on the operated side following ureteral transection and ligation and was more pronounced in the latter. Degree of hypertrophy of the control kidney showed a close correlation with degree and duration of the hydronephrosis of the kidney on the operated side. These observations have been interpreted as indicating the operation of a control system regulating kidney growth in response to changes in some way related to kidney size.

The authors would like to express their thanks for valuable technical assistance to Mr. R. Blabey, W. W. Flamembaum, N. Reicheck, and Mrs. M. L. Schreiber.

1. Smith, H. W., Oxford Univ. Press, New York, 1951.
2. Lowenstein, L. M., Stern, A., Science, 1963, v142, 1479.
3. Goldstein, D. J., Leech, 1960, v30, 115.
4. McKay, E. M., Cockrill, J. R., J. Nutrition, 1931, v4, 25.
5. Allen, R. B., Mann, F. C., Arch. Path., 1935, v19, 341.
6. Simpson, D. P., Am. J. Physiol., 1961, v201, 517.
7. Block, M. A., Wakim, K. G., Marin, F. C., Am. J. Physiol., 1953, v172, 60.
8. Snedecor, G. W., Statistical Methods, 5th Ed., Iowa State Univ., Ames, Iowa, 1958.
9. Deetjen, P., Kramer, K., Klin. Wochr., 1960, v38, 680.

Received July 2, 1965. P.S.E.B.M., 1965, v120.

Infusion of Sodium Bicarbonate-C^{14} in Sheep.* (30489)

T. L. HUBER, E. D. MAYFIELD, R. L. HUSTON AND B. CONNOR JOHNSON
Division of Nutritional Biochemistry, Department of Animal Science, University of Illinois, Urbana

The oxidative metabolism of various substrates in ruminants has been studied by single injection(1,2,3) and constant infusion techniques(4,5). These techniques usually require interpretation of expired carbon dioxide data to estimate the oxidative rate. Although the time rate of oxidation is not actually measured, it is assumed to be proportional to the rate of elimination of carbon dioxide in expired air. Existence of large carbon dioxide pools in the body with which metabolically produced carbon dioxide mixes to a variable extent before it is expired, could lead to erroneous oxidative rates. The advantages of continuous infusion over single injection methods has been discussed by Morris and Simpson-Morgan(6).

The purpose of this study was to study the carbon dioxide pool system of sheep using constant infusion of sodium bicarbonate-C^{14}.

Methods. Two yearling grade wethers were used for a total of 3 infusions, each 4 hours in duration. A recovery period of about 4 months was allowed between experiments when the same sheep was used for 2 infusions. Sodium bicarbonate-C^{14}, dissolved in physiological saline, was infused by means of an automatic infusion withdrawal pump through a polyethylene catheter (I.D. 0.062″, O.D. 0.082″) inserted into the right jugular vein. Blood samples were withdrawn through an indwelling catheter inserted into the left jugular vein. The catheters were threaded into the veins through an 11-gauge hypodermic needle the day before infusions were conducted.

The amount of acid-releasable CO_2 in whole heparinized blood was determined manometrically as described by Gupta *et al* (7). Hydrochloric acid with a normality of 0.5 was used to release CO_2 rather than 0.2% citric acid used by Gupta *et al.* For radioactivity assay, acid-releasable CO_2 was collected in 2 N sodium hydroxide and an aliquot removed and added to a scintillator solution described by Harlan(8), composed of Cab-O-Sil, ethanol, toluene, 2,5-diphenyloxazole (PPO), and 5-phenol-oxazole (POPOP), and counted in a Packard Tri-Carb Scintillation Spectrometer.

Rumen fluid was obtained through a ru-

* This work was supported in part by the National Science Foundation (NSF Grant GB-168) and by a Hatch grant.

men cannula. Total urine excretion was collected. Rumen fluid and urine were assayed for radioactivity by the procedure described by Bray(9).

To collect expired CO_2, a tracheal fistula was constructed by exposing the trachea through a 5-cm incision along the midline of the neck; 1.5 cm was removed from one tracheal ring, and a stainless steel tube (1.3 cm O.D.) was inserted. This tube was removed, cleaned and replaced daily. To collect expired gases, the metal tube was removed and a Magill catheter (No. 32) was inserted in its place. When the bulb was inflated, all respired gases—and only the respired gases—passed through the tube; contamination of expired air by rumen gases was thus prevented. A respiratory valve was connected and the expired gases were passed through a meter that simultaneously measured the volume and gave a continuous aliquot (0.2%) which was collected in football bladders. The specific activity of expired CO_2 was determined by the method of Opperman *et al*(10) which involves absorption of CO_2 in primene, titration of the primene-CO_2 complex and liquid scintillation counting.

Values obtained from blood data were calculated as follows:

Turnover rate (the amount of CO_2 disappearing from the CO_2 pool), which is equal to

$$\frac{\text{Infusion rate (d.p.m./min)}}{\text{Specific activity of blood } CO_2 \text{ (d.p.m./meq)}}$$

Turnover time (the time taken for the body to remove from the pool an amount of CO_2 equal to the total present), which is equal to

$$\frac{\text{Half-Life}}{\text{Ln } 2}$$

Half-Life of CO_2, which was calculated from the fall in specific activity of the blood CO_2 after infusion was stopped.

Pool size (the amount of CO_2 present in the body, exclusive of the rumen, at any given time), which is equal to

Turnover rate (meq/min) $\times$ turnover time (min)

CO_2 space (the volume of fluid in which the CO_2 pool is dissolved), which is equal to

$$\frac{\text{Pool size (meq)}}{\text{Concentration (meq/l)}}$$

Results. In all experiments the specific activity of blood CO_2 reached equilibrium during the final hour of infusion. Table I shows a typical record of level and specific activity of blood CO_2 taken from Experiment 3.

Equilibrium of blood CO_2 specific activity during the final hour of infusion would suggest that the amount of radiocarbon infused should equal the amount of radio carbon expired. Table II shows the number of d.p.m.'s infused and expired during the final hour of infusion.

The rate of fall of specific activity of blood CO_2 during the exponential part of the curve after infusion was stopped, is plotted in Fig. 1. For the 3 experiments conducted the average half-life for body pool CO_2 was 32.6 minutes. This corresponds to a mean turnover time of 46.8 minutes and mean turnover rate of 6.15 meq/min (Table III).

The recovery data in Table IV indicate that a large percentage of the infused radiocarbon passed into the rumen and a limited amount was excreted in the urine.

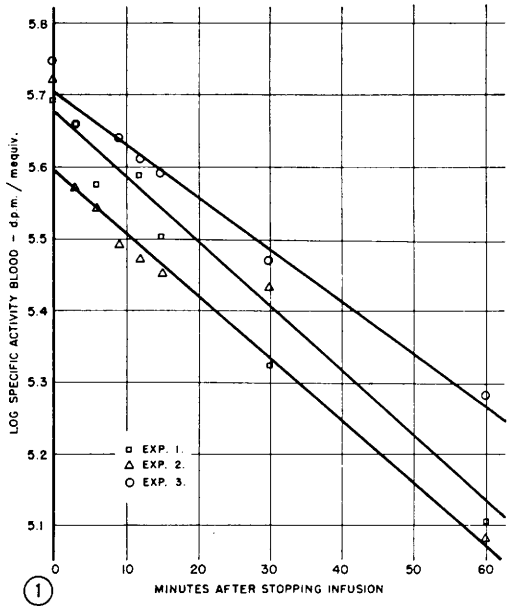
Fig. 2 shows the specific activity of expired CO_2 during the decay part of the curve. The mean half-life was 31 minutes which corresponds to the 32.6 minutes mean half-life for blood CO_2. Different levels of specific activ-

TABLE I. $C^{14}O_2$ in Blood During Infusion of Sodium Bicarbonate-C^{14}.

Time, min	CO_2, meq/l	Specific activity, 10^4 d.p.m./meq
0	14.0	—
30	15.0	23.6
60	14.4	32.8
90	17.6	37.0
120	17.6	41.3
150	17.8	50.1
180	16.9	55.6
210	16.8	57.9
240	17.0	56.0

TABLE II. d.p.m.'s Infused and Expired During Final Hour of Infusion.

Exp	Infused	Expired
	10^6	10^6
1	164.40	102.39
2	187.20	116.68
3	240.00	115.00



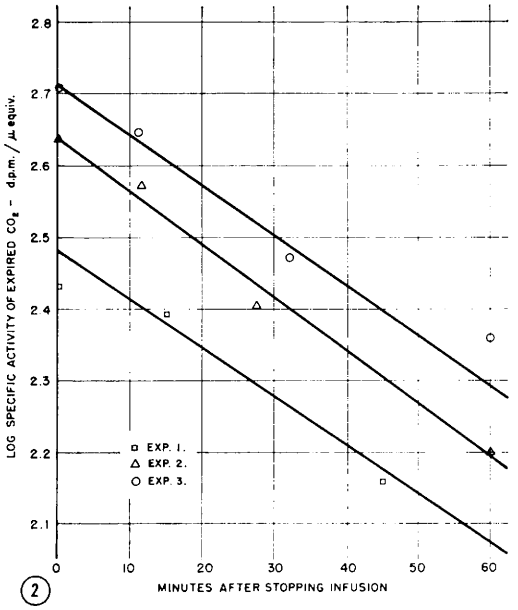


FIG. 1. Specific activity of blood CO_2 following infusion of sodium bicarbonate-C^{14}. Line computed by method of least squares.

FIG. 2. Specific activity of expired $^{14}CO_2$ following infusion of sodium bicarbonate-C^{14}. Line computed by means of least squares.

TABLE III. Sodium Bicarbonate Metabolism in Sheep.

Exp	Amt infused, 10^6	Turnover rate: meq/min	Turnover rate: meq/hr/kg body wt	Half-life, min	Turnover time, min	CO_2 pool, meq/animal	CO_2 space: Per animal	CO_2 space: % Body wt
1	3.12	6.58	9.40	27	39.1	257.3	15.9	37.8
2	2.74	4.98	9.50	34	47.8	238.0	10.9	34.4
3	4.00	6.89	9.84	37	53.6	369.4	21.9	52.1
Mean		6.15	9.58	32.6	46.8	288.2	16.2	41.4

ities between animals reflect different rates of infusion.

Discussion. Equilibration of blood CO_2 specific activity during the final hour of infusion indicates that at least 3 hours were required for a complete mixing of the infused bicarbonate with the CO_2 pools of the body. Although the blood CO_2 specific activity plateaued during the final hour of infusion, the difference between infused and expired carbon-14 during the final hour of infusion suggests variable rates of turnover for some CO_2 pools of the body. The rather large amount of carbon-14 found in the rumen at the end of the infusion period in Exp. 2, indicates that the rumen pool accounts for most of this difference. The epithelium of the reticulo-rumen sac is permeable to both HCO_3 and CO_2(11). However, once the CO_2 enters the rumen, it can enter into several reactions such as reduction to methane(12), and subsequent loss in eructated gases and several other biosynthesis pathways. Therefore, the turnover time for the rumen CO_2 pool may not be greatly different from other body pools, but products formed from CO_2 may turnover slower than CO_2 and consequently

TABLE IV. Percent Infused d.p.m.'s Recovered.

Exp	Time, hr	Expired	Rumen	Urine
1	4	43.28	—	—
	6	55.38	—	—
2	4	51.60	23.78	.75
	6	65.00	4.32	6.70
3	4	39.50	—	—
	6	52.30	—	—

partially account for the observed difference between infused and expired carbon-14.

About 33% of the radioactivity found in the urine was in the form of CO_2. However, identification of compounds containing the remaining radioactivity was not made. Rust *et al*(13) found radioactivity in urea of the urine of rats following injections of sodium bicarbonate-14.

The close agreement between the half-life of blood CO_2 and respiratory CO_2 calculated from the exponential part of the curve (Fig. 1 and 2) suggest that blood CO_2 data may be used in studying oxidative rates of substrates. This would eliminate the respiratory stress of the animal due to the apparatus needed to collect expired air.

The turnover rate was constant for the 3 experiments with a mean of 9.58 meq/hr/kg of body weight. The mean CO_2 space was 0.414 liter/kg body weight, which is about twice the extracellular volume. The amount of radioactivity found in the rumen would indicate that the rumen must be considered part of the CO_2 space.

Summary. The carbon dioxide pool system of sheep was evaluated by the constant infusion of sodium bicarbonate-C^{14} for a period of 4 hours. The specific activity of blood CO_2 plateaued during the final hour of infusion. Following infusion, the half-lives of blood and expired CO_2 were 32.6 and 31 minutes, respectively. Mean turnover rate, turnover time and CO_2 space were 9.58 meq/hr/kg body weight, 46.8 minutes and 41.4% of body weight, respectively. Considerable radioactivity was found in the rumen at the end of the infusion period. These observations suggest a time of at least 3 hours for complete labeling of the CO_2 pool, of which the rumen is a part, and that blood CO_2 data may be used as well as expired CO_2 data for substrate oxidation studies.

1. Annison, E. F., Lindsay, D. B., Biochem. J., 1961, v78, 777.

2. Black, A. L., Kleiber, M., Biochem. Biophys. Acta, 1957, v23, 59.

3. Kleiber, M., Smith, A. H., Black, A. L., Brown, M. A., Tolbert, B. M., J. Biol. Chem., 1952, v197, 371.

4. Sabine, J. R., Johnson, B. Connor, ibid., 1964, v239, 89.

5. Essig, H. W., Norton, H. W., Johnson, B. Connor, Proc Soc. Exp. Biol. and Med., 1961, v108, 194.

6. Morris, B., Simpson-Morgan, M. W., J. Physiol., 1963, 169, 713.

7. Gupta, J., Crump, M. H., Nichols, R. E., Am. J. Vet. Res., 1962, v23, 201.

8. Harlan, J. W., Atomlight (New England Nuclear Corp.), 1961, v19, 8.

9. Bray, G. A., Anal. Biochem., 1960, v1, 279.

10. Oppermann, R. A., Nystrom, R. F., Nelson, W. O., Brown, R. E., Intern. J. Appl. Radiation and Isotopes, 1959, v7, 38.

11. Ash, W., Dobson, A., J. Physiol., 1963, v169, 39.

12. Carroll, E. J., Hungate, R. E., Arch. Biochem. Biophys., 1955, v56, 525.

13. Rust, J. H., LeRoy, G. V., Spratt, J. L., Ho, G. B., Roth, L. J., Radiation Res., 1963, v20, 703.

Received May 10, 1965. P.S.E.B.M., 1965, v120.

Effects of Diphenylhydantoin upon the Plasma Free Fatty Acid Response Induced by Hormones.* (30490)

ANTHONY C. CHUNG† (Introduced by J. C. Houck)

Surgical Research Metabolic Laboratory, Georgetown University Hospital, Washington, D.C.

Administration of sodium bi-phenyl hydantoinate (Dilantin) to rats resulted in changes of dermal concentrations of collagen, scleroprotein and hexosamine(1,2). It was demonstrated that reductions of dermal triglyceride and phospholipid concentrations were a result of Dilantin administration(3).

* This investigation is supported in part by U.S.P.H.S. Grant HE 07395-02 and the Hartford Foundation.

† Present address: Adolescent Medicine, Children's Hospital, Washington, D.C.