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Received October 8, 1965. P.S.E.B.M., 1966, v123

Oxidation of Infused Acetate to Body Pool CO_2. (31390)

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Experiments with constant infusion of C^{14}-sodium bicarbonate into the jugular vein of sheep indicated turnover time for body pool bicarbonate of about 30 minutes(4). The turnover time of infused C^{14}-acetate to expired CO_2 has been shown to be 60 minutes for 1-C^{14} and 120 minutes for 2-C^{14} labeled acetate(9). This indicates a turnover time of approximately 30 minutes from pool acetate to pool bicarbonate for 1-C^{14} labeled acetate.

The present experiments were planned to study the turnover time involved in the oxidation of pool acetate to pool CO_2 and its turnover to expired CO_2. By determining the turnover time from the acetate pool to the bicarbonate pool, a better indication of the actual oxidation rate of acetate is obtained, since the bicarbonate pool turnover time is eliminated. In addition, the distress to the animal involved in the collection of CO_2, via a tracheal cannula, is obviated. 1-C^{14} and 2-C^{14} labeled acetate were used in these experiments.

Methods. Four yearling wethers were used for 4 infusions of sodium acetate. 1-C^{14} or 2-C^{14} sodium acetate dissolved in physiological saline was infused by means of a constant infusion pump through a polyethylene catheter (I.D. 0.062″-O.D. 0.082″) inserted into right jugular vein. Blood samples were withdrawn through a catheter inserted in the left jugular vein.

The amount of CO_2 released by acid in whole blood was determined as previously reported(4). For radioactivity assay, the CO_2 released by acid was trapped in 2N sodium hydroxide and an aliquot removed and counted in the scintillation solution described by Harlane(3).

Expired CO_2 was collected from a tracheal cannula by the method of Sabine and Johnson (9) and its specific activity determined by the method of Oppermann *et al*(7).

Blood acetate was measured by the method of Ramsey(8) modified slightly as follows: to one volume of heparinized blood were added 2 volumes of water, one volume of 10% sodium tungstate and one volume of 0.66N sulphuric acid. The mixture was centrifuged to remove the protein, the supernatant made alkaline with potassium hydroxide and evaporated to dryness at about 60°C under reduced pressure. The residue was dissolved in one to two ml of water, made acidic with sulphuric acid, mixed with silicic acid and transferred quantitatively to the top of a silicic acid column, previously prepared. The column was eluted with benzene, chloroform and 1% tert-butanol in chloroform. Ten ml fractions were collected in scintillation vials, titrated with 0.01 N alcoholic potassium hydroxide in a stream of nitrogen and dried at 60-70°. The residue was dissolved in 1 ml of absolute ethanol, 14 ml of scintillation fluid (5g of 2.5 diphenyloxazole (PPO) per liter of toluene) were added and the solution counted in a Tricarb liquid scintillation counter.

The specific activity, turnover time, turn-

TABLE I. Acetate in Blood During Infusion of 1- or 2-C^{14} Acetate.

Time of infusion, min	Exp 1* CH_3COOH, meq/l	Exp 1* Sp. ac., dpm/meq 10^6	Exp 2* CH_3COOH, meq/l	Exp 2* Sp. ac., dpm/meq 10^6	Exp 4† CH_3COOH, meq/l	Exp 4† Sp. ac., dpm/meq 10^6
0	.68	—	1.46	—	1.58	—
15	.58	2.50	1.61	1.80	1.80	.94
30	.59	.94	1.34	1.87	1.80	1.51
45	.70	.84	1.44	2.01	1.55	1.30
60	.82	.63	1.52	1.53	1.50	1.15
90	.93	.59	1.44	2.10	1.57	1.40
120	.68	1.56	1.42	2.40	1.63	.89
150	.68	2.11	1.21	2.12	1.32	1.38
180	.59	1.79	1.17	1.40	1.35	1.84
210	.77	.82	1.16	2.20	1.22	1.57
240	.59	2.07	1.12	2.30	1.74	1.48
270	.58	2.53	1.05	3.24	1.50	1.42
300	.55	2.60	.84	2.10	1.30	1.30
330	.70	1.52	1.07	2.50	1.41	1.23
360	.66	1.65	.99	2.40	—	—
390	.60	1.65	.82	2.70	—	—
420	.61	.92	.94	2.20	—	—
450	.62	1.24	.92	2.20	—	—
480	.52	2.40	—	—	—	—

Infusion rate: Exp 1, 5.07×10^6 dpm/min. Exp 2, 5.55×10^6 dpm/min. Exp 4, 5.75×10^6 dpm/min.

* 1-C^{14} acetate.
† 2-C^{14} " .

over rate, pool size and pool space of acetate were calculated as described by Sabine and Johnson(9).

Results. The blood acetate infusion and analysis data for 3 infusions are given in Table I and II. In Fig. 1 are given the rates of fall of specific activity of blood acetate after stopping infusion. These data show an average half life of body pool acetate of 1.2 minutes, corresponding to a turnover time of 1.7 minutes and this turnover time is not affected by the site of labeling of the acetate.

The rates of fall of specific activity of blood CO_2 in the experiments conducted with acetate are given in Fig. 2. The data calculated from Fig. 2 are presented in Table III and show an average half life of 76.5 minutes for acetate labeled in the one position. This corresponds to a mean turnover time (acetate-carboxyl to CO_2) of 110 minutes. With acetate labeled in the 2 position, the corresponding values from Table III are 135 minutes for the half life and 195 minutes for the mean turnover time of acetate-methyl to CO_2.

The half life of one labeled acetate to expired CO_2 as determined from the rate of fall of specific activity after infusion, was 78 minutes which means a turnover time of 113 minutes (Fig. 4).

Discussion. Annison and Lindsay(1) by means of a single acetate injection experiment suggested a half life of pool acetate of about 3 to 4 minutes. Lee and Williams(6) obtained half lives of 1.6 and 3.8 minutes estimated from single injection and continuous infusion experiments. The results obtained during the present investigation showed a half life of pool acetate of 1.2 minutes which agrees with

TABLE II. Metabolism of Sodium Acetate (CH_3COONa) in Sheep.

Exp	Wt of sheep, kg	Infusion rate 10^6	Turnover rate, meq/min	Turnover time, min	Half life, min	Pool size
1*	23.6	5.07 dpm/min	3.23	1.83	1.27	5.8
2*	34.1	5.55 "	2.56	1.65	1.14	4.2
3*	32.0	5.70 "	—	—	—	—
4†	30.0	5.75 "	4.3	1.86	1.30	8.0

* 1-C^{14} acetate. † 2-C^{14} acetate.

the earlier reports of Essig *et al*(2), and Sabine and Johnson(9). The turnover time of pool acetate is not affected by the site of labeling.

Huber *et al*(4) reported that at least 3 to 4 hours are required for complete mixing of infused sodium bicarbonate. In the present investigation, 4 hours were allowed for equilibration of pool bicarbonate specific activity (Fig. 3).

By means of a single injection of 1-C^{14} acetate, Johnson *et al*(5) reported the half

TABLE III. Metabolism of Blood and Expired CO_2.

Exp	Wt of sheep, kg	Infusion rate 10^6	Blood CO_2 Turnover time, min	Blood CO_2 Half life, min	Expired CO_2 Turnover time, min	Expired CO_2 Half life, min
1*	23.6	5.07 dpm/min	121	84	121	84
2*	34.1	5.55 "	100	69	104	72
3*	32.0	5.70 "	100	69	—	—
4†	30.0	5.75 "	195	135	—	—

* 1-C^{14} acetate. † 2-C^{14} acetate.

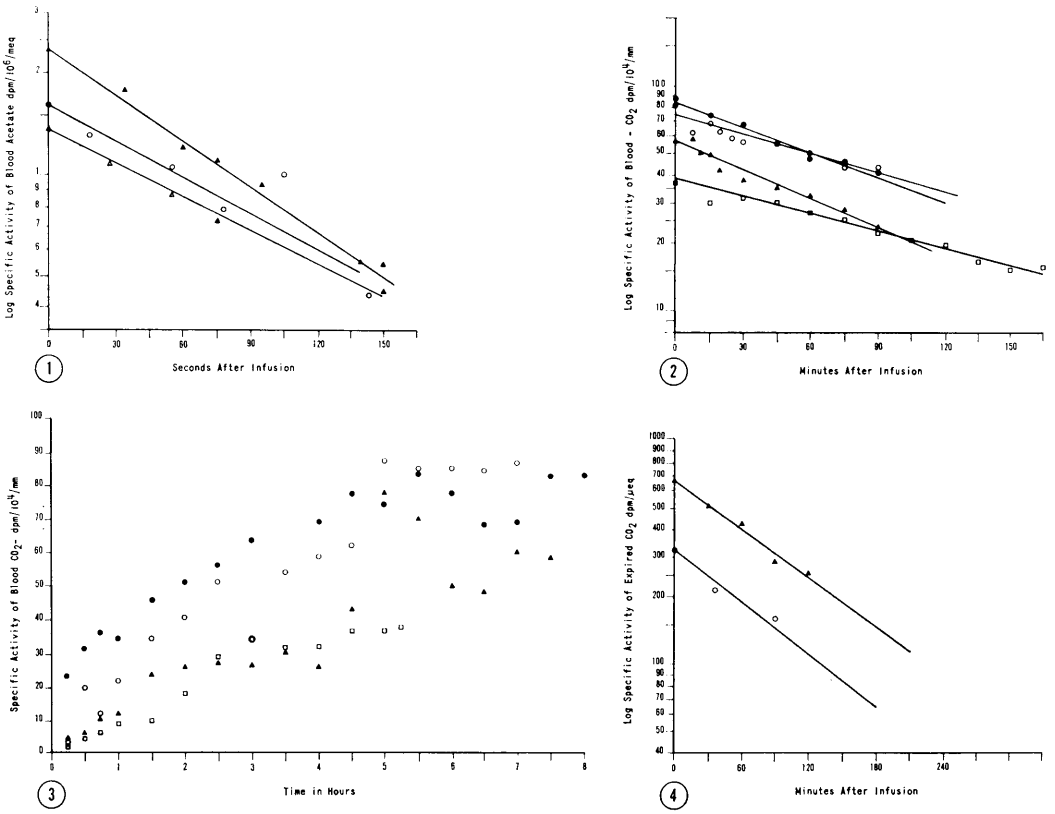

FIG. 1. Fall in specific activity of blood acetate after infusion of 1- or 2-C^{14} labeled acetate was stopped. Lines computed by method of least squares. Exp. 1 is represented by ○; Exp. 2 ▲; and Exp. 4 △.

FIG. 2. Fall of specific activities of blood CO_2 after infusion of C^{14} acetate was stopped. Lines computed by method of least squares. Exp. 1 ○; Exp. 2 ▲; Exp. 3 ●; Exp. 4 □.

FIG. 3. Specific activity of blood CO_2 during infusion of C^{14} acetate. Exp. 1 ○; Exp. 2 ▲; Exp. 3 ●; Exp. 4 □.

FIG. 4. Specific activity of expired $C^{14}O_2$ following infusion of 1- or 2-C^{14} labeled acetate. Lines computed by least square. Exp. 1 ○; Exp. 2 ▲.

time from pool acetate to expired CO_2 as 60 minutes which is slightly shorter than the half life from the carboxyl carbon of acetate to CO_2 obtained in this investigation of 76.5 minutes. This result following equilibration during an infusion period is probably a better figure than that obtained following a single injection. The longer half life (135 min) of conversion from the methyl carbon of acetate to pool CO_2 found when 2-C^{14} acetate was infused, emphasizes the difference in the pathway of oxidation of the methyl carbon and of the carboxyl carbon of acetate.

The biological half lives, as calculated from the fall of specific activity of body pool bicarbonate and from expired CO_2, are in excellent agreement. Thus, blood data may be used for studying the oxidative rates of acetate, eliminating the turnover time of the bicarbonate pool which otherwise masks the picture of oxidation in the animal body.

Summary. The rates of oxidation of acetate to CO_2 were estimated by using 1-C^{14} and 2-C^{14} labeled acetate and measuring both blood and expired $C^{14}O_2$. Following 1-C^{14} labeled acetate infusion, the half life of blood and expired $C^{14}O_2$ was about 76 minutes indicating a mean turnover time of acetate carboxyl to pool CO_2 of about 110 minutes. The half lives of 2-C^{14} labeled acetate were 135 minutes and 195 minutes respectively. The half life of the pool acetate was 1.2 minutes; turnover time 1.7 minutes; turnover rate 3.4 meq per hour and pool size 6.0 meq per sheep.

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Received November 1, 1965. P.S.E.B.M., 1966, v123.

Absorption and Excretion of Furosemide-S^{35} in Human Subjects. (31391)

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Furosemide (Lasix^R) is an anthranilic acid derivative that is at least as efficient as organomercurial agents in provoking water and sodium diuresis(1-3). It has the advantage over the commonly used mercurial diuretics that it is effective when given by mouth and that its action is independent of alterations in acid-base balance(4,5). The absorption, distribution and elimination of S^{35}-labeled furosemide has been studied in several species of animals(6). The present study was undertaken to investigate some of these parameters in human subjects.

Material. This investigation comprised 20 individual acute studies which were conducted during a 6-month period in a group of 16 subjects; these included 14 healthy human volunteers between 22 and 59 years of age and weighing between 54 and 99 kg and 2 patients over 55 years of age who were free of any discernible cardiorenal disease.

The initial specific activities of the 2 batches of furosemide-S^{35}* ($t_{1/2}$ = 87.1 days) were 8 and 40 mc/g, and the purity was ascertained

* Hoechst Pharmaceuticals, Inc., Cincinnati, Ohio.