CARBON DIOXIDE ENTRY RATE AS AN INDEX OF ENERGY EXPENDITURE IN LAMBS

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Summary

The rate of entry of CO, into the body pool(s) of CO_2-HCO_3^- in lambs of 6 h to 14 days of age was measured by radioisotope techniques involving single injections and continuous infusions of $NaH^{14}CO_3$ solution. Rates of CO_2 expiration and O_2 consumption were measured at the times of injection and infusion by open circuit calorimetry. The rate of CO_2 expiration was significantly related to CO_2 entry rate over the range of from 15 to 194 ml CO_2/min, and entry rate was significantly related to energy expenditure (30 to 264 kcal/m^2/h).

I. INTRODUCTION

Although the energy expenditure of newborn lambs has been studied under controlled environmental conditions (Alexander 1962a, b, c), there have been no reports of similar studies on animals in the field, probably largely due to the lack of suitable techniques. Recently, Young et *al.* (1967) reported a relationship between the rate of expiration of CO_2 by sheep of various ages measured by open-circuit calorimetry, and CO_2 entry rate estimated by a radiotracer technique. This paper presents further evidence for the closeness of the relationships of the rate of CO_2 expiration, and the rate of energy expenditure, with CO_2 entry rate of restrained lambs. Possible application to lambs in the field is discussed.

II. MATERIALS AND METHODS

(a) Animals

Merino lambs between 6 h and 14 days of age, weighing between 2.5 and 8.7 kg, and prepared with jugular vein catheters, were placed in a controlled temperature room. They were held at temperatures of either 27, 20 or 0°C for from 30 min to 14 days before studies began. Starved lambs were all held at approximately 27°C until 30 min before an experiment and were then treated in the same manner as the non-fasted lambs.

(b) Administration of radioisotope

Carrier-free $NaH^{14}CO_3$ was dissolved in a solution of 0.9% (w/v) NaCl and 0.8% (w/v) Na_2CO_3. The solution was either infused intravenously at a constant rate within the range of 200 to 800 mμCi/min over a 5 h period or was given as a single injection of 5 to 10 μCi over a period of 1 min. In preliminary continuous infusion experiments, blood samples were taken at 20 min intervals from the start but in subsequent experiments they were taken only at 20 min intervals after 3 h of infusion. In single injection experiments, blood samples were taken at 10, 20, 40, 60, 80 and 100 min, and at 30 min intervals thereafter to 5 h.

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(c) Measurement of energy expenditure

Lambs were either confined in a small perspex chamber or were fitted with a fibreglass mask. Air was drawn across the animal, through the chamber or mask, at a rate such that the CO_2 concentration in the analyser system was between 0.3 and 1.9% (v/v). Water vapour was removed by ice-salt and calcium chloride traps and the volume of air was recorded with a dry gas meter. Samples of dry air were withdrawn from the main air stream for continuous monitoring of O_2 with a paramagnetic oxygen analyser (Beckman Inc.) and of CO_2 with an infra-red carbon dioxide analyser (Onera SO).

Energy expenditure was calculated from the equation given by Marston (1948) for conditions where urinary nitrogen excretion is not known.

$$E = b\left(4.686 + 1.23\left(\frac{a}{b} - 0.707\right)\right) \quad \text{.....(1)}$$

where E = rate of energy expenditure (cal/min)

a = rate of CO_2 expiration (ml/min)

b = rate of O_2 consumption (ml/min)

(d) Measurement of *radioactivity*

The specific radioactivity (SR) of blood CO_2 was estimated either by the method of Leng and Leonard (1965) or of Hinks, Mills and Setchell (1966). Radioactivity in expired gas was assayed with a 4.31 ion chamber attached to a vibrating-reed electrometer (Carey Model 31) and the SR was obtained by dividing the rate of expiration of carbon-l 4 by the rate of expiration of CO_2.

(e) Calculation of *CO_2 entry rate*

(i) Continuous infusion experiments

Division of the rate of infusion of radioisotope (mμCi/min) by the plateau SR of blood CO_2 in mμCi/mg carbon (mμCi/mgC) gave values for CO_2 entry rate (mgC/min).

(ii) Single injection experiments

Pool size (P) and entry rate of CO_2 (ER) were calculated by the method of Baker *et al.* (1959):

$$P\ (\text{mgC}) = \frac{I}{(A_1 + A_2 + \text{.....})} \quad \text{.....(2)}$$

where I = injected dose of radioisotope (mμCi)

A_1, A_2 etc = zero time intercepts of the separate components

$$ER\ (\text{mgC/min}) = \frac{P(A_1m_1 + A_2m_2 + \text{.....})}{(A_1 + A_2 + \text{.....})} \quad \text{.....(3)}$$

where m_1, m_2 etc = decay constants of the separate components:

Entry rate in the units mgC/min was converted to ml/min of CO_2 by dividing by the atomic weight of carbon (12.01) and multiplying by the standard molar gas volume (22.3 l/mole).

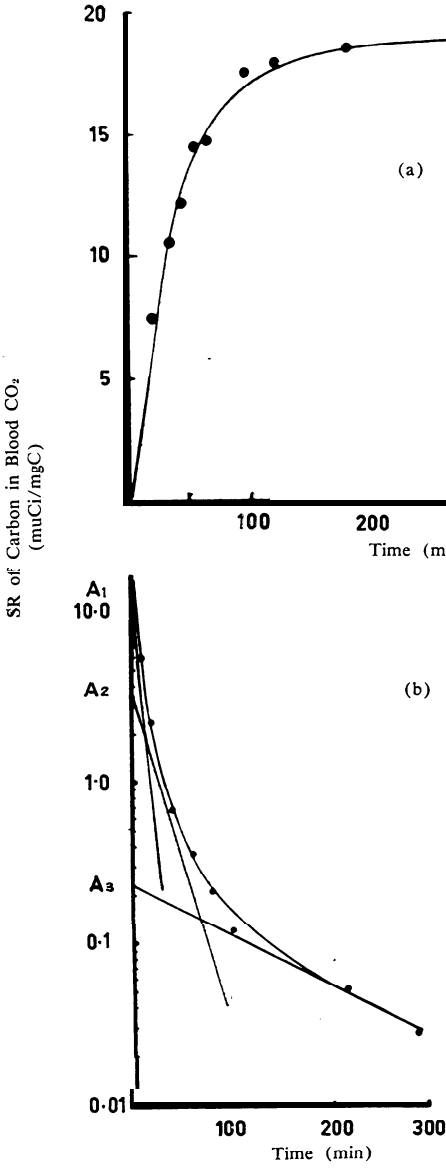

Fig. 1 — Specific radioactivity (SR) of carbon in blood CO_2
(a) during a continuous infusion (0.735μCi/min)
and (b) following a single injection (9.80μCi) of $NaH^{14}CO_3$
CO_2 entry rate from (a) was 72.4 ml/min and from (b) was 118 ml/min.
Pool size from the single injection experiment (b) was 424 mgC.
The single injection curve was described by the equation.

$$SR_t = 17.00e^{-0.173t} + 3.37e^{-0.049t} + 0.24e^{-0.002t}$$

III. RESULTS

(a) Entry rate of CO_2

(i) Continuous infusion experiments

Figure 1 (a) shows the change in the SR of CO_2 in blood with time for a typical experiment. After a period of 150 min, the SR of CO_2 in blood remained approximately constant.

(ii) Single injection experiments

Figure l(b) shows a typical relationship between log SR of CO_2 in blood, and time after the injection of $NaH^{14}CO_3$. The line was described by three exponential components as were the lines for the two other lambs comprising this group.

TABLE 1

Gaseous exchanges and CO_2 entry rates in lambs

Lamb	Age	Treatment	Ambient Temp. (°C)	Live-weight (kg)	Rate of Expiration of CO_2 (ml/min)	CO_2 Entry Rate (ml/min)	Rate of Energy Expenditure (kcal/m²/h)†
J	6h	starved	0-3	4.83	169	195	264
M	14d	fed	0-3	8.71	159	193	168
E	1d	fed	6-8	3.14	54	67	130
F	4d	fed	25	3.35	24	30	65
G	1d	starved	0-3	3.15	37	30	94
G		starved	0-3		22	22	69
G		starved	25		11	15	30
K	5d	fed	0-3	4.64	146	131	238
N	14d	fed	20	8.00	104	100	103
O	14d	fed	25	4.73	58	64	99
I	5d	fed	20	7.60	60	73	65
C	9h	fed	20	—	33	41*	—
H	1d	fed	20	2.55	88	107*	129
I	8h	starved	0-3	3.18	135	118*	153

*Entry rate measurements from single injection experiments.

†Surface area (m^2) was calculated from liveweight (W) in kg according to Lines and Peirce (1931): $(0.121W^{0.5})$.

Starved lambs were taken from ewes soon after birth and were not allowed to drink; all other lambs were taken from ewes 1-2 h before the start of experiments.

(b) Comparison of CO_2 entry rate with the rate of expiration of CO_2

CO_2 entry rate (Table 1), as estimated by the continuous infusion technique, was significantly correlated with the contemporary rate of expiration of CO_2 (Figure 2). Except for two lambs, where only one blood sample was taken, mean CO_2 entry rate was calculated from at least three estimates of SR of CO_2 in blood.

A range in the rate of CO_2 expiration of from 11 to 169 ml/min was obtained and CO_2 entry rate varied from 15 to 194 ml CO_2/min. The lowest values were from a comatose one day old lamb held in an environmental temperature of 27°C following 4 h at O-3 °C and starved from birth (Table 1). The highest rate of CO_2 expiration was from a lamb of 1 day of age held in an ambient temperature between 0 and 3°C.

The rate of expiration of CO_2 (Y, ml/min) was generally less than CO_2 entry rate (E, ml/min) and was given by the equation:

$$Y = 3.34 + 0.88E \qquad r = +0.979,\ RSD = 12.3$$
$$(\pm 0.060) \qquad \text{.....(4)}$$

The residual standard deviation (RSD) of the equation is 16.0% of the mean rate of CO_2 expiration.

Figure 2 also shows the relationship between CO_2 entry rate measured by the single injection technique and the mean rate of CO_2 expiration over the first 3 h of the experiment. Values for the single injection experiments lie within the range of those from continuous infusion experiments.

(c) Energy expenditure

The mean respiratory quotient (RQ) for these animals was 0.91 (range 0.65 to 1.17) for 11 observations. Energy expenditure *(Z,* cal/min) was significantly

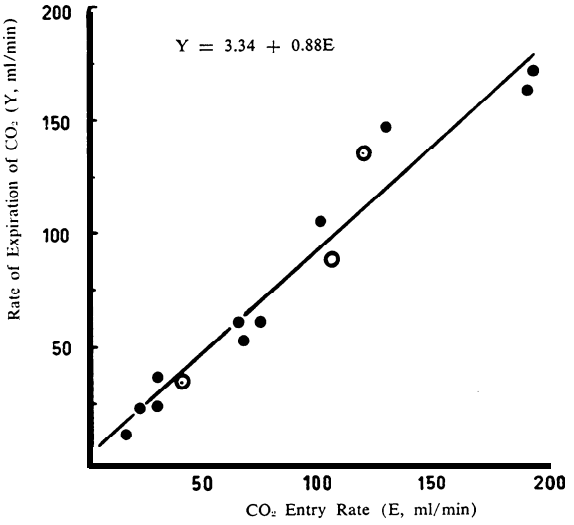

Fig. 2 — Relationship between rate of expiration of CO_2 and CO_2 entry rate.
- Continuous infusion experiments.
- Single injection experiments.

correlated ($P<0.001$) with CO_2 entry rate (E, ml/min) in continuous infusion experiments (Fig. 3):

$$Z = 50.25 + 4.26E \qquad r = +0.973 \text{ RSD} = 68.8$$
$$(\pm\ 0.353) \qquad \text{.....(5)}$$

The RSD of equation (5) is 16.9% of the mean energy expenditure.

Figure 3 shows that values for the three single injection experiments lie within the range of those from continuous infusion experiments.

IV. DISCUSSION

Radiotracer techniques have been used to study the metabolism of CO_2-HCO_3^- in many species including sheep (Huber et ***al.*** 1965; Annison ***et al.*** 1967) but no relationship between CO_2 metabolism and CO_2 expiration or metabolic rate was reported by these workers. Entry rate, measured by the continuous infusion technique, is an estimate of the overall rate of entry of CO_2 into the body pool(s) of CO_2-HCO_3^-. In steady-state conditions, CO_2 entry rate is equal to the sum of all irreversible losses of CO_2 from the CO_2-HCO_3^- pools plus CO_2 which recycles through these pools. The main avenues of irreversible loss of CO_2 are expiration, excretion in the urine as HCO_3^- and urea and incorporation into essentially static body constituents such as bone. Recycling of Carbon-14, i.e. the incorporation of carbon- 14 from $H^{14}CO_3^-$ into some product which is itself metabolised to produce $^{14}CO_2$, may result in a continual increase in the SR of the precursor, $H^{14}CO_3^-$, with time. Recycling would therefore lead to spurious estimates of entry rate (Steele ***et al.*** 1956).

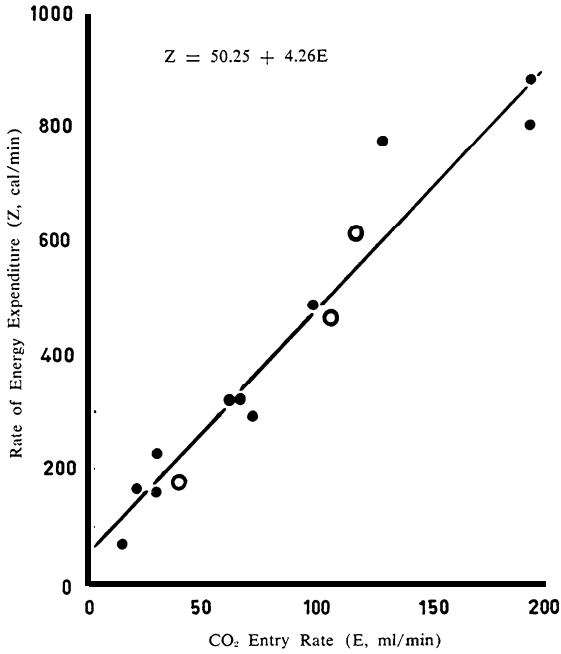

Fig. 3. — Relationship between energy expenditure and CO_2 entry rate.
● Continuous infusion experiments.
○ Single injection experiments.

In our continuous infusion. experiments, no significant increase was noted in the SR of blood CO, with time other than that which could be accounted for by a decrease in metabolic rate. The third component of single injection experiments (see Figure 2b), which is probably due to recycling, accounted for only 0.08% of the entry rate. Hence, recycling of carbon – 14 was probably insignificant in these experiments.

CO_2 entry rate was highly correlated with, and generally was in excess of, the rate of CO_2 expiration. This finding confirms the report of a similar relationship for lambs and adult sheep (Young ***et al.*** 1967). The magnitude of difference between the rates of CO_2 entry and expiration changed only slightly with the level of energy expenditure (Figure 2a). The range in rate of CO_2 expiration shown in Figure 1 was equivalent to a range in energy expenditure of 30 to 264 kcals/m²/h. The lowest value was from a comatose lamb and the highest from a cold stressed lamb held at 0°C. The two highest rates of energy expenditure measured in these experiments (Table 1) lie within the range reported for lambs of similar liveweight during, summit metabolism (Alexander 1962c). Although our climate room facilities could not be readily manipulated to produce summit metabolism, it is suggested that the technique will be applicable to these conditions.

In situations where O_2 consumption is not known an RQ must be assumed to calculate energy expenditure. Depending on the RQ selected, a bias may result in values for energy expenditure. Justification for using an RQ of 0.83 under

normal conditions has been outlined by Brody (1945) who showed that variation in the RQ of from 0.71 to 1.0 resulted in changes in the caloric value of oxygen of only 7%. The RQ's in this work were in the upper part of the range and slightly in excess of those reported by Alexander (1962a, b). Hence, energy expenditure estimated from CO_2 entry rate and an RQ of 0.83 would be in excess of values from the calorimetric studies.

The advantage of the single injection technique for prediction of the rate of CO, expiration is the simplicity of administration of the radioisotope; the disadvantage is that the decline in SR of CO_2 with time must be determined precisely on blood samples taken serially while the animal is in an unrestrained state. The continuous infusion -of radioisotope in the field may not be difficult as rapid developments have been made for routine infusion of radioisotope into adult sheep (Young ***et al.*** 1967; Young and Corbett 1968). Provided lambs can be caught quickly, samples of blood can be taken with a small, though probably measurable, change in the SR of blood CO_2. A device for taking blood samples at predetermined times is being developed in this laboratory (D. J. Farrell, pers. comm.) to overcome such metabolic disturbances during mustering.

A serious limitation in the application of the continuous infusion technique is the time taken for the SR of blood CO_2 to reach equilibrium. It may be possible to reduce the time from 2½ h to 15 min by using the primed-infusion technique (Steele ***et al***. 1956). However, more single injection experiments over a wide range of rates of energy expenditure are required to allow accurate selection of a suitable ratio of priming dose to infusion rate for the routine use of the primed infusion technique.

V. ACKNOWLEDGMENTS

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