612.235:612.261

THE SOURCE OF CO_2 EXPIRED AND THE SITE OF ITS RETENTION.

By LAURENCE IRVING, J. K. W. FERGUSON and F. B. PLEWES.

(*From the Department of Physiology, University of Toronto.*)

After evisceration the respiratory quotient (R.Q.) of a cat maintained by artificial ventilation and receiving glucose injections rises to about one (Burn and Dale). These experiments were repeated by Kilborn (2), using eviscerated cats without extra sugar, showing that the R.Q. rose to one or even above. But he showed that the eviscerated animals were being over-ventilated, and he considered that on that account there was no proof for a rise in true R.Q. In a previous paper (1) we have shown that in the eviscerated cat over-ventilation diminished the muscle CO_2 content as well as that of the blood, and that the observed loss in CO_2 from blood and muscle accounted for from 10 to 30 p.c. of the extra CO_2 calculated by Kilborn's method as that blown off by over-ventilation.

By demonstrating that the CO_2 reserve in the muscles contributed CO_2 during over-ventilation after evisceration, Kilborn's view that the rise in R.Q. did not represent a change in metabolism was sustained. The actual source of all of the extra CO_2 was not, however, determined. In examining the tissues for possible sources of the unexplained extra CO_2, it became apparent that only the bones contained a sufficient amount of CO_2 to supply the unexplained extra quantity expired, if it originated in a preformed source. But the bones were so rich a source of CO_2 that only very close analytical comparisons could have revealed the loss of the unexplained extra CO_2, and for this reason the results were not conclusive.

The problem has a general as well as particular interest when we wish to determine the extent of the carbonate reserves of the animal which are labile enough to respond rapidly to alterations in alveolar CO_2 tension. As the decapitated eviscerated cat is a rather variable preparation, other preparations have been selected which would be better fitted to indicate the extent and situation of the labile body carbonates.

The over-ventilated cat shows a similar adjustment to diminished

alveolar CO_2 tension in giving off extra CO_2. If the degree of over-ventilation is not great, no severe symptoms appear. Recovery is prompt, and there is no reason to suspect an alteration in the type of metabolism. The converse type of experiment is shown where cats are curarized and caused to breathe CO_2 rich mixtures. In these experiments Shaw (5) has shown that after a period of CO_2 retention a return to the original R.Q. is observed. The animal apparently adjusted itself to a state of higher CO_2 tension without any effect on the type of metabolism. In over-ventilated cats and those ventilated with CO_2 rich mixtures there is no reason for suspecting a change in the true R.Q. We have therefore repeated experiments with cats during over-ventilation and ventilation with CO_2 rich mixtures, examining (1) the expired air as an indicator of CO_2 lost or gained, and (2) blood, muscle and bone for changes in CO_2 content to explain the source of extra CO_2 expired, or site of its retention.

Experimental procedure.

For over-ventilation experiments cats under amytal anæsthesia were prepared with tracheal and carotid cannulæ. Normal samples of expired air were then taken by inserting a Pearce animal membrane valve between the tracheal cannula and the 5-litre rubber collecting bag. From the bag about 50 c.c. were at once transferred to a Brodie gas pipette and stored, under mercury seal, until analysis. A sample of arterial blood was allowed to flow directly into an Ostwald pipette and transferred to the Van Slyke apparatus. Muscles were then excised from one leg and kept in liquid air until analysed. Bones were removed from the same leg, bleeding was arrested, and the cat covered on the warmed table.

Over-ventilation was effected by a pump with sliding metal valves. The pump was shown to deliver accurately all the air taken in, and was adjustable as to volume per stroke and stroke per minute. The inspired air was metred. At about half-hour intervals 5-litre samples of expired air were collected and about 50 c.c. transferred to the Brodie gas pipettes. Immediately after taking the last air sample, arterial blood was drawn and the muscles and bones removed from the other leg.

Analytical procedure.

Expired air samples were analysed for CO_2 and O_2 in a series of Haldane gas analysis apparatus. From these analyses the CO_2 produced and O_2 consumed per 100 c.c. of expired air were calculated by comparison with inspired air corrected on the basis of nitrogen content. The total CO_2 produced and O_2 consumed for any period was obtained by

multiplying the p.c. CO_2 produced and p.c. O_2 used by the volume of air expired during the period. This in turn was calculated from the volume of inspired air. The arterial blood CO_2 contents were determined according to the methods of Van Slyke and Neill(6); CO_2 combining power of blood was determined (after equilibration at 38° C.) in the Van Slyke apparatus. Muscle CO_2 was also determined in a Van Slyke blood gas apparatus adapted for receiving solid pieces of muscle(3). The most suitable muscles were small ones which could be quickly dissected free from fascia and used whole or in definite corresponding parts, such as Extensor Digitorum, Peroneus Longus and Tibialis Anticus. Normal right and left muscles agree in CO_2 content to within 5 p.c. with an average percentage deviation from the mean of each pair of muscles of 1·4 p.c. For bone CO_2 determinations the whole fibula or part of a tibia was dissected out, cleaned and weighed. The CO_2 was expelled by acid and collected in weighed absorption tubes. The average discrepancy in CO_2 content shown between right and left corresponding bones was less than 2 p.c.

Results of over-ventilation.

Typical results of an experiment are listed as Exp. 1, and four experiments are summarized in Table I. These show a CO_2 loss during over-ventilation, assuming that no change in true R.Q. occurs, averaging 378 c.c. per kilo. The figure is somewhat greater than for the CO_2 lost from eviscerated cats. Blood and muscle consistently lose CO_2 in over-ventilation, but the amount so accounted for is only 10 p.c. of the total loss calculated from respiratory measurements.

Exp. 1. Over-ventilated cat. 29. i. 29.	
Weight	1920 g.
Time over-ventilated	2 hr. 50 min.
Respiration.	
Before over-ventilation	0·555 litres per min.
During over-ventilation	1·10 „ „
R.Q. before	0·72
R.Q. after	0·81, 0·90, 1·0, 1·0, 1·39, 1·36, 1·19
CO_2 expired	2554 c.c.
CO_2 calculated from O_2 consumption at R.Q. = 0·72	1610
Extra CO_2 expired	940
Blood.	
Initial CO_2 content	39 vol. p.c.
Final CO_2 content	22 „
CO_2 loss = 0·17 × 0·05 × 1920 =	17 c.c.
Muscle.	
Initial CO_2 content	25 vol. p.c.
Final CO_2 content	16 „
CO_2 loss = 0·09 × 0·44 × 1920 =	76 c.c.
Total loss from blood and muscle =	93 c.c.

TABLE I. Summary of Experiments on over-ventilated cats.

Date	Weight g.	Respiration during over-ventilation: Time of over-ventilation period h. m.	Initial R.Q.	CO_2 expired c.c.	O_2 consumed c.c.	Average R.Q.	Excess CO_2 c.c. per kilo	CO_2 lost: From muscle c.c. per kilo body weight	CO_2 lost: From blood c.c. per kilo body weight	P.c. of CO_2 loss accounted for
17. xii. 28	1550	4 51	0·72	4749	5542	0·86	480	35	6·6	8·6
18. xii. 28	2350	4 15	0·82	3345	3024	1·10	370	17	8·0	7·0
29. i. 29	1920	2 50	0·72	2554	2240	1·14	490	40	8·8	9·9
30. i. 29	1250	3 0	0·74	2279	2777	0·82	175	31	7·5	21·8

The CO_2 loss from blood cannot possibly be very great. The total CO_2 in the blood of a cat, assuming a concentration of 40 volumes p.c. is only 20 c.c. per kilo, and the actual loss from blood is about 16 volumes p.c. in each experiment, or 8 c.c. per kilo, which is only 2 p.c. of the total loss calculated. The muscles listed in Table II lost an average of about 7 volumes p.c. of CO_2, or 31 c.c. per kilo, *i.e.* 8 p.c. of the total CO_2 loss. Here again it is plain that the loss of all muscle CO_2, 88 c.c. per kilo, would not go far toward explaining the calculated extra CO_2 expired. To calculate the total muscle CO_2, the muscles of a cat have been taken as 44 p.c. of the whole weight (1).

TABLE II. Muscle and blood changes during over-ventilation.

Experiment	Total CO_2 in muscle (in vols. p.c.): Before	After	Total CO_2 in arterial blood (in vols. p.c.): Before	After	Change in muscle / Change in blood
17. xii. 28	18·8	11·0	36·9	24·0	0·58
	19·9	12·6			
18. xii. 28	16·9	13·0	35·7	19·8	0·28
	17·4	12·2			
5. ii. 29	21·7	14·1	41·9	26·4	0·49
29. ii. 29	27·3	18·6	39·7	23·0	0·56
	27·1	17·1			
30. i. 29	26·1	16·6	35·7	19·8	0·48
	24·3	18·5			
				Average	0·48

In three experiments the final CO_2 tension of arterial blood had fallen from an initial 40, 49 and 35 mm. Hg to 13, 15 and 12 mm. Hg respectively. From four experiments in which there were three or more determinations of total CO_2, it appeared that the blood had reached its minimum CO_2 content during over-ventilation within a half-hour. The R.Q.'s usually continued to rise for from 2 to 4 hours. There was no sign of approaching equilibrium or a minimum CO_2 content at a new low CO_2

tension in other tissues than blood, although it might have been reached if the over-ventilation had been less severe.

There remains a balance of about 90 p.c. of the calculated extra CO_2 to be accounted for, about 340 c.c. per kilo body weight. In three over-ventilated cats the tibias contained about 10 c.c. of CO_2 per gram, but the difference between normal and over-ventilated bones did not exceed 3 p.c. The bones make up about 25 p.c. of the cat's weight (1) and contain, on the basis of the single bones analysed, about 2500 c.c. of CO_2 per kilo. If the extra CO_2 had been derived from bones, they would have lost 13 p.c. of their CO_2. The loss of even half that amount should have been detectable.

VENTILATION WITH CO_2 RICH MIXTURES.

The converse of over-ventilation experiments are the following ones in which cats under amytal were caused to breathe mixtures containing from 6 to 9 p.c. CO_2. CO_2 mixtures were prepared in a spirometer, usually with oxygen added to approximate the oxygen tension of atmospheric air. After collecting normal blood, air, muscle and bone samples, the animals were curarized and the spirometer mixture led through a metre and the respiration pump. As before, samples of expired air were taken at intervals throughout the experiment. Samples of arterial blood, muscle and bone were secured at the termination of the experiment. From the oxygen consumption calculated from the results of the analysis of each sample, the CO_2 of metabolism was calculated as if the original R.Q. prevailed. The p.c. CO_2 expired in the sample was considered to obtain for the preceding period. Subtracting the expired CO_2 from the CO_2 of metabolism plus the CO_2 inspired showed the amount of CO_2 retained. Actually, as the CO_2 content of expired air rose in successive samples, the amount expired in the early periods is less than the amount indicated by the sample terminating the period, making the calculated retained CO_2 too low.

A typical experiment is outlined as Experiment 2. At the start of ventilation with 7·02 p.c. CO_2, the CO_2 of the expired air was less than that of inspired air. Gradual accumulation of CO_2 in the tissues produced a CO_2 tension sufficient to cause the normal CO_2 elimination, and the R.Q. returned to its original value. No observed characteristic of metabolism was different at the end of the experiment from the start, and the tissue CO_2 tension was evidently in equilibrium with the new higher CO_2 tension. Blood and muscle CO_2 content were consequently increased.

Exp. 2. Cat breathing 7·02 p.c. CO_2. 21. i. 29.

Weight	2850 g.
Respiration.	0·585 litre per min. (22°)
R.Q. before breathing CO_2 mixture	0·75
R.Q. after breathing CO_2 mixture	0·69[1], 0·29, 0·39, 0·37, 0·47, 0·64, 0·76, 0·75
Average	0·51
CO_2 expired (excess over inspired)	3570 c.c.
CO_2 calculated from O_2 consumption at R.Q. = 0·75	5250 c.c.
CO_2 retained	1680 c.c.
Blood.	
Initial CO_2 content	33·7 vol. p.c.
Final CO_2 content	49·4 „
CO_2 retained = 0·16 × 0·05 × 2850 =	23 c.c.
Muscle.	
Initial CO_2 content	19·5 vol. p.c.
Final CO_2 content	34·1 „
CO_2 retained = 0·15 × 0·44 × 2850 =	188 c.c.
Retention by blood and muscle	211 c.c.

Four such experiments are summarized in Table III. Two of the experiments, lasting for over 4 hours (January 21 and March 19) show an exact return to the original R.Q. In one experiment (March 18) on a decapitate cat the R.Q. returned to 0·78 in 2 hours 35 minutes, compared with a normal of 0·90. The normal rather high R.Q. is the average of two samples of which the second was the lower at 0·86, so that the true preliminary R.Q. was perhaps less than 0·90. The experiments on January 21 and March 19 indicate certainly that final equilibrium has been attained at the higher CO_2 tension. In the experiment of March 18 we may say that equilibrium is practically attained. The first experiment (December 3) lasted for only 2 hours, during which time the R.Q. did not return to the starting value. Evidently equilibrium was not reached.

TABLE III. Summary of experiments on cats ventilated with CO_2 rich air.

Experiment	Weight g.	Duration of tank air breathing h. m.	Initial R.Q.	Respiratory measurements: CO_2 expired c.c.	O_2 consumed c.c.	CO_2 retained c.c. per kilo	Average R.Q.	Tissue CO_2 gain: In muscle c.c. per kilo body weight	In blood c.c. per kilo body weight	CO_2 retention accounted for p.c.
23. xii. 28	1550	2 0	0·64	660	3470	1000	0·19	26·4	2·96	2·9
21. i. 29	2850	4 40	0·75	3570	6920	568	0·50	66·0	8·0	12·5
18. iii. 29	3180	2 35	0·90	1425	2156	162	0·66	59·0	7·8	42
19. iii. 29	4720	4 0	0·83	5900	8540	250	0·69	74·5	4·0	31

[1] CO_2 of expired air was less than inspired by an amount giving this ratio to the oxygen consumed.

In the last three experiments, in which equilibrium was attained, the average blood CO_2 content increase was only 6 c.c. per kilo of cat; not enough to be significant. The CO_2 retained by muscle was quite large, averaging 67 c.c. per kilo, and with that absorbed by the blood accounting for 13 to 42 p.c. of the amount retained.

As from 58 to 87 p.c. of the retained CO_2 cannot be located, it is well to consider the rate of CO_2 absorption by blood and muscle. In two experiments where several blood samples were taken, the maximum CO_2 content was already present in the first samples analysed, after ½ hour and 1 hour respectively. Consequently the arterial blood maximum was reached rapidly. It seems unnecessary to figure on the attainment of the maximum CO_2 content in venous blood, for the quantity of CO_2 involved by the blood changes was always relatively small. Venous blood equilibrium would only be reached when the R.Q. returned to its starting value.

We have not been able to follow the rate of increase in muscle CO_2 content in many experiments. However, by taking successive muscle samples in two experiments it was evident that the muscle CO_2 content reached its maximum after blood and before the process of CO_2 absorption was complete. Establishment of muscle saturation preceding return to respiratory equilibrium would indicate an absorbing system for the unexplained CO_2 distinct from blood and muscle.

The critical factor in determining CO_2 absorption by tissues is the CO_2 tension. In three experiments the arterial blood CO_2 tension increased

TABLE IV. Muscle and blood changes during ventilation with CO_2 rich air.

Experiment	Total CO_2 in muscle (in vols. p.c.) Before	After	Total CO_2 in arterial blood (in vols. p.c.) Before	After	Change in muscle / Change in blood
3. xii. 28	27·2 26·1	33·7 33·9	36·1	42·4	1·13
4. xii. 28	15·4 15·8	26·6 26·3	26·1	42·1	0·68
9. xii. 28	27·4 28·5	36·6 36·0	44·8	53·0	1·02
21. i. 29	20·9 21·1	36·9 36·5	33·7	49·4	1·0
19. ii. 29	28·8 30·9	48·5 42·3	47·3	55·2	1·96
18. iii. 29	18·8 19·5	33·5 35·1	30·2	47·3	0·88
19. iii. 29	19·6 18·5	36·6 37·6	34·3	42·2	2·28
				Average	1·28

from 20, 25 and 30 mm. to 30, 67 and 51 mm. respectively. If we knew the true CO_2 tension in muscle we could make a CO_2 dissociation curve, but arterial blood represents only the lowest possible muscle CO_2 tension. Comparison of the amounts of CO_2 absorbed per unit of blood and muscle (Table IV) shows that muscle gains on the average 1·2 times as much CO_2. Consequently muscle CO_2 capacity exceeds that of blood toward increasing CO_2 tension, while for over-ventilation the relation was reversed, and muscle CO_2 lost only half as much CO_2 per c.c. as blood.

The changes in CO_2 content of these two tissues account for only one-third of the absorbed CO_2. As in the eviscerated and over-ventilated cats, where bone was regarded as the likely source of CO_2, in the high CO_2 cats it was considered as a possible site of retention. Results of analysis of a number of bones are given in Table V. The analysis of corresponding bones before and after CO_2 breathing showed more frequently than not a gain in CO_2 content. The average amount of CO_2 gained, allowing 25 p.c. for bone weight, was about 60 c.c. per kilo. After allowing for blood and muscle in the three experiments in which equilibrium was reached, an average of 260 c.c. of CO_2 per kilo remained to be explained. The retention of 60 c.c. of CO_2 by the bones is enough to suggest that bones constitute an important site of retention of the unexplained absorbed CO_2.

TABLE V. Bone CO_2 content after ventilation with CO_2 rich air.

Experiment	Bone	c.c. CO_2 per g.			Ca mg. per g.		c.c. CO_2 per g. Ca		Loss (−) or gain of CO_2 per g. Ca
		Before	After	Δ	Before	After	Before	After	
13. ii. 29	Tibia proximal	9·91	10·18	0·27	135	141	73·4	72·2	−1·2
	10th and 11th ribs	12·02	11·36	−0·68	162	155	74·3	73·4	−0·9
19. ii. 29	Fibula	15·31	16·72	1·41	—	—	—	—	—
18. iii. 29	Distal Tibia	12·22	12·51	0·29	184	196	66·5	64·0	−2·5
	Fibula	14·50	14·33	−0·17	224	224	64·7	64·1	−0·6
19. iii. 29	Distal Tibia	13·20	12·99	−0·21	196	192	67·5	67·7	0·2
25. iii. 29	Distal Tibia	12·06	12·46	0·40	186	188	64·9	66·3	1·4
27. iii. 29	Distal Tibia	13·24	13·34	0·10	193	204	68·7	65·5	−3·2
	Fibula	14·62	15·21	0·49	220	228	66·5	66·8	0·3

These analytical differences are not, however, convincing. It has been shown that the ratio $\frac{Ca}{CO_2}$ in bones of rats is a characteristic of their age (4). In an alteration of the CO_2 content the calcium content is a natural reference point, and changes in CO_2 content might appear more clearly when expressed in terms of CO_2 per g. Ca. Referring bone CO_2 to calcium does not show a gain in CO_2 but, on the contrary, more frequently implies a

loss. As in the eviscerate cat, it appears that the bones may serve as reservoirs of labile CO_2, but the evidence is not conclusive.

Possible alternative sites of retention or loss of CO_2 may be considered together, as they probably involve similar processes. Entrance of significant amounts of extraneous CO_2 into these over-ventilated cats breathing outside air is not possible. But CO_2 might escape from the cats breathing CO_2 rich air. In two experiments cats were sealed in a metal box and ventilated with about 7 p.c. CO_2 through a tracheal cannula sealed in the box. If CO_2 escaped through the skin or small exposed area of the trachea, it would be caught in the box. CO_2 free air was then drawn through the box and passed through sulphuric acid and weighed absorption tubes. During 6 hours 33 mg. of CO_2 were collected, *i.e.* a loss from the skin of about 17 c.c. of CO_2. In the second experiment one leg was amputated, in order to see if CO_2 escaped more readily when the conditions were similar to those of our experiments. 8 mg. or 4 c.c. were collected in 2 hours, while the blood CO_2 content increased from 36·7 to 44·7 volumes p.c. Apparently the small quantity of CO_2 that can be lost through the skin has no significance in contrast to the large amount of CO_2 retained. These results agree with the findings of Shaw (7).

Examination of several viscera in two normal animals showed that their CO_2 content was only slightly higher than that of muscle, as is indicated in Table VI. As all of the viscera weigh less than half as much as all of the muscles, a CO_2 retention in the viscera corresponding to that in muscle would amount to about 30 c.c. per kilo. It is probable that changes in the viscera would account for nearly 10 p.c. of the retained CO_2, but the unexplained balance would not be significantly reduced. The loss from the viscera during over-ventilation, if comparable to the muscle loss, would be somewhat less.

TABLE VI. CO_2 content of soft tissues of normal cats. Average for two cats.

Tissue	CO_2 in c.c. per 100 g.
Liver	32
Brain	27
Kidney	24
Ileum	31

Urea formation might be considered a cause of CO_2 retention. Shaw (5) considered it ruled out by finding CO_2 retention unchanged after tying off the ureters, but that proceeding would not prevent the formation of urea. We have made no attempt to rule out possible excretion because the rôle of CO_2 in the process of urea formation is uncertain[1].

[1] This possibility has now been investigated.

Conclusions.

It is apparent that an increase or a decrease in alveolar CO_2 tension causes respectively a decrease or an increase in CO_2 elimination. The altered rate of CO_2 elimination can cause a great change in R.Q. for several hours at least. There is no reason for suspecting a change in the kind of material oxidized, although the possibility is not ruled out. It is well known that changes in blood CO_2 content follow closely after alteration in the alveolar CO_2 tension, but the total amount of blood CO_2 is not sufficient to cause much change in R.Q. The muscle CO_2 is labile, like that of blood, and contributes roughly four times as much as blood to the change in R.Q. following altered alveolar CO_2 tension. But the combined changes in blood and muscle do not account for half of the change in R.Q.

When it is considered that CO_2 may be either retained or given off according to the alveolar CO_2 tension, it is likely that the tissue carbonates and bicarbonates constitute the buffer or reserve system. After proper allowance is made for the blood, muscles and viscera, bones are the only tissue with a sufficient CO_2 content to serve as the remaining part of the reserve system. Loss or gain in bone CO_2 was not established during these acute experiments, but the large amount of CO_2 present in bone makes it difficult to say to what extent the small changes required in bone carbonates would be detectable. It is still possible that other physiological adjustments of the tissues, such as urea formation, are partly responsible for the change in R.Q.

The extent of the blood and muscle CO_2 reserves has been traced, the visceral reserves indicated, and the bones shown adequate for the remainder. There is no evidence for a change in oxidative metabolism, and good evidence that a tissue reserve or buffering system causes changes in the R.Q. following every alteration in the alveolar CO_2 tension.

The picture of changes in R.Q. and in the part contributed to those changes by blood and muscle is similar in eviscerated and over-ventilated cats. Consequently it does not seem proper to conclude that the R.Q. of the eviscerated preparation is a true indicator of metabolism until the tissue CO_2 reserves are examined and either appropriate corrections can be made or the experimental procedure prevents loss of tissue CO_2.

SUMMARY.

During over-ventilation of cats, 375 c.c. of CO_2 per kilo may be expired in excess of the amount which would be necessary to maintain the original type of metabolism. Of this extra CO_2, about 8 c.c. per kilo comes from blood, about 31 c.c. from muscle.

During ventilation with CO_2 rich mixtures about 375 c.c. of CO_2 per kilo may be retained. Blood retains about 6 c.c. per kilo, muscle about 67 c.c.

It is possible that bone may account for much of the unexplained retained CO_2, but the results of our analyses give no convincing support to this view.

The large changes in R.Q. following artificially altered alveolar CO_2 tension have not been entirely assigned to definite tissues by analytical methods. Judging by the characteristics of the tissue changes observed, the R.Q. changes originate in alteration of the tissue CO_2 reserves.

REFERENCES.

1. Ferguson, J. K. W., Irving, L. and Plewes, F. B. This Journ. 68. p. 265. 1929.
2. Kilborn, L. G. Ibid. 66. p. 403. 1928.
3. Ferguson, J. K. W. and Irving, L. J. Biol. Chem. 84. p. 143. 1929.
4. Kramer, B. and Shear, M. J. J. Biol. Chem. 79. p. 147. 1928.
5. Shaw, L. A. Amer. J. Physiol. 79. p. 91. 1926.
6. Van Slyke, D. D. and Neill, J. M. J. Biol. Chem. 61. p. 523. 1924.
7. Shaw, L. A. Amer. J. Physiol. 85. p. 158. 1928.