Ionized calcium in acidosis: differential effect of hypercapnic and lactic acidosis

Schaer, H ; Bachmann, U

Abstract: The effects of various forms of acidosis on ionized calcium concentrations were investigated in vivo in rabbits and in vitro in human plasma. Calculation of least square regression equations of ionized calcium (mM) on pH yielded the following regression coefficients in human plasma: hypercapnic acidosis —0.53±0.07; hydrochloric acidosis —0.65±0.06; lactic acidosis —0.27±0.05. These findings in human plasma are roughly paralleled by those in rabbits. From stability constants it was calculated that the formation of Ca-lactate complexes accounts for the difference between lactic and hydrochloric acidosis. It is concluded that differences in the behaviour of ionized calcium between hypercapnic and lactic acidosis might contribute to the known differences in cardiovascular effects

DOI: https://doi.org/10.1093/bja/46.11.842

Br. J. Anaesth. (1974), **46**, 842

IONIZED CALCIUM IN ACIDOSIS: DIFFERENTIAL EFFECT OF HYPERCAPNIC AND LACTIC ACIDOSIS

H. SCHAER AND U. BACHMANN

SUMMARY

The effects of various forms of acidosis on ionized calcium concentrations were investigated in vivo in rabbits and in vitro in human plasma. Calculation of least square regression equations of ionized calcium (mM) on pH yielded the following regression coefficients in human plasma: hypercapnic acidosis -0.53 ± 0.07; hydrochloric acidosis -0.65 ± 0.06; lactic acidosis -0.27 ± 0.05. These findings in human plasma are roughly paralleled by those in rabbits. From stability constants it was calculated that the formation of Ca-lactate complexes accounts for the difference between lactic and hydrochloric acidosis. It is concluded that differences in the behaviour of ionized calcium between hypercapnic and lactic acidosis might contribute to the known differences in cardiovascular effects.

The effects of acidosis on the heart and circulation have been subjects of continuing interest. According to the present concept, acidosis modifies cardiac function both by direct and indirect mechanisms: by a depression of contractility, by a diminished responsiveness in the adrenaline-inotropic response mechanism, by a release of catecholamines, and by an increase in the concentration of ionized calcium (Schaer, 1974a). However, the implications of the last of these have not received proper consideration, despite the importance of calcium ions for myocardial contraction and for the release of neurotransmitter substances (Rubin, 1970).

It is generally recognized that the level of ionized calcium is affected by pH alterations. Due to a decreased protein-binding, the lowering of the pH from 7.4 to 6.9 leads to an increase in ionized calcium of 0.2–0.4 mM/litre (Moore, 1970; Schwartz, McConville and Christopherson, 1971; Pedersen, 1971; Höffken et al., 1971; Lindgärde, 1972), which is large enough to increase contractile force by about 30% in an isolated heart preparation. Whether this increase in ionized calcium is dependent on the way in which acidosis is produced has not been thoroughly investigated. The recent development of an accurate calcium-specific potentiometric method permits the determination of ionized calcium in small volumes of serum under anaerobic conditions (Moore, 1970). In this report, the effect of various forms of acidosis on ionized calcium are described. Preliminary studies consisted of in vivo experiments with rabbits, while the main part of this work is based on in vitro experiments with human plasma.

H. SCHAER,* M.D., P.D.; U. BACHMANN, M.D.; Department of Anesthesiology, University Clinics and Department of Pharmacology, University of Zurich, Switzerland.

*Present address: Department of Anesthesia, Kreisspital Männedorf, CH 8708 Männedorf—Zürich, Switzerland.

METHODS

Experiments with rabbits.

Rabbits of either sex weighing from 2.5 to 3.5 kg were anaesthetized with aprobarbitone (Numal, 1 mg/kg i.v.) and a tracheostomy was performed. A carotid artery was cannulated for the monitoring of arterial pressure and for blood sampling, and a jugular vein was cannulated for administering drugs. After intravenous injection of atropine sulphate 0.25 mg and alcuronium (Alloferin) 1 mg, the animals were hyperventilated with 100% oxygen using a Starling pump. For the induction of a respiratory pH-shift, the breathing mixture was consecutively changed to gases with increasing carbon dioxide concentration (5%, 10%, 20%, 30% in oxygen), each period lasting about 15 min. For the production of a metabolic acidosis, a molar solution of lactic acid was infused, using a Harvard infusion pump, at a rate of 0.764 ml/min. Arterial pressure signals were obtained with a Statham Pb23 transducer and, together with the e.c.g., were recorded on a direct-writing Offner dynograph. These two signals were monitored for control of the vital status of the animals and were not evalu-

ated. For the determination of pH, and ionized and total calcium, 2.5 ml of blood were needed. Blood for ionized calcium was heparinized with heparin 4 units/ml. It was stored and centrifuged under paraffin oil to avoid any loss of carbon dioxide and analysed immediately after termination of the animal experiments.

Experiments with human plasma.

Human plasma was obtained by centrifugation of heparinized blood (heparin 4 units/ml) drawn from healthy humans. Aliquots of plasma from the same person were used to perform one complete set of experiments comprising carbonic, hydrochloric and lactic acidosis. For every experiment, 2-ml aliquots of plasma were equilibrated with a gas mixture of 5% carbon dioxide and 95% oxygen at 37°C. For simulation of a metabolic acidosis, 10–60 μl of molar hydrochloric acid or molar lactic acid were added; for production of a respiratory acidosis, the plasma sample was equilibrated with 10%, 20% and 30% carbon dioxide in oxygen. Appropriate amounts of distilled water were added in the carbon dioxide experiments in order to obtain the same dilution of plasma as in the metabolic series.

Calcium (Ca^{++}) and pH determinations.

The pH of blood or plasma was measured with a capillary pH-electrode (Methrom BM1-08, pH-meter E388). Ionized calcium was determined with a potentiometric method (calcium-selective flow-through electrode No. 99-20, pH-meter model 801; Orion Research Corp., Cambridge, Mass.) (Moore, 1970; Studer, Knob and Binswanger, 1972). For the calibration of the electrode, standards with known concentrations of ionized calcium were used, as previously described (Schwartz, McConville and Christopherson, 1971; Schaer, 1974b). The total calcium was determined with the atomic absorption method (Perkin Elmer).

Heparin is known to complex calcium so that ionized calcium in heparinized blood is lower than in the corresponding serum (Moore, 1970; Ladenson and Bowers, 1973). This problem has been restudied by adding to 1 ml of serum 0.04 ml of heparin solutions of various strengths. The measured concentrations of ionized calcium were plotted against the concentration of heparin as shown in figure 1. This demonstrates that the concentrations of ionized calcium in serum and heparinized plasma are virtually identical as long as the concentration of heparin does not exceed 10 units/ml.

Calcium electrodes tend to become sensitive to hydrogen ions in the acid pH range. Ross (1967), however, had shown that, with the particular electrode employed, this would occur only at pH values below 5.5. In addition, the independence of the calcium readings from the hydrogen ion concentration actually occurring in this work was verified by equilibrating aqueous bicarbonate buffered physiological solutions with different carbon dioxide concentrations. It could be demonstrated, that calcium readings were not affected down to a pH of 6.6 (Schaer, 1974b).

Least square regression equations and tests for covariance were calculated according to Snedecor (1956) on a desk computer (Olivetti Programma 101).

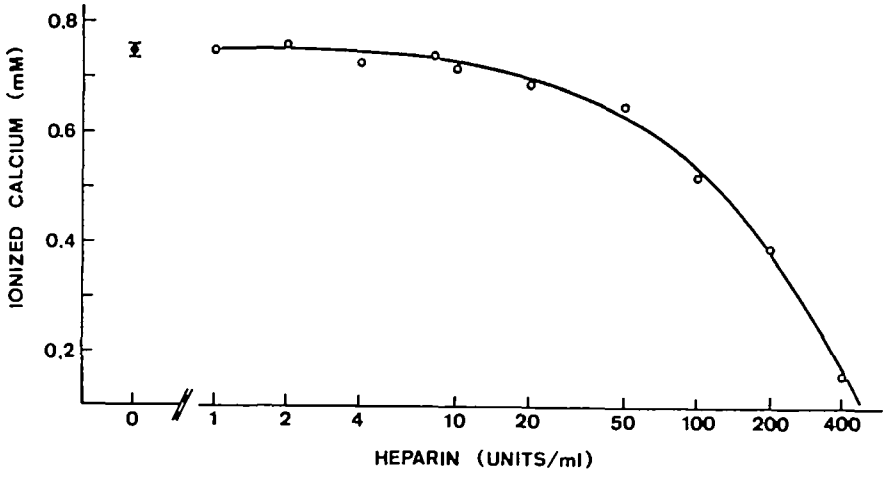


FIG. 1. Effect of heparin on ionized calcium. ●=serum O=plasma.

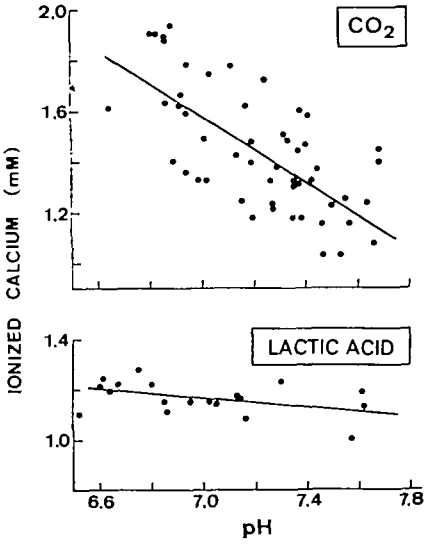
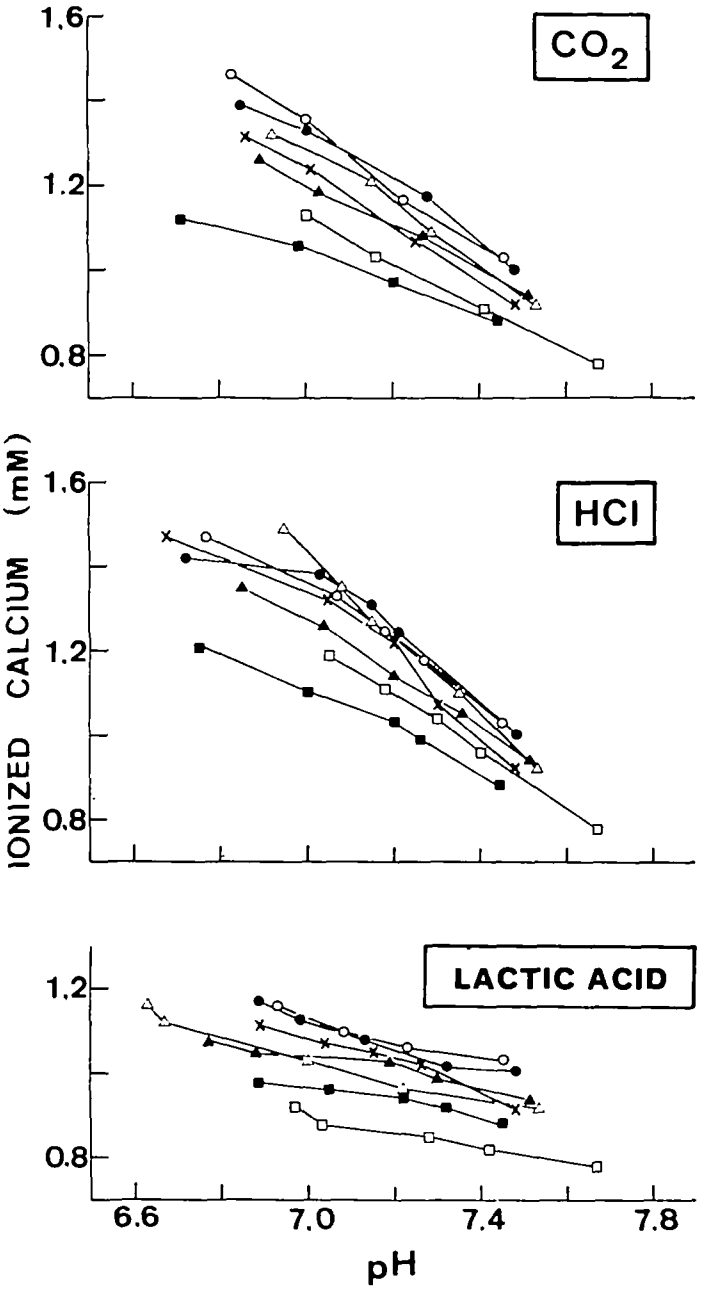


FIG. 2. (*above*). Effects of hypercapnic and lactic acidosis on plasma-ionized calcium in rabbits (mM=mM/litre).

FIG. 3. (*right*). Effects of hypercapnic, hydrochloric and lactic acidosis on ionized calcium in human plasma.



RESULTS

Effects of hypercapnic and lactic acidosis in rabbits.

The effect of a decreasing pH on ionized calcium is shown in figure 2. The following least square regression equations were calculated (Ca^{++} in mM/l). (Sample standard deviation of the regression coefficient s_b, correlation coefficient r.)

Hypercapnic acidosis, n=51 (7 rabbits):

$$[Ca^{++}] = -0.65\ pH + 6.14$$
$$s_b = 0.09 \qquad r = -0.69 \qquad P \ll 0.01$$

Lactic acidosis, n=19 (4 rabbits):

$$[Ca^{++}] = -0.09\ pH + 1.78$$
$$s_b = 0.04 \qquad r = -0.48 \qquad P < 0.05$$

The experiments with lactic acid deserve some comment. First, some haemolysis during acid infusion always occurred and could not be avoided even by using slower infusion rates or more dilute acid solutions. Secondly, the dilution effect of the infused fluid volume, which does not occur in the respiratory series, is likely to introduce a systematic error into the calcium/pH relationship. Therefore, these experiments should be considered as preliminary and are only presented to show the similarity of experimental findings obtained with those subsequently described in vitro.

Effects of various forms of acidosis on ionized calcium in human plasma.

The influence of a simulated respiratory and metabolic acidosis on ionized calcium is shown in figure 3. The following sample regression equations were obtained.

Hypercapnic acidosis, n=28:

$$[Ca^{++}] = -0.53\ pH + 4.97$$
$$s_b = 0.07 \qquad r = -0.82 \qquad P \ll 0.01$$

Hydrochloric acidosis, n=35:

$$[Ca^{++}] = -0.65\ pH + 5.83$$
$$s_b = 0.06 \qquad r = -0.86 \qquad P \ll 0.01$$

Lactic acidosis, n=35:

$$[Ca^{+}] = -0.27\ pH + 2.94$$
$$s_b = 0.05 \qquad r = -0.69 \qquad P \ll 0.01$$

The analysis of covariance among the three sample regressions of ionized calcium on pH have shown that the regression coefficient calculated from the lactic acid experiments was significantly smaller ($F = 11.1$; $P < 0.005$) than the regression coefficients obtained from hydrochloric or carbonic acidosis. A comparison between the effects of a respiratory acidosis in vivo (fig. 2) and in vitro (fig. 3) showed no significant difference between the two regression coefficients.

Total calcium concentration in these plasma samples amounted to 2.21 ± 0.06 mM/litre. Reducing the pH from 7.4 to 6.9 increased the fraction of ionized calcium from $44.7\% \pm$ SE 0.7 to $57.8\% \pm$ SE 0.9 in the carbon dioxide experiments, from $45.6\% \pm$ SE 0.9 to $60.4\% \pm$ SE 1.1 in the hydrochloric acid experiments, and from $43.2\% \pm$ SE 0.7 to only $48.1\% \pm$ SE 0.5 in the lactic acid experiments.

DISCUSSION

The effect of pH on the calcium binding of plasma proteins has been the subject of various investigations. Some of the more recent results are summarized in table I. Since there are different methods of presenting the results, to allow comparison, the approximate increase in the concentration of ionized calcium resulting from a pH-shift from 7.4 to 6.9 has been calculated from the published data. Our own findings in carbonic and hydrochloric acidosis are within the range of previously published values. Whether the pH-shift is induced in vivo or in vitro appears to make no difference. The most prominent finding of this study, however, is the much smaller decrease in ionized calcium associated with lactic acidosis in vivo as well as in vitro. Further implications of this will be discussed later.

We have found a linear regression of ionized calcium on pH, whereas others (Schwartz, McConville and Christopherson, 1971; Lindgärde, 1972) have used semilogarithmic scales. On theoretical grounds, the regression line of ionized calcium on pH is inversely S-shaped. It is understandable, therefore, that with the use of a semilogarithmic plot, a closer correlation is found when the pH extends to the alkaline range. On the other hand, it appears that the pH range from 7.6 to 6.9 lies within the linear part of the whole calcium-protein dissociation curve.

Our findings in rabbits and those of Höffken et al. (1971) in rats and of Hinkle and Cooperman (1971) in humans show that pH alterations affect the concentration of ionized calcium in vitro and in vivo in a similar fashion. These experiments were all completed in less than 120 min and can be considered as models of acute types of acidosis. On

TABLE I. *Summary of recent investigations on ionized calcium in acidosis.*

Author	Methods of Ca-determinations	Type of acidosis	Regression of ionized calcium (mM/litre) on pH	Increase in ionized calcium per 0.5 pH unit (mM/litre)
Moore, 1970	Humans; potentiometric	Not stated	Linear	0.21[1]
Pedersen, 1971	Dialysed serum; spectrophotometric	HCl and CO_2	Linear	0.25[1]
Schwartz et al., 1971	Serum; potentiometric	Not precisely stated	Semilogarithmic; slope −0.30	0.41[1] (pH 7.4 to 6.9)
Höffken et al., 1971	Rats in vivo; potentiometric	HCl	Linear; slope −0.41	0.20[1]
		CO_2	slope −0.46	0.23[1]
Hinkle and Cooperman, 1971	Humans in vivo; potentiometric	CO_2	Linear; slope -0.64 ± 0.12[1]	0.32[1]
Lindgärde, 1972	Serum in vitro; potentiometric	HCl and CO_2	Semilogarithmic; slope −0.279	0.40[1] (pH 7.4 to 6.9)
Present paper	Rabbits in vivo; potentiometric	CO_2	Linear; slope -0.65 ± 0.09	0.32
	Plasma in vitro; potentiometric		Linear; slopes	
		CO_2	-0.57 ± 0.07	0.28
		HCl	-0.65 ± 0.06	0.32
		Lactic acid	-0.27 ± 0.05	0.14

[1]These values were calculated by the present authors from data given in the relevant papers.

the other hand, it is generally accepted that the concentration of ionized calcium is regulated within very narrow limits under physiological conditions. The variability of ionized calcium is much smaller than that of total calcium and the variations of total calcium are almost fully accounted for by corresponding variations in its protein-bound fraction (Moore, 1970; Pedersen, 1972). This raises the question of whether ionized calcium would be regulated at the same level under all pH conditions or if, in chronic types of acidosis, an increased level of ionized calcium would be maintained by the calcium control mechanisms. On the basis of available data, any assumptions about this are purely speculative. In preliminary hyperventilation experiments in humans, Lindgärde (1972) found a smaller decrease in ionized calcium in vivo than in vitro and concluded that the pH-mediated increased protein-calcium binding would be partly counteracted by calcium ion homeostatic control mechanisms. This opinion is contradicted by the findings of Höffken et al. (1971) in rats that the pH-mediated alterations in ionized calcium were paralleled by alterations in total calcium.

A drawback, of theoretical rather than practical importance, is the lack of temperature control of the calcium-electrode. A decrease in temperature leads to an increase in pH of 0.0118 pH units/C° (Rosenthal, 1948), while at the same time the protein binding of calcium increases (Gupta, 1967). However, these two factors, having opposite effects on ionized calcium, largely compensate for each other, so that ionized calcium measured at 37°C is only about 0.02–0.03 m.mol/litre less than at 25°C (Hansen and Theodorsen, 1971; Ladenson and Bowers, 1973). We have, therefore, adopted the practice of all the other authors quoted in table I and have used the pH values measured at 37°C and the values of ionized calcium determined at room temperature. There is no doubt that this procedure has introduced a systematic error in our results and in all those reported in table I also. This error, however, is quite small and will not affect the conclusion drawn from our data.

Lactic acidosis is a clinically important form of metabolic acidosis, appearing whenever the oxygen supply to tissues is impaired (Huckabee, 1958). The severest form of this condition occurs in cardiac arrest. In the customary acid-base terminology, the expression "negative base excess" (−BE) is used to designate an excess of non-volatile acid (Siggaard-Andersen, 1965). In order to bring the data determined in lactic acidosis closer to the clinical situation, pH was plotted against the concentration of lactic acid and against the base excess (fig. 4). In the same figure, pH values taken from the "blood acid-base alignment nomogram" (Siggaard-Andersen, 1963) were drawn. It can be seen

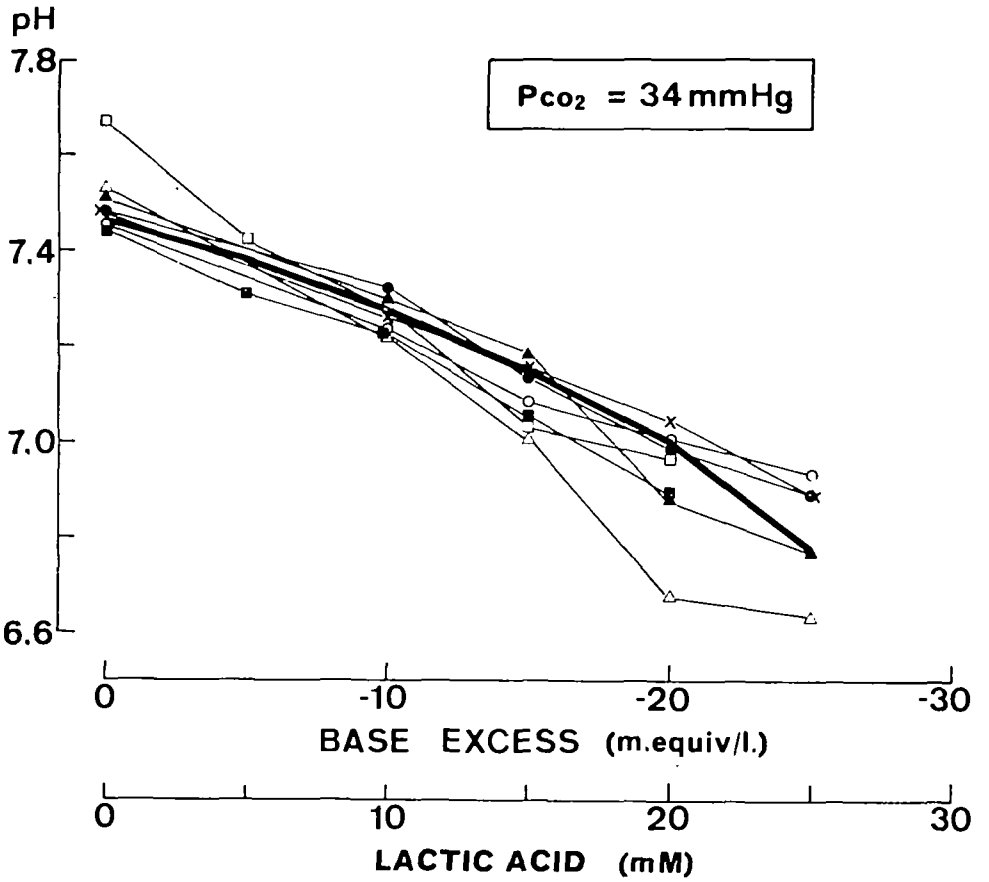


FIG. 4. In-vitro experiments with human plasma. Comparison of the experimental pH/lactate relationship (thin line) with the dependence of pH on base excess read from the alignment nomogram from Siggaard-Andersen (1963) (thick line).

that our experimental points correspond closely with pH values read from the nomogram.

The stability constants for the calcium-lactate (CaL) complexes have been determined (Verbeek and Thun, 1965). These authors found stability constants $K_1=8.0$ litre/mol ($\log \beta_1=0.90\pm0.01$) for the CaL^+ complex, and $K_2=17.4$ litre/mol ($\log \beta_2=1.24\pm0.015$) for the CaL_2 complex. This indicates that CaL^+ complexes will be formed almost exclusively. In figure 5 regressions of ionized calcium on base excess (added hydrochloric or lactic acid) were drawn, and the concentration of the calcium-lactate complexes calculated from the stability constants are indicated by the vertical columns. This presentation shows that the formation of calcium lactate complexes accounts approximately for the difference in ionized calcium between hydrochloric and lactic acidosis.

As has been mentioned in the introduction, acidosis affects the heart and circulation by both

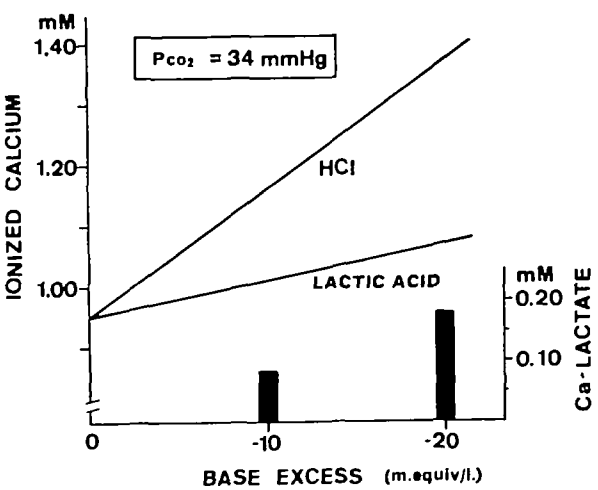


FIG. 5. In-vitro experiments with human plasma. Dependence of ionized calcium on base excess in hydrochloric and lactic acidosis. The columns indicate the concentration of calcium-lactate complexes calculated from the stability constants.

direct and indirect mechanisms. The numerous descriptions of the overall circulatory effects of acidosis lack uniformity. It appears that most of the discrepancies may be attributed to differences in the experimental methods. However, the few comparative investigations on the effects of hypercapnic and lactic acidosis in intact animals are consistent (Ligou and Nahas, 1960; Carson et al., 1965). A respiratory acidosis of a moderate degree (pH 7.0) leads to an increase in arterial pressure and cardiac output, whereas, in a comparable lactic acidosis, cardiovascular functions are unchanged or depressed. The difference between the circulatory actions of hypercapnic and lactic acidosis was at least partially explained by a greater excitation of the sympathetic nervous system and release of catecholamines in hypercapnic than in lactic acidosis (Ligou and Nahas, 1960; Nahas et al., 1967). The present study points to another important difference in humoral background, namely, the concentration of ionized calcium between these two forms of acidosis. Implications of this condition can be illustrated by one example. Acidosis decreases myocardial contractile force in isolated heart preparations irrespective of the way it is produced (Schaer, 1974a). In intact animals this depressant effect of hydrogen ions would be more than compensated by the concomitant increase in ionized calcium during hypercapnic acidosis, whereas the much smaller increase in ionized calcium during lactic acidosis would be insufficient to overcome the myocardial depression.

In view of the importance of calcium ions for myocardial contraction and catecholamine secretion (Kirpekar and Misu, 1967; Rubin, 1970; Stjärne, 1973), differences in the behaviour of ionized calcium appear to contribute to the different cardiovascular actions of respiratory and lactic acidosis.

ACKNOWLEDGEMENTS

The authors are indebted to Dr U. Binswanger, Dept. of Medicine, University of Zurich, for the use of the calcium-electrode and to PD Dr H. Rosenmund, Central Laboratory, Kantonsspital, Zurich, for the determinations of total calcium.

The authors are also indebted to Miss E. Fahrnbühl and to Mrs V. Müller-Kilchenmann for their expert technical assistance, to Dr U. Binswanger for advice concerning the determinations of ionized calcium and to Dr A. Hofmann for valuable discussions.

REFERENCES

Carson, S. A. A., Chorley, G. E., Hamilton, F. N., Lee, D. C., and Morris, L. E. (1965). Variation in cardiac output with acid-base changes in the anesthetized dog. *J. Appl. Physiol.*, **20**, 948.

Gupta, G. S. (1967). Effect of temperature on calcium interactions in normal human plasma. *Ind. J. Biochem.*, **4**, 58.

Hansen, S. O., and Theodorsen, L. (1971). The usefulness of an improved calcium electrode in the measurement of ionized calcium in serum *Clin. Chim. Acta*, **31**, 119.

Hinkle, J. E., and Cooperman, L. H. (1971). Serum ionized calcium changes following citrated blood transfusion in anaesthetized man. *Br. J. Anaesth.*, **43**, 1108.

Höffken, B., Parkinson, D. K., Storms, P., and Radde, I. C. (1971). Effects of alterations of blood pH on calcium ion activity in rat plasma. *Clin. Orthop.* **78**, 30.

Huckabee, W. E. (1958). Relationships of pyruvate and lactate during anaerobic metabolism. III: Effect of breathing low-oxygen gases. *J. Clin. Invest.*, **37**, 264.

Kirpekar, S. M., and Misu, Y. (1967). Release of noradrenaline by splenic nerve stimulation and its dependence on calcium. *J. Physiol.*, **188**, 219.

Ladenson, J. H., and Bowers, G. N. (1973). Free calcium in serum. I: Determination with the ion-specific electrode, and factors affecting the results. *Clin. Chem.*, **19**, 565.

Ligou, J. C., and Nahas, G. G. (1960). Comparative effects of acidosis induced by acid infusion and CO_2 accumulation. *Am. J. Physiol.*, **198**, 1201.

Lindgärde, F. (1972). Potentiometric determination of serum ionized calcium in a normal human population. *Clin. Chim. Acta*, **40**, 477.

Moore, E. W. (1970). Ionized calcium in normal serum, ultrafiltrates, and whole blood determined by ion-exchange electrodes. *J. Clin. Invest.*, **49**, 318.

Nahas, G. G., Zagury, D., Milhaud, A., Manger, W. M., and Pappas, G. D. (1967). Acidemia and catecholamine output of the isolated canine adrenal gland. *Am. J. Physiol.*, **213**, 1186.

Pedersen, K. O. (1971). The effect of bicarbonate, P_{CO_2} and pH on serum calcium fractions. *Scand. J. Clin. Lab. Invest.*, **27**, 145.

—— (1972). On the cause and degree of intraindividual serum calcium variability. *Scand. J. clin. Lab. Invest.*, **30**, 191.

Rosenthal, T. B. (1948). The effect of temperature on the pH of blood and plasma in vitro. *J. Biol. Chem.*, **173**, 25.

Ross, J. W. (1967). Calcium-selective electrode with liquid ion exchanger. *Science*, **156**, 1378.

Rubin, R. P. (1970). The role of calcium in the release of neurotransmitter substances and hormones. *Pharmacol. Rev.*, **22**, 389.

Schaer, H. (1974a). Influence of respiratory and metabolic acidosis on epinephrine-inotropic effect in isolated guinea pig atria. *Pflügers Arch.*, **347**, 297.

—— (1974b). Decrease in ionized calcium by bicarbonate in physiological solutions. *Pflügers Arch.*, **347**, 249.

Schwartz, H. D., McConville, B. C., and Christopherson, E. F. (1971). Serum ionized calcium by specific ion electrode. *Clin. Chim. Acta*, **31**, 97.

Siggaard-Andersen, O. (1963). Blood acid-base alignment nomogram. *Scand. J. Clin. Lab. Invest.*, **15** 211.

—— (1965). *The Acid-base Status of the Blood*, 3rd, edn. Copenhagen: Munksgaard.

Snedecor, G. W. (1956). *Statistical Methods*, 5th edn. Ames, Iowa: State University Press, Iowa.

Stjärne, L. (1973). Michaelis-Menten kinetics of secretion of sympathetic neurotransmitter as a function of external calcium: effect of graded alpha-adrenoceptor blockade. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **278**, 323.

Studer, O., Knob, M., and Binswanger, U. (1972). Kalziumkonzentration im Serum. *Schweiz. med. Wochenschr.*, **102**, 305.

Verbeek, F., and Thun, H. (1965). The stability constants of the alkaline earth lactate and α-hydroxyisobutyrate complexes. *Anal. Chim. Acta*, **33**, 378.