The per cent of the administered dose excreted and half time for each of the three rate processes

Patient	T_1 (1/2)	Per cent	T_2 (1/2)	Per cent	T_3 (1/2)	Per cent	Total per cent excreted
	hours		*hours*		*hours*		
1	3	36	31.2	27	223	29	92
2	2.7	32	40	13	163	38	83
3	2.5	13	36	21	336	52	86
4	3.3	22	16	17	240	49	88

min./mg. $BaCO_3$. It is probable that there is a slower component, the radioactivity of which could be detected by more sensitive methods (10, 11); however, this could represent only a small fraction of the administered dose.

The relation of these three different rate processes to known biochemical processes is not known. However, the measurement of the uptake of C^{14} in the plasma proteins and their turnover time suggests that the slowest component may represent the rate constant for protein synthesis and turnover (12).

The finding that the slowest measured rate of excretion is of the same order of magnitude in man as in mice should permit the extrapolation to man of animal investigations with similar C^{14} labeled compounds. This may allow the use of such animal data as the basis for the determination of the hazard of using C^{14} in clinical investigation. In addition to the 83 to 92 per cent excreted via the lungs, some 5 per cent of the injected dose is excreted in the urine during the first 14 days, and a negligible amount in the feces (12). An additional 2 per cent is present in the red blood cells as hemoglobin and will not be eliminated until the red cells are destroyed at the end of approximately 120 days in normals (8).

Analysis of C^{14} content of the tissues obtained at autopsy from Patient 3 shows that approximately 10 per cent of the injected activity was still present in the tissues 106 days after administration of the labeled glycine. If this remaining activity is eliminated from the body by the lungs by a single first order rate process, the elimination half time for this component can be estimated by use of the equation $N = 1.44\ N_0\ T\frac{1}{2}$ where N (2.2×10^7 dis./min.) is equal to the total C^{14} to be excreted by this rate process and N_0 is the zero time extrapolated value of the component. N_0 can be estimated to be no greater than 120 dis./min. (0.05 dis./min./mg. $BaCO_3$) (2.4×10^3 mg. $BaCO_3$/min. breathing) since the effect of this component cannot be seen in the graph in Figure 3, which is typical of the four patients.

As calculated in this manner, the half time is approximately 90 days. This represents the minimum value that this component could take since what is probably the maximum value for the zero time extrapolated value of this component has been chosen.

Sprinson and Rittenberg (13) have calculated that there is a protein turnover component in man with a half time of 158 days. The data presented here suggest that there is a C^{14} turnover component associated with labeled glycine administration of at least 90 days, which is in fair coincidence with that of Sprinson and Rittenberg, considering the assumptions made.

Since approximately 90 per cent of the administered C^{14} is rapidly eliminated by processes which can at the present time be measured directly, the radiation dose from the small fraction which is retained for a longer period is negligible at the present administered dosage level of 100 microcuries, and thus there is no hazard involved in this use of C^{14}. Finally, it should be pointed out that even if there were no elimination of the administered C^{14}, 100 microcuries can still be considered a safe tracer dose since it will deliver on the average less than 0.006 r per day of radiation to the tissues.

SUMMARY AND CONCLUSIONS

1. The rate of excretion of C^{14} via the lungs when administered as Glycine-2-C^{14} in three patients has been presented.

2. This pulmonary excretion has been described in terms of three rate processes, the slowest of which has a half time of seven to 14 days.

3. In 55 days approximately 83 to 92 per cent of the C^{14} is excreted via the lungs, 5 per cent via the urine, and 2 per cent is retained in the red blood cells during their life span.

4. Autopsy data in Patient 3 suggests that there is at least a fourth component of breath excretion with a half time of 90 days or greater.

5. The lack of hazard involved in the use of C^{14} in the manner described is discussed from the standpoints of rapid excretion of 90 per cent of the dose and retention of approximately 10 per cent in chemical compounds having a longer turnover time.

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ADDENDUM

Patient 1 (*W. F.*) is a 29 year old white male who has had chronic lymphatic leukemia of three years duration

and who at the time of these studies was in almost complete clinical remission.

Patient 2 (*H. M.*) is a 52 year old white male with polycythemia vera of 12 years duration. At the time of these studies he was in hematological remission, but shortly thereafter began to relapse hematologically.

Patient 3 (*G. J.*) was a 52 year old white female with chronic myelogenous leukemia of one year's duration. At the time of these studies the white blood count was in the 300,000 range, the platelet count was 600,000 and the red blood count 3.0 and the hemoglobin 6.9 grams. This patient pursued, despite treatment, a down hill course and expired in August 1950.

Patient 4 (*M. D.*) is a 50 year old white female with polycythemia vera of 14 years duration. At the time of these studies the red blood count was 6.5, hemoglobin 15 grams, white blood count 6,500, platelets 280,000. The patient has maintained this hematological picture until the present time.

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