Respiration Physiology (1977) **31**, 387–395

POST-HYPERCAPNIA RECOVERY IN THE DOG: ARTERIAL BLOOD ACID–BASE EQUILIBRIUM AND GLYCOLYSIS[1]

C. SAUNIER, P. HORSKY, B. HANNHART, T. GARCIA-CARMONA[2] and D. HARTEMANN

Physiopathologie Respiratoire U 14 INSERM, Vandoeuvre-les-Nancy, France

Abstract. Changes in acid–base equilibrium and blood lactate and pyruvate concentrations were studied during recovery (breathing room air) after three days hypercapnia ($FI_{CO_2} = 0.10$) in awake dogs. Fast return to $FI_{CO_2} = 0$ produced a slight alkalosis in arterial blood and an increase in lactate and pyruvate concentrations which seemed to be maximum at the 15th minute. These changes were inhibited by previous injection of acetazolamide (50 mg/kg body weight). During progressive return to $FI_{CO_2} = 0$, over 1 hour, the peak value of blood lactate and pyruvate was delayed until the end of that hour, at the same time as a slight blood alkalosis appeared. These phenomena are most probably explained by a stimulation, due to alkalosis, of glycolysis at the level of phosphofructokinase.

Acid–base equilibrium	Lactate
Alkalosis	Pyruvate
Glycolysis	Recovery after hypercapnia

Changes in the medium H^+ ion concentration are known to influence red blood cells glycolysis *in vitro* (Minakami *et al.*, 1964): alkalosis enhances it, acidosis inhibits it. CO_2 concentration *per se* has also been shown to have an inhibitory action (Zborowska-Sluis *et al.*, 1972). *In vivo*, red blood cells glycolysis is inhibited by hypercapnic acidosis too (Hartemann *et al.*, 1976) despite the release of catecholamines (Nahas *et al.*, 1968) and on the contrary it is enhanced by hyperventilation alkalosis (Engel *et al.*, 1969).

An interesting point to study lies in the possible changes in blood glycolysis during acid–base equilibrium variations brought about by air-breathing recovery from prolonged hypercapnia. In such a situation, in which Pa_{CO_2} decreases more

Accepted for publication 24 August 1977.

[1] Supported by grant CLR 715 1086 from INSERM Paris, France.

[2] Present address: Clinica Puerta de Hierro, San Martin de Porres, 4, Madrid, Spain.

or less rapidly, one could expect to find an alkalosis. During the period of chronic hypercapnia, renal compensation for respiratory acidosis has resulted in accumulation of excess base in extracellular fluid. If then Pa_{CO_2} is abruptly reduced, the underlying nonrespiratory alkalosis is unmasked, because the rate of excretion of bicarbonate by the kidney is slower than the rate of excretion of carbon dioxide by the lung (Robin, 1963). The arterial pH rises rapidly. To prevent this occurrence after chronic hypercapnia it is necessary to consider a gradual and slow return to a normal Pa_{CO_2}. On the other hand, one could also think that the almost instantaneous return to $FI_{CO_2} = 0$ would in itself lead to a metabolic change, by sudden modification in cellular P_{CO_2}. Therefore, it was necessary to explore also the progressive return to atmospheric air, either by slowly decreasing FI_{CO_2}, or by giving a carbonic anhydrase inhibitor before taking the animal outside the hypercapnic atmosphere.

We successively studied for 4 hours in the awake dog (1) the fast return to atmospheric air, (2) the progressive return to air, (3) the fast return to air after administration of acetazolamide.

Material and methods

Experiments were performed in 42 mongrel dogs of either sex weighing 18–50 kg (average: 26.8 kg). On the day before the experiment, under general anesthesia, a catheter was inserted into a femoral artery; the distal end of the catheter was brought outside through a skin incision on the external part of the leg, and was covered with a large bandage. On the next day the awake dog was suddenly brought into a large conditioned chamber (constructed by the 'Compagnie Française des Produits Oxygénés'), with a volume of about 45 m^3 ($4.8 \times 3.5 \times 2.65$ m); temperature was regulated at 21 C, humidity 50%, and the atmosphere was kept at 21% oxygen and 10% CO_2 (33 experiments) or 0% CO_2 (9 control experiments).

During the time he was in the chamber, the dog was alone. Water and standard food (granules) were given *ad lib.* Once or twice a day, an operator went into the chamber to flush the catheter, to feed and take care of the dog, and to clean the place; he was breathing compressed air from outside through a facial mask connected to one of the plugs available on the walls of the chamber.

At the end of the 72-hour sojourn in the chamber (time 0) arterial blood was sampled while the dog was still inside the chamber; the following variables were measured: pH using a Radiometer electrode, partial pressure of CO_2 (Pa_{CO_2}) by Astrup's method, with calculation of the bicarbonate concentration in plasma (HCO_3^-) and of the base excess in blood (BE), oxygen saturation with Kipp Hemoreflector, lactate and pyruvate concentration by an enzymatic method (Bergmeyer, 1965). Potassium concentration in plasma was measured with a flame photometer.

To study the recovery period, three experimental and one control groups were observed:

Group 1, fast recovery. At the 72nd hour of hypercapnia, the animals were suddenly

taken to room air. 17 dogs were observed for 1 hour, only 6 for 4 hours (but n = 4 at 45 min).

Group 2, slow recovery. After 72 hours of hypercapnia, CO_2 percentage in the chamber was gradually decreased over one hour until zero, then the animal was taken out and further observed for 3 hours in room air (n = 7).

Group 3, fast recovery after acetazolamide. Before suddenly taking the animal into ambient air, acetazolamide was given i.v. (50 mg/kg). 9 dogs were studied for 1 hour, only 4 for 4 hours.

Group 4, control. After the 72-hour sojourn in the chamber in air the animals were then taken out the chamber and studied for another 4 hours (n = 9, but at 45 min n = 6).

During recovery, the same blood measurements were done as at the 72nd hour of sojourn in the chamber (time 0), at the 15, 30, 45, 60, 120, 180, and 240th minute after return to room air (or beginning of FI_{CO_2} decrease).

As a rule each dog was used for one experiment only; however, 4 animals from group 2 were also used in another experiment, namely fast recovery (group 1) for 3 of them, and control study for the last one (group 4).

Differences between average values from diverse experiments were tested using the Student Fisher *t*-test. Changes between values measured at different times during the same experiment were tested by *t*-test on paired data.

Results

Changes in hydrogen ion concentration, Pa_{CO_2}, BE, lactate and pyruvate concentration in arterial blood are shown in fig. 1 for group 1 (fast return to room air) and 2 (slow return to $FI_{CO_2} = 0$). Figure 2 represents the same variables changes for groups 3 (fast return to air after acetazolamide injection) and 4 (control group: room air).

1. Fast recovery (fig. 1)

At the end of the hypercapnic period (time 0) blood reaction was still slightly acid: $[H^+] = 53.4 \pm 0.8$ nmol/l (pH = 7.273) and $Pa_{CO_2} = 72.9 \pm 1.5$ torr. Bicarbonate concentration in arterial plasma was 32.3 ± 0.69 mmol/l, BE = $+3.4 \pm 0.5$ mmol/l. Lactate and pyruvate concentrations were respectively of 0.78 ± 0.06 mmol/l and 61 ± 5 μmol/l.

When the animal was taken back to room air, the return of Pa_{CO_2} towards normal was fast: at the 15th minute of recovery, $Pa_{CO_2} = 41.53$ torr ± 1.45 and H^+ ions concentration was below normal: 33.63 ± 0.88 nmol/l, pH = 7.449, $P < 0.001$; although there was a significant decrease ($P < 0.001$) in bicarbonate concentration, it was still elevated: 27.6 ± 0.7 mmol/l. BE showed an increase which was maximum around the 45th–60th minute ($P < 0.001$ at 60 min), then its value was steady and still elevated. There was a slight 'reventilation alkalosis'. At the same time lactate and pyruvate concentrations increased significantly ($P < 0.01$): at the 15th minute

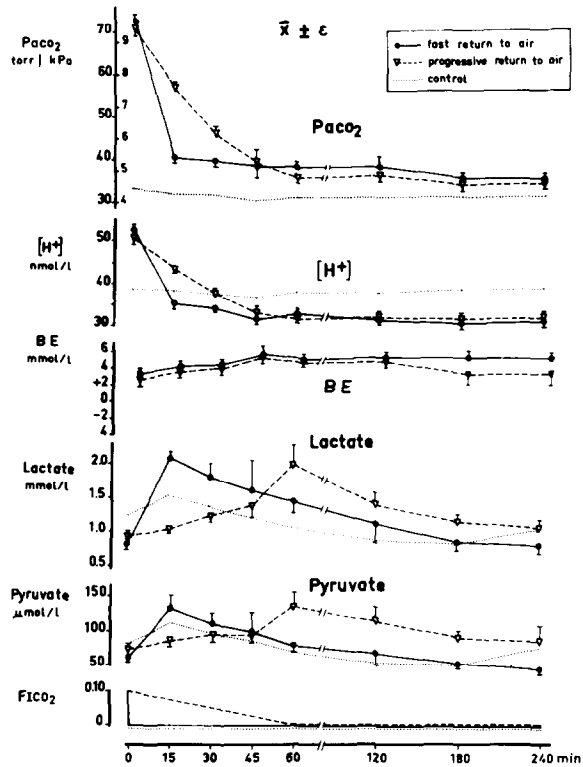

Fig. 1. Changes in H^+ ions, lactate and pyruvate concentrations, CO_2 partial pressure (Pa_{CO_2}), and base excess (BE), in arterial blood of dogs suddenly taken from $FI_{CO_2} = 0.10$ to $FI_{CO_2} = 0$ (continuous line) and progressively taken from $FI_{CO_2} = 0.10$ to 0 over 1 hour (interrupted line). Mean values ± 1 standard error (ε). For comparison control dogs (dotted line).

of recovery they were respectively of 2.07 ± 0.24 mmol/l and 136 ± 18 µmol/l.

Subsequently Pa_{CO_2} continued to slowly decrease, to 37.9 ± 1.26 torr after 4 hours recovery. $[H^+]$ diminished until the 2nd hour, then remained steady around 32 nmol/l. After the initial peak value, lactate and pyruvate concentrations decreased gradually to their value at time 0 within 4 hours. There was no hypoxemia during the experiment (fig. 3). Pa_{O_2} was higher ($P < 0.001$) at the end of the period of hypercapnia (time 0) because of hyperventilation in these awake dogs breathing a gas mixture with 10% CO_2. Arterial oxygen saturation was above 96% in all experiments. Potassium concentration in arterial plasma decreased slightly and gradually ($P < 0.001$ at 60 min, fig. 3).

2. Slow recovery (fig. 1)

The main changes in acid–base data, lactate and pyruvate concentrations after progressive return (within one hour) to $FI_{CO_2} = 0$ are also represented in fig. 1. One can see that Pa_{CO_2} and H^+ ion concentration decreased more slowly but still significantly ($P < 0.001$). BE changes were similar during the first 2 hours; from the

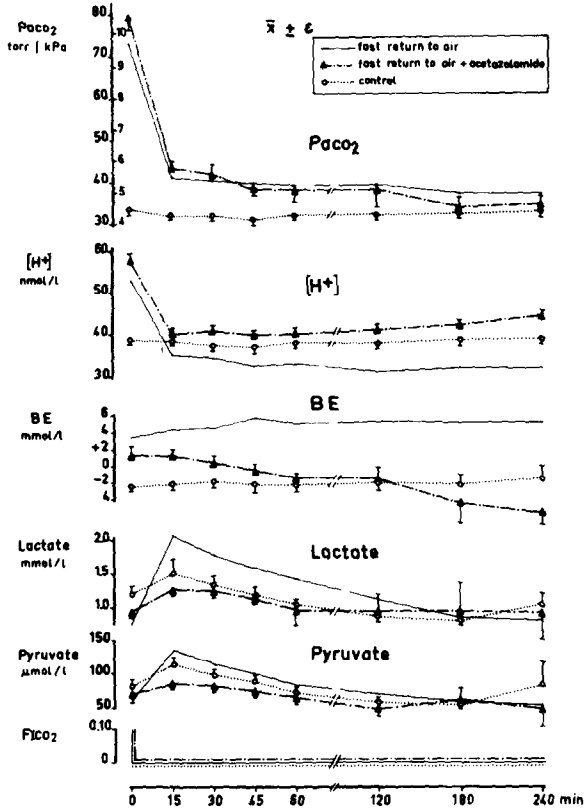

Fig. 2. Changes in H^+ ions, lactate and pyruvate concentrations, CO_2 partial pressure, and base excess in arterial blood of dogs suddenly taken from $FI_{CO_2} = 0.10$ to $FI_{CO_2} = 0$, after acetazolamide injection (50 mg/kg) (dash-dotted line) and control dogs (dotted lines). Changes after fast recovery from hypercapnia without acetazolamide are shown for reference (continuous lines).

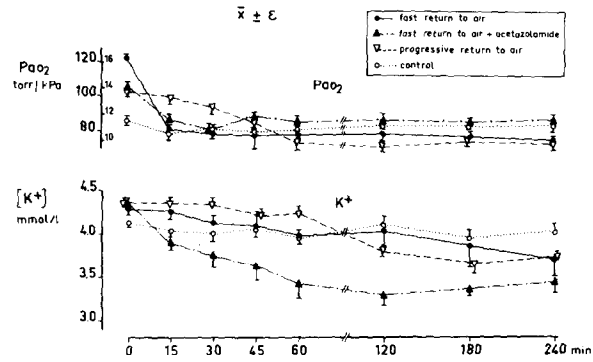

Fig. 3. Changes in arterial oxygen partial pressure and potassium concentration in arterial plasma, in the 4 experimental groups.

2nd to the 3rd hour the decrease was not significant. The peak value for lactate and pyruvate was observed at 60 min ($P < 0.01$ and 0.05, respectively), at the time when FI_{CO_2} was back to zero.

In this group Pa_{O_2} was lower than the value found in group 1 at the end of the hypercapnic period (time 0) and it decreased more slowly. Potassium concentration in plasma began to diminish at the 2nd hour of recovery only ($P < 0.001$, fig. 3).

3. Fast recovery after acetazolamide injection (fig. 2)

Acetazolamide injection before sudden return to room air did not prevent the acid–base changes observed without the drug; however $[H^+]$ was a little higher ($P < 0.001$), but this value was already higher at the 72nd hour of hypercapnia ($P < 0.01$) as well as Pa_{CO_2} ($P < 0.05$). Pa_{O_2} was lower at time 0 ($P < 0.001$, fig. 3).

Differently from the return in room air without drug, after acetazolamide BE decreased gradually, and there was no lactate nor pyruvate peak value after 15 minutes of recovery. There was still a very significant ($P < 0.001$) increase, though, but this was not different from the control group. The differences between the group with fast recovery alone and the group with fast recovery plus acetazolamide were significant for lactate ($P < 0.02$) and pyruvate ($P < 0.05$) at the 15th minute of recovery. Pa_{O_2} decreased quickly, back to normal values. The decrease in plasmatic $[K^+]$ was more marked ($P < 0.01$) than during fast recovery without acetazolamide (fig. 3).

4. Control group (fig. 2)

As expected, acid–base variables were steady; however, there was a slight, non-significant, increase in blood lactate and pyruvate concentrations when the dogs were taken out the chamber. According to the *t*-test, the difference between control animals and dogs with fast post-hypercapnia recovery was significant ($P < 0.01$) for lactate concentration at the 15th minute of recovery, but not for pyruvate. There was no significant difference between control group and fast recovery with acetazolamide group as far as lactate and pyruvate were concerned.

Discussion

Some differences between groups were observed at the 72nd hour of hypercapnia (time 0) for Pa_{CO_2}, $[H^+]$ and Pa_{O_2}; they are difficult to explain, except by small variations in CO_2 exposition, or maybe by different individual CO_2 sensitivity of respiratory centers among the groups.

The increase in blood lactate was simultaneous with the decrease in Pa_{CO_2} and even more with the decrease in H^+ ions concentration. During fast recovery the peak value appears to be at 15 min when the average value of H^+ concentration was slightly decreased = 35.6 nmol/l (pH = 7.449). Actually, as the first lactate measurement was done at the 15th minute of recovery, we do not know the changes

before this point, and it is possible that the increase began earlier. This would be compatible with the small alkalosis already seen at the 5th minute of recovery when the mean values of 7 experiments were for $[H^+]$ 36.9 ± 1.90 nmol/l (pH = 7.436) and for Pa_{CO_2} 43.0 ± 3.2 torr (not shown in fig. 1). Moreover, after 1 hour of slow recovery the lactate peak value seems to appear when the mean $[H^+]$ was 32.2 nmol/l (pH = 7.493), which is close to the value cited above. In both cases alkalosis was slight but obvious. However, despite the persistence of blood alkalosis, later on blood lactate diminished towards normal values. The enhancement of glycolysis by alkalosis was certainly due to activation of phosphofructokinase inside the red blood cells (Minakami *et al.*, 1964). From this, one can assume that lactic acidosis must rather quickly restore intracellular red blood cell (RBC) pH, and thus secondarily depresses glycolysis, although extracellular pH remains alkalotic. This hypothesis needs to be tested. On the other hand, the enhancement of glycolysis could also be favoured by the fact that during chronic hypercapnia with low arterial pH, blood phosphofructokinase activity is depressed (Jacey and Schaefer, 1972). When arterial blood pH returns to a hardly alkaline value, this enzyme activity could be normalized, with an only transient phase of hyperactivity.

However it may be, extracellular alkalosis must be largely influenced by bicarbonate transfer from the interstitial space, which is less buffered than blood (Brown and Clancy, 1965; Dell and Winters, 1970) and maybe from heart intracellular space (Strome *et al.*, 1976). This leads to an increase in blood BE, of the same order of magnitude as during acute hyperventilation (Engel *et al.*, 1969). However, during hyperventilation, the sustained increase in blood lactate is a factor of rapid disappearance of this phenomenon. As the blood lactate increase was quite transient during recovery from hypercapnia blood BE remained elevated, at least during the 4-hour period of the experiment. Events concerning BE were quite similar during slow recovery.

The amount of acetazolamide given to the dogs was sufficient to block carbonic anhydrase in RBC (Maren, 1967) and it should have depressed CO_2 elimination and transiently slowed down the fall in tissular P_{CO_2}. But after return to room air one should have observed a lower Pa_{CO_2} than in group 1 (fast recovery), since inhibition of carbonic anhydrase lowers alveolar P_{CO_2} (Cain and Otis, 1961). Actually this phenomenon was not perceptible, and the changes in Pa_{CO_2} and in $[H^+]$ in group 3 were identical to those in group 1. The problems of measuring blood P_{CO_2} after carbonic anhydrase inhibition are well known. The reaction of CO_2 dehydration slowly continues inside the sampling syringe, and the arterial blood P_{CO_2} measured at equilibrium is then higher than *in vivo* (Maren, 1967). All P_{CO_2} measurements by Astrup's method have been carried out within the quarter of an hour following the blood sampling, so that the dehydration reaction was probably completed in the syringe before the measurement (Roughton, 1964). However, although arterial $[H^+]$ changes were identical in group 1 (without) and in group 3 (with acetazolamide), arterial $[H^+]$ values in group 3 were always above 40 nmol/l, hence there was no arterial alkalosis: therefore glycolysis was not any more enhanced

by the increase in RBC phosphofructokinase activity, and it is not surprising that arterial lactate and pyruvate concentration did not increase. It is possible that acetazolamide led to this result by delaying CO_2 elimination from RBC, and by maintaining some intracellular acidosis. It could also bring about an increase in bicarbonate loss by a renal mechanism. Arterial plasma bicarbonate concentration after acetazolamide fell from 32.84 ± 1.34 to 22.26 ± 0.77 mmol/l within 1 hour of recovery, whereas after hypercapnia alone the fall was from 32.34 ± 0.69 to 27.78 ± 0.37 mmol/l only ($P < 0.01$). At the same time, K^+ concentration in arterial plasma decreased much more ($P < 0.01$) after acetazolamide, seemingly owing to a renal loss of this ion (fig. 3). These events, probably due to an inhibition by carbonic anhydrase do not seem to last longer.

Acknowledgements

We are grateful to F. Schrijen for commenting on and translating the manuscript. This work was partly done with the valuable collaboration of R. Hennequin and Th. Colas. We wish to thank them for their help. We also thank E. Deloison and P. Ulmer for typing the manuscript and M. C. Rohrer for drawing the figures.

References

Bergmeyer, H. U. (ed.) (1965). Methods of Enzymatic Analysis. 2nd print. New York, Academic Press, p. 1064.

Brown, E. B., Jr. and R. L. Clancy (1965). *In vivo* and *in vitro* buffer curves. *J. Appl. Physiol.* 20: 885–889.

Cain, S. M. and A. B. Otis (1961). Carbon dioxide transport in anaesthetized dogs during inhibition of carbonic anhydrase. *J. Appl. Physiol.* 16: 1023–1028.

Dell, R. B. and R. W. Winters (1970). A model for the *in vivo* CO_2 equilibration curve. *Am. J. Physiol.* 219: 37–44.

Engel, K., P. Kildeberg and R. W. Winters (1969). Quantitative displacement of blood acid–base status in acute hypocapnia. *Scand. J. Clin. Lab. Invest.* 23: 5–17.

Hartemann, D., P. Horsky, T. Garcia Carmona, B. Hannhart and C. Saunier (1976). Intermédiaires de la glycolyse érythrocytaire au cours d'une hypercapnie de trois jours chez le chien. *Bull. Europ. Physiopath. Respir.* 12: 185–197.

Jacey, M. J. and K. E. Schaefer (1972). The effects of chronic hypercapnia on blood phosphofructokinase activity and the adenine nucleotide system. *Respir. Physiol.* 16: 267–272.

Maren, T. H. (1967). Carbonic anhydrase: chemistry, physiology, and inhibition. *Physiol. Rev.* 47: 595–581.

Minakami, S., T. Saito, C. Susuki and H. Yoshikawa (1964). The hydrogen ion concentration and erythrocyte glycolysis. *Biochem. Biophys. Res. Commun.* 17: 748–751.

Nahas, G. G., C. F. Poyart and L. Triner (1968). Acid–base equilibrium changes and metabolic alterations. *Ann. N.Y. Acad. Sci.* 150: 562–576.

Robin, E. D. (1963). Abnormalities of acid–base regulation in chronic pulmonary disease, with special reference to hypercapnia and extracellular alkalosis. *New Engl. J. Med.* 268: 917–922.

Roughton, F. J. W. (1964). Transport of oxygen and carbon dioxide. In: Handbook of Physiology. Section 3. Respiration. Vol. 1, edited by W. O. Fenn and H. Rahn. Washington D.C., Am. Physiol. Soc., pp. 767–825.

Strome, D. R., R. L. Clancy and N. C. Gonzales (1976). Myocardial CO_2 buffering: role of transmembrane transport of H^+ or HCO_3^- ions. *Am. J. Physiol.* 230: 1037–1041.

Zborowska-Sluis, D. T. and G. A. Klassen (1972). The effect of carbon dioxide and H^+ on canine erythrocyte glycolysis. *Respir. Physiol.* 15: 96–103.