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CARBON DIOXIDE AND CONTROL OF RESPIRATION DURING HYPOTHERMIA

By W. I. CRANSTON, MARGOT C. PEPPER and D. N. ROSS

From the Department of Physiology, St Mary's Hospital Medical School and Department of Thoracic Surgery, Guy's Hospital Medical School, London

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When the temperature of the non-hibernating mammal is artificially reduced, there is generally a fall in the pH of its arterial blood (Hegnauer & Penrod, 1950; Swan, Zeavin, Holmes & Montgomery, 1953; Fleming, 1954; Osborn, 1953). It has been suggested that this increase in hydrogen-ion concentration may be one of the causes of an observed tendency to ventricular fibrillation, but conclusive evidence on this point is lacking. Since hypothermia is proving increasingly useful in surgery it seemed important to investigate the changes in hydrogen-ion concentration further.

METHODS

Twenty-two adult mongrel dogs, weighing from 15·6 to 28·1 kg were used. All were premedicated with morphine 10 mg and atropine 0·6 mg subcutaneously, 30 min before the experiment began. Anaesthesia was induced by intravenous thiopentone sodium, 25–30 mg/kg and maintained by intermittent injection of small doses (0·5–2 mg/kg). This anaesthetic was used because it permitted observation of the animals in a state of light anaesthesia before and after cooling, while allowing fairly deep anaesthesia during the actual cooling period, which largely inhibited shivering. On the other hand, its disadvantage was that it was not possible to be sure that the same depth of anaesthesia was maintained before and after cooling. Depth of anaesthesia was judged largely by observation of superficial reflexes; on this assessment, all animals were considered to be in a comparable state of light anaesthesia, before and after cooling. A cuffed endotracheal tube was introduced in each case, and cooling was accomplished by using an extracorporeal cooling coil (Ross, 1954). Blood from the femoral artery was led through a coil immersed in a cooling fluid at −3 to 0° C, and returned to the circulation through the femoral vein. Using this method, the body temperature could be reduced to about 26° C in from 50 to 140 min. The animal's temperature was recorded with a mercury thermometer inserted 5–10 cm into the rectum. In six dogs, expired air volume was measured in a 6 l. balanced spirometer, using Bailey-type celluloid mercury valves; the dead space of the valves and endotracheal tube was 55 ml. Blood samples were withdrawn from the brachial artery into oiled syringes, containing 3 drops of heparin-fluoride mixture (1000 units of heparin, and 40 mg sodium fluoride per ml.). Arterial blood pH was measured on a Cambridge pH meter, using a Stadie cell maintained at a temperature within ±0·5° C of the animal's rectal temperature at the time of taking the specimen. The mean difference between duplicates in eighty-nine determinations of pH was 0·011, S.D. ±0·009 unit. The greatest single

difference, found on two occasions, was 0·04 unit. Arterial blood CO_2 content was measured manometrically (Van Slyke & Neill, 1924) in fifteen animals; the mean difference between duplicates in seventy-five estimations was 0·12, S.D. $\pm$0·11 mM/l. The largest single difference, found on one occasion, was 0·3 mM/l. In the first seven animals whole blood CO_2 content was measured, and the plasma CO_2 content calculated from the nomogram of Van Slyke & Sendroy (1928). In the remainder, the plasma CO_2 content was measured directly. The results from the two methods did not appear to differ materially so they have been considered together. In three dogs plasma sodium and potassium were estimated by flame photometry, and chloride by a potentiometric method (Sanderson, 1952).

The distribution of plasma CO_2 as bicarbonate and dissolved CO_2 (calculated as carbonic acid) was determined, using the Henderson-Hasselbalch equation, with the pK′ correction of Cullen, Keeler & Robinson (1925); these authors found that the correction was the same for dog and human plasma, and that the pK′ increased by 0·005 for each degree centigrade fall in temperature of the system. Plasma carbaminoprotein has been neglected, and it has been assumed that the plasma CO_2 content is entirely made up of bicarbonate and dissolved CO_2. The CO_2 tension at different temperatures was calculated from the plasma solubility of CO_2 as given by Dill & Forbes (1941). Strictly speaking calculation of CO_2 tension from the data obtained is dependent on the maintenance of a steady plasma water content. Plasma proteins have not been measured in this series, but according to other authors (Swan *et al.* 1953) the plasma protein does not show any systematic trend during hypothermia, and does not alter by more than an amount indicating a change of plasma volume of 5%. An uncorrected change of this order would result in an error of less than 1% in the calculated CO_2 tension.

Estimations were made during a control period, the period of cooling, and shortly thereafter. The rewarming period was not studied. Spontaneous ventilation was present in all animals at temperatures down to 25° C.

RESULTS

The pH and plasma CO_2 contents are shown in Table 1, along with the derived values for dissolved CO_2, bicarbonate, and CO_2 tension, calculated as described above. In dogs 1–7 the arterial pH alone was measured, and in dogs 8–14 the plasma CO_2 content was obtained from the haematocrit and whole blood CO_2 content, using the nomogram of Van Slyke & Sendroy (1928).

In Fig. 1 the pH values are plotted against rectal temperature, and the decrease in pH with falling temperature is obvious. From the few values obtained below 25° C, it appears that the pH falls more rapidly below this temperature, a finding which may be accounted for by the tendency for spontaneous respiration to cease at temperatures between 20 and 25° C.

Fig. 2 shows the relationship between hydrogen-ion concentration and dissolved CO_2 concentration. The points indicated by closed circles were obtained at body temperatures above 30° C, and those indicated by open circles at temperatures below 30° C. The solid regression line refers to values above 30° C, and the interrupted line to values below 30° C. The slopes and positions of the lines do not differ significantly. Thus rises in hydrogen-ion concentration are associated with increases in the amount of dissolved CO_2 in plasma, and the relationship does not change significantly over the temperature range studied.

In Fig. 3 the concentration of dissolved CO_2 is plotted against temperature. There is an inverse relationship, which may, for practical purposes, be

TABLE 1. Arterial pH and total plasma CO_2 values in all dogs, with derived values for dissolved CO_2, bicarbonate, and CO_2 tension. In dogs 1–7 pH alone was measured, and in dogs 8–14 whole blood CO_2 was measured, and converted to plasma CO_2 using the nomogram of Van Slyke & Sendroy (1928)

Dog 1: 38·5° C, 7·38; 32·5° C, 7·34; 27·6° C, 7·29.
Dog 2: 38·0° C, 7·38; 29·8° C, 7·30; 25·4° C, 7·225.
Dog 3: 38·5° C, 7·37; 27·5° C, 7·285; 23° C, 6·93.
Dog 4: 36·5° C, 7·40; 23·4° C, 7·09; 24·7° C, 7·175.
Dog 5: 36·7° C, 7·365; 25·7° C, 7·28; 22·5° C, 7·07.
Dog 6: 37·1° C, 7·395; 34·5° C, 7·38; 27·2° C, 7·28; 24·2° C, 7·215.
Dog 7: 37·4° C, 7·375; 29·1° C, 7·22; 23·0° C, 7·05; 24·6° C, 7·235.

Dog	Rectal temp. (° C)	pH	Plasma CO_2 (mM/l.)	Dissolved CO_2 (mM/l.)	$BHCO_3$ (mM/l.)	CO_2 tension (mm Hg)
8	36·0	7·30	29·0	1·79	27·2	56·5
	26·5	7·235	27·1	2·10	25·0	52·3
9	36·7	7·365	26·5	1·41	25·1	45·8
	25·0	7·28	29·0	2·06	26·9	48·5
10	38·0	7·365	28·8	1·49	27·3	49·5
	25·1	7·25	29·1	2·22	26·9	52·1
11	36·5	7·365	25·5	1·34	24·2	44·2
	29·0	7·30	26·0	1·70	24·3	45·0
	25·2	7·19	23·4	2·02	21·4	47·5
12	38·1	7·395	26·2	1·26	24·9	42·0
	25·2	7·24	24·8	1·93	22·9	45·5
13	36·5	7·365	25·5	1·31	24·2	43·5
	27·0	7·225	27·3	2·12	25·2	53·0
14	39·0	7·375	23·3	1·18	22·1	39·2
	25·5	7·285	24·4	1·72	22·7	40·7
15	38·5	7·26	25·2	1·63	23·6	53·5
	38·5	7·285	22·3	1·37	20·9	45·5
	28·0	7·225	27·1	2·10	24·9	54·2
	29·0	7·19	23·2	1·91	21·3	50·5
16	38·5	7·34	26·9	1·47	25·4	48·8
	25·0	7·29	25·2	1·76	23·4	41·6
	25·1	7·22	26·2	2·19	24·0	52·0
17	38·5	7·36	26·8	1·39	25·4	46·3
	38·5	7·34	23·6	1·22	22·4	40·6
	25·0	7·265	24·8	2·22	22·6	52·8
	25·3	7·32	21·0	1·37	19·6	32·4
18	38·2	7·355	26·2	1·38	24·8	45·7
	38·2	7·35	23·3	1·24	21·1	41·3
	33·1	7·17	27·8	2·31	25·5	66·0
	26·3	7·245	28·0	2·12	25·9	51·5
	27·4	7·255	26·2	1·93	24·3	48·0
19	38·0	7·345	25·3	1·36	23·9	45·2
	38·0	7·435	22·3	0·98	21·3	33·6
	31·0	7·28	25·8	1·72	24·1	47·5
	28·0	7·30	26·6	1·76	24·8	45·2
	26·5	7·285	24·6	1·70	22·9	42·0
20	38·5	7·39	26·5	1·29	25·2	43·0
	38·5	7·39	26·0	1·27	24·7	42·2
	32·5	7·20	25·5	2·00	23·5	57·0
	26·3	7·24	27·4	2·08	25·3	50·5
	26·5	7·27	25·3	1·84	23·5	44·9
21	38·5	7·355	22·9	1·20	21·7	40·0
	38·5	7·35	20·3	1·08	19·2	36·0
	25·8	7·11	23·0	2·32	20·7	56·5
	26·4	7·195	23·3	1·96	21·3	47·7
22	37·2	7·31	27·5	1·58	25·9	51·6
	37·2	7·31	26·0	1·49	24·5	48·7
	26·5	7·23	26·8	2·07	24·7	51·7
	27·0	7·215	24·5	1·95	22·5	49·0

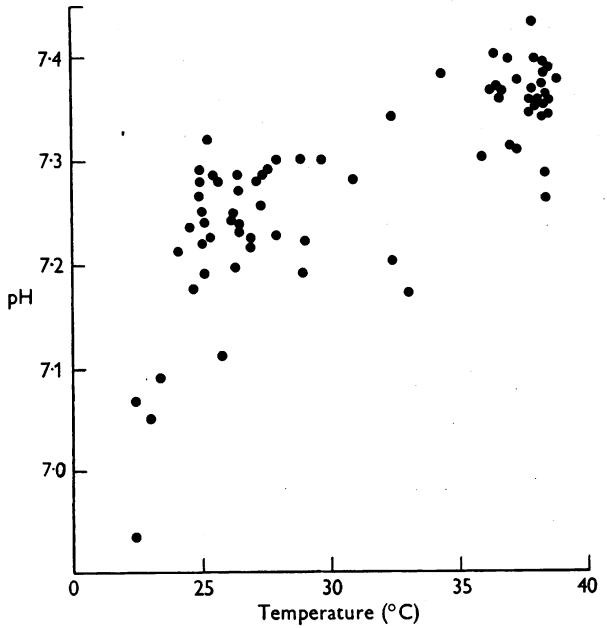

Fig. 1. Relation between arterial blood pH and temperature.

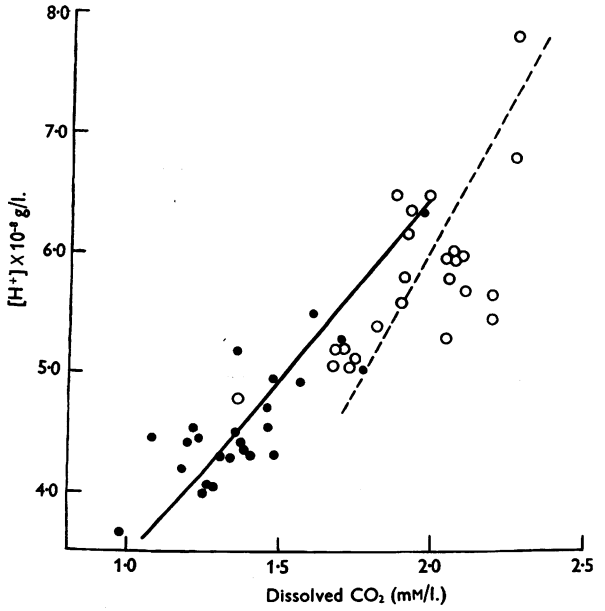

Fig. 2. Relationship between arterial hydrogen-ion concentration and concentration of dissolved CO_2 measured as H_2CO_3. Closed circles represent values obtained at temperatures above 30° C, and open circles values obtained below 30° C. The respective regression lines are represented by the solid and interrupted lines.

considered as linear, between these two variables, at temperatures down to 25° C ($r = -0{\cdot}812$, $P < 0{\cdot}001$). It is seen that the mean concentration of dissolved CO_2 rises by 0·68 mM/l., or 50% of its control value, between 38 and 25° C. In this figure the interrupted line indicates the concentration of dissolved CO_2 which would be expected at different temperatures under a steady tension of 45 mm Hg (Dill & Forbes, 1941). This line bears a similar slope to that of the calculated regression line, so that when CO_2 tensions are calculated

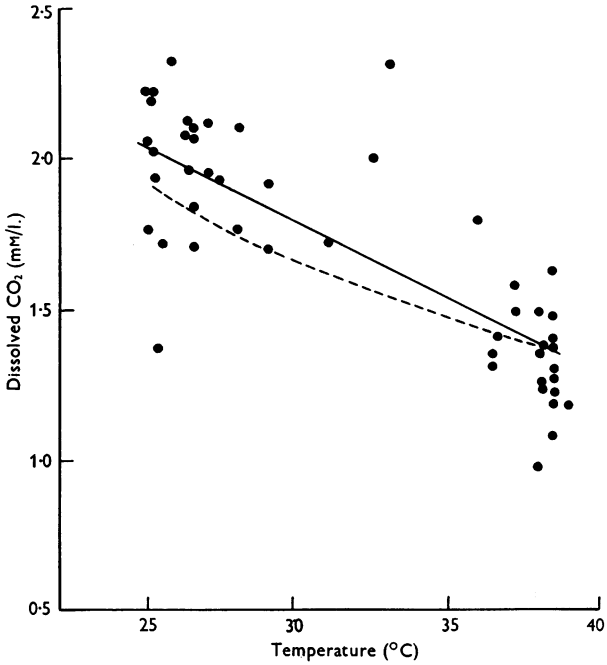

Fig. 3. Relationship between dissolved CO_2 concentration and temperature. The interrupted line indicates the theoretical concentration of dissolved CO_2 at a constant tension of 45 mm Hg (Dill & Forbes, 1941).

and plotted against temperature it is found (Fig. 4) that the relationship only just reaches the 5% significance level ($r = -0{\cdot}275$, $P = 0{\cdot}05$). The mean CO_2 tension increases by 5·3 mm Hg, or 12% of its control value, between 38 and 25° C. This suggests that in these animals the CO_2 tension was maintained at a more steady level than the hydrogen-ion concentration or concentration of dissolved CO_2.

As pH is dependent on the concentration of dissolved hydrated CO_2, this means that the CO_2 tension must be decreased if the pH is to be maintained at a steady level. In this series it would have been necessary to reduce alveolar CO_2 tension from a mean value of 46·5 mm Hg at 38° C to 33·5 mm Hg at 25° C, if the concentration of dissolved CO_2 was to be kept steady.

The minute volume of respiration during spontaneous breathing was measured in six dogs at normal and reduced temperatures. The mean of thirteen observations at normal temperature was 4640, S.E. ± 371, ml./min (BTPS). The mean of fourteen observations at temperatures of 25–27° C was 2150, S.E. ± 359, ml./min. The mean minute volume thus decreased to 46% of its value at normal temperature. Oxygen uptake was not measured, and it is difficult to compare the change in minute volume in this series, with changes in oxygen uptake in other reported series (Penrod, 1949; Ross, 1954) because of differences in anaesthesia and shivering. In the presence of slight shivering, as was often seen in our dogs, the oxygen uptake at 26–27° C would appear to be reduced to 30–50% of its value at normal temperatures. It may well be that the minute volume decreases commensurately.

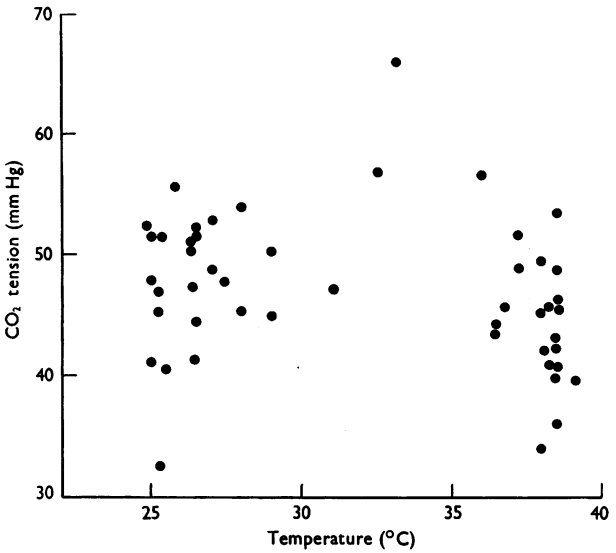

Fig. 4. Relationship between arterial CO_2 tension and temperature.

To determine whether the respiratory centre was still sensitive to CO_2, the effect was observed on six dogs, of inhaling 6% CO_2 in air. The gas was given over periods of 5–8 min, and the minute volume of respiration during the last 3 min of inhalation was expressed as a percentage of the mean of the control values before, and 5–10 min after, the inhalation. The results are shown in Table 2. It is seen that, although there is a slight diminution in response at low body temperatures, the difference is not significant ($t = 0{\cdot}695$, $P > 0{\cdot}10$).

The animals appeared to respond in a normal fashion to an increase in CO_2 tension, so that it could be assumed that the observed plasma values were the results of the action of a functioning control mechanism.

When the plasma bicarbonate content was plotted against temperature, the

changes were not significantly related ($r = +0{\cdot}109$, $P > 0{\cdot}10$). There was, however, a fairly typical pattern of bicarbonate change in all animals. The changes in six animals are shown in Fig. 5. During anaesthesia, and before cooling began, there was a fall of plasma bicarbonate; so far as could be determined,

TABLE 2. Increase in respiratory minute volume when respiring 6% CO_2 in air at normal and reduced temperatures. The increase is expressed as the percentage of the mean of the control values before and after inspiration of CO_2

	Temperature	
	36·5–38·5° C	25–27° C.
	142	106
	166	144
	167	123
	136	196
	161	169
	164	169
	133	119
		123
Mean	151·3	143·6

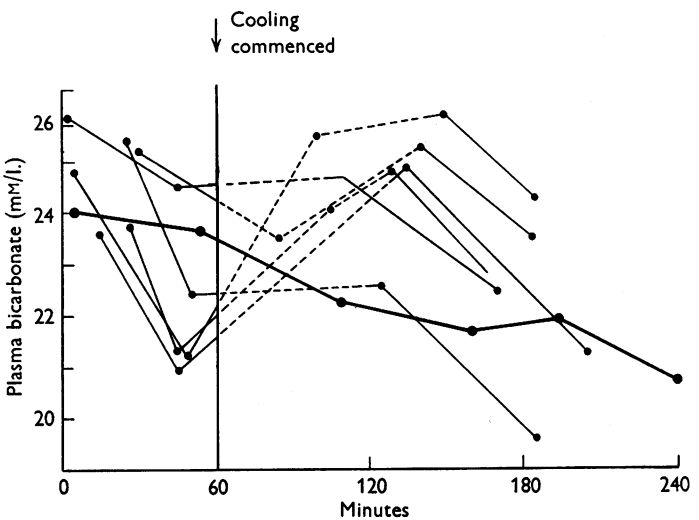

Fig. 5. Arterial plasma bicarbonate during cooling in six dogs. Vertical line shows the time at which cooling began, and the heavy line represents values obtained during a control experiment in which temperature was not reduced. The interrupted lines indicate the period of cooling.

this was not related to the depth of anaesthesia, because there was no corresponding pH rise. During cooling, the bicarbonate level rose, only to fall again when a steady low temperature was reached. The rise during the period of decreasing temperature was presumably due to the decrease of protein base-binding capacity, under the influence of falling temperature and pH. The net result was that the bicarbonate levels after cooling did not differ

significantly from those observed before cooling. One animal was anaesthetized, and a coil was introduced between femoral artery and vein. The coil was immersed in water at 39° C, so that the animal's temperature remained steady between 37 and 38° C. This dog showed a steady fall of bicarbonate throughout the procedure, as is indicated in Fig. 5 by the heavy line. A fall in alkali reserve during anaesthesia has been previously described (Reimann & Bloom, 1918; Ronzoni, Koechig & Eaton, 1924), and we have no evidence that cooling accentuates the process; it may well be that a considerable metabolic acidosis develops during rewarming, when protein base-binding capacity increases, and shivering may take place.

In three animals in which they were followed, plasma sodium and potassium levels showed no significant change. There was a slight increase of up to 2 m-equiv/l. in plasma chloride in two of the dogs, and no change in the third. Thus, assuming little change in the calcium or magnesium levels, the unmeasured anion fraction tended to fall in these three animals, but the change was small, and it seems reasonable to assume that there was no significant degree of metabolic acidosis or alkalosis which might vitiate the conclusions drawn from the CO_2 tensions.

DISCUSSION

The findings reported here show that the body tends to maintain a constant tension of carbon dioxide in arterial blood, although the concentration of dissolved carbon dioxide increases considerably during cooling. The respiratory response to inspiration of 6 % CO_2 indicates that this is not simply a fortuitous manifestation of failure of the respiratory centre.

It was established many years ago (Haldane & Priestley, 1905) that an increase in the tension of carbon dioxide in inspired air resulted in stimulation of respiration. The exact mode of action of carbon dioxide in stimulating respiration has been the subject of considerable speculation and controversy. It was considered (Haldane, 1922; Banus, Corman, Perlo & Popkin, 1944) that increase in arterial CO_2 tension led to an increase in the concentration of arterial carbonic acid, and that this affected respiration by altering pH. This hypothesis was challenged by Nielsen (1936) on the grounds that the respiratory response to a given arterial pH change induced by ammonium chloride was considerably smaller than the response to a smaller pH change produced by carbon dioxide. This view was supported by Schmidt & Comroe (1941).

Although it is possible to follow changes in extracellular fluid composition with a fair degree of accuracy, very little is known about the state of affairs within the cells, where the ultimate response may be determined. This investigation supports the view that, as far as extracellular fluid is concerned, CO_2 tension is the factor controlling respiration, rather than pH or concentration of dissolved CO_2. This does not prove that there is a stimulating effect

specific to carbon dioxide, as it has been suggested that the extracellular CO_2 tension represents the force tending to retain CO_2 in the cells, where its hydration as carbonic acid is responsible for respiratory stimulation. Under normal circumstances this is true, as the CO_2 tension bears a fixed relationship to the amount of CO_2 in solution, and the concentration gradient of dissolved CO_2 controls its movement out of cells. When the body temperature is reduced, a greater amount of CO_2 is dissolved at the same tension, in extracellular fluid, and it appears reasonable to assume that the same thing happens within the cells. If this is so, and if CO_2 within cells behaves in the same way as extracellular CO_2, with respect to hydration, then it seems unlikely that change of intracellular pH, as a result of increasing concentration of CO_2, plays a large part in the control of respiration. If it did so, one would expect respiratory stimulation to occur when the CO_2 concentration began to rise; as a result of this the extracellular CO_2 tension should tend to fall, as the temperature decreases. The mechanism for control of respiration appears to be intact, because the animal responds to increasing the content of CO_2 in the inspired air. For the same reason, it is unlikely that there is any interference with the passage of CO_2 across cell membranes at low temperatures, or with its hydration within cells. It appears, therefore, that CO_2 has a specific property of stimulating respiration, and that this property is in some way bound up with the tension of the gas in solution. Because of this, a 'respiratory' acidosis with decreasing temperature is inevitable, as the dissolved CO_2 concentration in plasma is bound to increase, if CO_2 tension is kept steady. If the arterial pH must be kept at a normal level, ventilation must be increased to such an extent as to reduce the tension of CO_2 in alveolar air and arterial blood.

SUMMARY

1. Arterial blood pH and carbon dioxide content were followed in anaesthetized dogs in which hypothermia was induced by blood stream cooling. A fall in pH with decreasing temperature was observed. This change was shown to be due to retention of carbon dioxide. The carbon dioxide tension showed only a very slight tendency to rise, the fall in pH being largely owing to increased solubility of carbon dioxide at low temperatures.

2. Inhalation of carbon dioxide in air produced a similar respiratory response in animals at normal and reduced temperatures.

3. It is suggested that carbon dioxide has a specific effect, independent of pH, in controlling respiration, and that this effect is associated with its tension rather than with its concentration in solution.

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