Malaya in Singapore for making available blood samples from Macaca cynomolgus, to Mr. J. T. FORBES of the Singapore Turf Club for providing the horse blood, and to Mr. STEPHEN PANG for his technical assistance.

F. VELLA

Department of Biochemistry, Faculty of Medicine, University of Malaya, Singapore, June 25, 1959.

Résumé

Le traitement des érythrocytes de bœuf (*Bos banteng*), de singe (*Macaca cynomolgus*), de cheval et de sujets humains avec du nitrite de sodium n'a pas d'influence sur la rapidité de l'hémolyse spontanée.

Bicarbonate Excretion after Prolonged Exposure to Carbon Dioxide in the Normal Dog

SULLIVAN and DORMAN[1] have shown that exposure of dogs to an atmosphere containing approximately 10% CO_2 during periods extending from 2 to 77 days, markedly increased the tubular reabsorption of bicarbonate. This increment was much more important than in experiments where arterial pCO_2 was acutely raised to similar levels. No satisfactory explanation was given to this phenomenon.

Previous work from this laboratory[2,3] demonstrated that hypochloremia increases tubular bicarbonate reabsorption. On the other hand, hypochloremia is a common component of the plasma electrolyte pattern of chronic respiratory acidosis in the human[4]. These facts seemed to afford a clue to the understanding of the process which increases the reabsorbtion of bicarbonate by the renal tubules during prolonged exposure to CO_2. The data to be presented demonstrate that the further increase in bicarbonate reabsorption observed during chronic respiratory acidosis as compared to acute respiratory acidosis, is intimately linked to the hypochloremia which characterises the chronic condition.

Methods. 9 female dogs, weighing from 11 to 29 kg, were used in 3 types of experiments.

(1) *Controls* (5 experiments, 54 clearance periods): intact dogs were infused with a solution of 1·2% $NaHCO_3$ in 0·1% creatinine at rates ranging from 8 to 10 ml/min, after priming doses of 6 g $NaHCO_3$ and 1 g creatinine. Plasma bicarbonate concentration was increased in a steplike manner by 3 successive priming doses of 3 to 9 g bicarbonate, according to the size of the animal, injected intravenously at 35–45 min intervals. Between these injections, the sustaining infusion was pursued at constant rate, and arterial blood and urine were collected anaerobically.

The usual clearance method was used throughout with urine collection periods ranging from 8 to 12 min. From 10 to 15 clearance periods were obtained for each experiment.

(2) *Acute respiratory acidosis* (5 experiments, 60 clearance periods): the same schedule was used as in the control experiments, except that the animals were breathing 8–12% CO_2 in oxygen under very light Pentothal anesthesia during the whole procedure. Plasma bicarbonate concentration was similarly raised by 3 successive priming doses of 3 to 9 g $NaHCO_3$.

(3) *Chronic respiratory acidosis* (9 experiments): the dogs were maintained during 4 to 6 days in an oxygen tent containing approximately 50% oxygen and from 7 to 10% CO_2. These animals were divided into 2 groups:

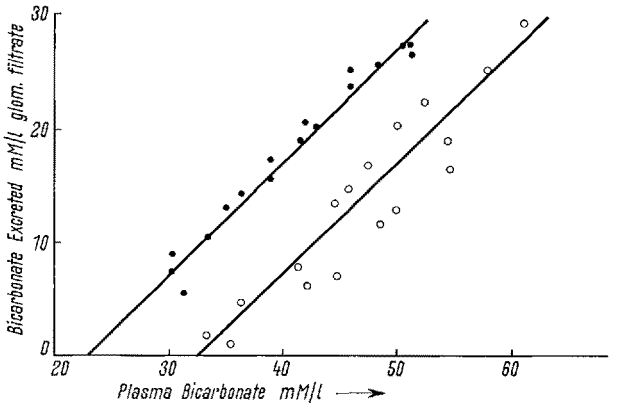

Fig. 1

• Controls	mean PCl = 99 mEq/l (89–110) mean pCO_2 = 46 mmHg (42–51)
○ Chronic CO_2-exposure	mean PCl = 81 mEq/l (76–90) mean pCO_2 = 54 mmHg (39–68)

Fig. 1.—Bicarbonate excretion rate in intact control dogs (black circles) and in dogs previously submitted to 4–6 days CO_2 exposure but breathing room air while infused with bicarbonate solution (open circles). Each symbol represents the mean of 3 successive urine collection periods. The mean values of pCO_2 and plasma chloride concentration (PCl) for each group are presented.

(a) *in the first group* (4 experiments, 54 clearance periods) after their removal from the tent, the dogs were submitted to the same procedure as in the controls,

(b) *in the second group* (5 experiments, 66 clearance periods), 1 to 3 min after their removal from the tent, the animals were lightly anesthetised with Pentothal and submitted to the same procedure as in the acute respiratory acidosis experiments.

Results. (1) *Controls* (see Fig. 1): In Figure 1, the excretion rate of bicarbonate in the intact dogs has been expressed in *mM*/l of glomerular filtrate (*mM*/l GF) and the mean values of this ratio, calculated from 3 successive clearance periods, plotted with corresponding plasma bicarbonate concentration mean values. The linear correlation between bicarbonate excretion rate and plasma bicarbonate concentration is similar to that observed by PITTS and LOTSPEICH[5] in the same type of experiments. The relationship meets the abcissae at a plasma concentration value of 22·5 *mM*/l which constitutes the theoretical threshold for bicarbonate. The slope of the curve is given by the ratio (bicarbonate excretion rate, *mM*/l GF)/plasma bicarbonate concentration *mM*/l-22·5). This ratio has a mean value of 0·970 for the 5 experiments instead of a theoretical value of 1·00. It should be noted that plasma chloride concentration has a mean value of 99 mEq/l (range 89–110) during these control experiments.

[1] W. J. SULLIVAN and P. J. DORMAN, J. clin. Invest. *34*, 268 (1955).

[2] CH. TOUSSAINT, M. TELERMAN, and P. VEREERSTRAETEN, Exper. *14*, 417 (1958).

[3] CH. TOUSSAINT, M. TELERMAN, and P. VEREERSTRAETEN, Exper. *15*, 232 (1959).

[4] J. R. ELKINTON and T. S. DANOWSKI, *The Body Fluids, Basic Physiology and Practical Therapeutics* (Williams and Wilkins Co., Baltimore 1955).

[5] R. F. PITTS and W. D. LOTSPEICH, Amer. J. Physiol. *147*, 138 (1946).

(2) *Acute respiratory acidosis* (see Fig. 2): Figure 2 shows in a similar manner the correlation between bicarbonate excretion rates and plasma bicarbonate concentrations in experiments where arterial pCO_2 was acutely raised in otherwise intact animals. The slope of the curve (0·942) is virtually the same as in the controls but the bicarbonate threshold value has been increased to 27·5 *mM*/l, due to the rise of arterial pCO_2 mean value from 46 to 73 mm Hg. During these experiments, plasma chloride concentration has a mean value of 94 mEq/l (range 83–106).

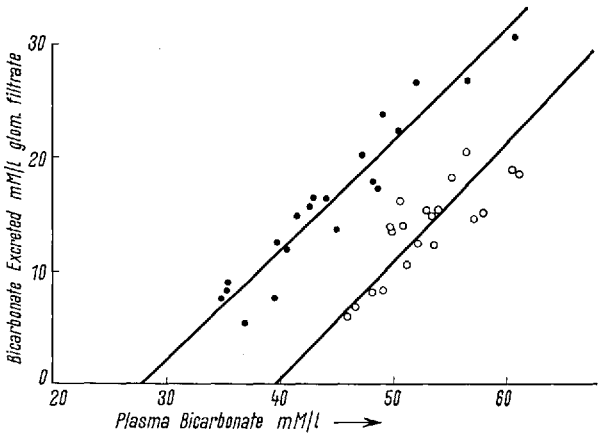

Fig. 2

• Acute Resp. Acidosis	mean PCl = 94 mEq/l (83–106) mean pCO_2 = 73 mmHg (59–86)
o Acute Resp. Acidosis After chronic CO_2 exposure	mean PCl = 81 mEq/l (13–92) mean pCO_2 = 76 mmHg (63–96)

Fig. 2. — Bicarbonate excretion rate during acute respiratory acidosis (black circles) and in dogs previously submitted to 4–6 days CO_2 exposure and breathing 8–12%. CO_2 while infused with bicarbonate solution (open circles). Same presentation as in Figure 1.

(3) *Chronic respiratory acidosis* (Fig. 1 and 2):

(a) *In animals breathing room air:* within a few minutes after their removal from the 'CO_2 tent', these dogs show a precipitous fall in arterial pCO_2 due to the high diffusibility of the gas. At this moment, plasma bicarbonate concentration is still at a high value because of the slowness of the renal adjustment mechanism to the acute decrease in arterial pCO_2. Figure 1 shows that the bicarbonate excretion rate-plasma concentration relationship has a slope (0·900) which is not significantly different[6] from that observed during bicarbonate infusion in intact dogs. Indeed the data are somewhat more scattered due to the larger dispersion of the pCO_2 values in the chronic experiments. It is however apparent that the bicarbonate threshold value has been increased to 32·0 *mM*/l by the previous exposure to CO_2. The mean value of plasma chloride concentration observed in these experiments is 79 mEq/l (range 72–90). In a first stage, chloremia is decreased from 112 (range 111–115) to 98 mEq/l (range 96–104) by the prolonged exposure to CO_2. In a second stage, it is further depressed to the 79 mEq/l level by the bicarbonate infusion.

(b) *In animals breathing 8–12% CO_2:* it is readily apparent from Figure 2 that for a given arterial pCO_2 value of approximately 70 mm Hg tubular bicarbonate reabsorption has been increased by the prolonged exposure to CO_2 in the tent: the plasma threshold value rises from 27·5 in acute respiratory acidosis to 39·7 *mM*/l in the chronic condition. Here again the slope of the bicarbonate excretion rate-plasma concentration relationship remains constant (1·029). As for the preceeding group, plasma chloride concentration decreases simultaneously to a mean value of 81 mEq/l (range 73–92).

Discussion. Our data confirm the important increase in tubular bicarbonate reabsorption observed by SULLIVAN and DORMAN[1] after prolonged exposure of dogs to CO_2.

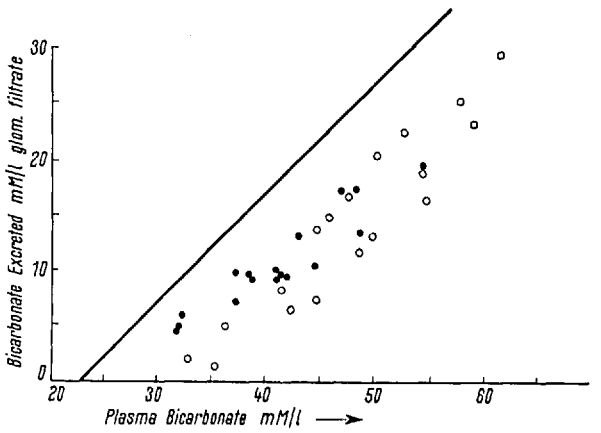

Fig. 3

• Hypochlor. alk.	mean PCl = 84 mEq/l (71–95) mean pCO_2 = 37 mmHg (29–48)
o Chronic CO_2 exposure	mean PCl = 81 mEq/l (76–90) mean pCO_2 = mmHg 54 (39–68)

Fig. 3. — Bicarbonate excretion rate in dogs made hypochloremic by hemodialysis or peritoneal lavage (black circles) and in dogs previously submitted to 4–6 days CO_2 exposure while infused with bicarbonate solution (open circles). Same presentation as in Figure 1.

The observations made on the animals breathing room air after removal from the 'CO_2 tent' further show that bicarbonate reabsorption is also increased at normal pCO_2 levels.

The increment of tubular bicarbonate reabsorption could be explained by one of the following hypotheses: (1) a fall of GFR would increase the renal bicarbonate threshold value; (2) prolonged exposure to CO_2 could enhance the activity of the carbonic anhydrase system of the tubules; (3) prolonged exposure to CO_2 could lead to some potassium depletion in the tubular cells; (4) the hypochloremia following prolonged exposure to CO_2 would be of a sufficient magnitude to account for the increase in bicarbonate reabsorption.

Hypothesis 1: THOMPSON and BARRETT[7] have shown in dogs that an acute fall in GFR is followed by an increase in bicarbonate threshold value because bicarbonate reabsorption by the tubules remains unabated despite the reduction of the bicarbonate filtered load. To rule out the possibility that a fall in GFR during prolonged exposure to CO_2 would be responsible for the increase in bicarbonate threshold, GFR was estimated by the endogenous creatinine clearance, using 24 h urine collection periods, in dogs observed in metabolic cages before and during prolonged exposure to CO_2. In none of 6 such experiments was a drop of GFR observed. Moreover, GFR constantly rises during the acute experiments performed

[6] Statistical calculation actually shows that there are no significant differences between the slopes of the bicarbonate excretion rate-plasma concentration relationship obtained in the 4 different types of experiments.

[7] D. D. THOMSON and M. J. BARRETT, Amer. J. Physiol. *176*, 201.

after prolonged CO_2 exposure as it is usual to observe in dogs by the rapid infusion of any electrolyte solution[8]. It can be concluded that a decrease in GFR does not account for the increment of bicarbonate threshold values in these experiments.

Hypothesis 2: A change in the activity of the carbonic anhydrase system of the renal tubules seems improbable, since CARTER *et al.*[9] could not find any change in carbonic anhydrase concentration in the kidney of rats submitted to prolonged exposure to CO_2.

Hypothesis 3: Potassium depletion of the tubule cells would constitute a better explanation for the enhancement of bicarbonate reabsorption following prolonged CO_2 exposure, since the reabsorption of this ion was found to be increased during potassium depletion in the dog[10] and in the human[11]. That prolonged exposure of dogs to CO_2 does not induce negative potassium balance is readily apparent from metabolic studies conducted in 6 dogs during chronic CO_2 exposure. It should be noted, however, that the usual drop in plasma potassium concentration following $NaHCO_3$ infusion is always more pronounced in animals previously submitted to prolonged CO_2 exposure. However, a direct action of hypokaliemia itself on bicarbonate reabsorption seems to be ruled out by the lack of effect of insulin or bicarbonate-induced hypokaliemia on bicarbonate reabsorption[11].

Hypothesis 4: Hypochloremia induced by renal adjustment to chronic respiratory acidosis[12] seems to constitute the main cause of the enhancement of tubular bicarbonate reabsorption following prolonged exposure to CO_2 atmosphere. Comparison between control dogs and animals breathing room air but having previously inhalated CO_2 for 4 to 6 days (Fig. 1) shows the following points: (1) arterial pCO_2 has practically the same mean value in both groups (46 and 51 mm Hg, respectively); (2) plasma chloride concentration values are very different: 99 mEq/l in the controls and 79 mEq/l in the experiments involving previous exposure to CO_2; (3) bicarbonate threshold values vary accordingly: 22·5 *mM*/l in the controls and 32·0 *mM*/l in the second group of experiments.

Comparison between the acute respiratory acidosis experiments, and similar experiments involving previous exposure of the animals to CO_2 atmosphere for 4 to 6 days, yields identical results (Fig. 2): (1) arterial pCO_2 has the same mean value in both groups (73 and 76 mm Hg); (2) plasma chloride concentration values are 94 and 81 mEq/l respectively; (3) bicarbonate threshold values are 27·5 and 39·7 *mM*/l respectively. It can be seen that for the two groups of chronic experiments a mean decrease of some 17 mEq/l in plasma chloride concentration is accompanied by a mean increase of approximately 11 *mM*/l in bicarbonate threshold value for similar arterial pCO_2 levels.

In Figure 3, bicarbonate excretion rates at normal pCO_2 levels in dogs previously submitted to prolonged CO_2 exposure are compared to bicarbonate excretion rates (51 clearance periods) at slightly higher arterial pCO_2 levels in 13 animals made acutely hypochloremic by hemodialysis[2] or peritoneal lavage[3]. Plasma chloride concentration is decreased to approximately the same mean value (84 and 81 mEq/l respectively) in both groups.

There is obviously no difference between the bicarbonate excretion rate-plasma concentration relationships in the two types of hypochloremia investigated. Thus, despite entirely different experimental procedures and marked difference in durations of induction, an equal drop in plasma chloride concentration is accompanied by a rise in tubular bicarbonate reabsorption of an equal magnitude.

On the other hand, an increase in plasma chloride concentration has been shown to decrease bicarbonate reabsorption in the dog, during acute respiratory acidosis[13] or at normal pCO_2 levels[5].

It is concluded that hypochloremia is the main cause, perhaps the only cause, of the supplementary increase in tubular bicarbonate reabsorption observed after prolonged exposure to CO_2. The mode of action of the Cl ion on the bicarbonate reabsorption mechanism is unknown.

CH. TOUSSAINT, M. TELERMAN, and P. VEREERSTRAETEN

Laboratoire de Médecine expérimentale, Université Libre de Bruxelles et Fondation Médicale Reine Elisabeth, Bruxelles (Belgique), May 15, 1959.

Résumé

L'excrétion du bicarbonate a été étudiée chez des chiens ayant préalablement séjourné pendant des périodes de 4 à 6 jours dans une atmosphère contenant de 7 à 10% de CO_2.

Ce procédé a pour effet d'accroître fortement la réabsorption tubulaire du bicarbonate par un mécanisme distinct de l'action de l'accroissement de la pression partielle du CO_2 et analogue à celui qui augmente la réabsorption du bicarbonate au cours de l'hypochlorémie aiguë réalisée par des procédés de vividialyse.

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[9] N. W. CARTER, D. W. SELDIN, and H. C. TENG, J. clin. Invest. *38*, 949 (1959).

[10] Unpublished data.

[11] K. E. ROBERTS, H. T. RANDALL, H. L. SANDERS, and M. HOOD, J. clin. Invest. *34*, 666 (1955).

[12] H. LEVITIN, W. BRANSCOME, and F. H. EPSTEIN, J. clin. Invest. *37*, 1667 (1958).

[13] J. G. HILTON, N. E. CAPECI, G. T. KISS, O. R. KRUESI, V. V. GLAVIANO, and R. WEGRIA, J. clin. Invest. *35*, 481 (1956).

Oxydation von Bernsteinsäure durch Leber akut urämischer Ratten[1]

5. Mitteilung
über Untersuchungen der Rest-N-fraktionen bei Urämie

In der akut urämischen Rattenleber sind Desaminierung und Transaminierung von DL-Alanin signifikant gesteigert[2]. Dieser Befund ist für den urämischen Organismus in zweifacher Hinsicht bedeutungsvoll.

Aminosäuren werden entweder zu Ketosäuren desaminiert bzw. transaminiert oder zu Aminen dekarboxyliert. Bekanntlich haben Amine an Mensch und Tier pharmakologisch-toxische Wirkungen. Da bei akuter experimen-

[1] Die vorliegende Arbeit wurde mit Unterstützung des Schweizerischen Nationalfonds durchgeführt.

[2] R. BOSSHARDT, H. THÖLEN und F. ENDERLIN, Exper. *13*, 497 (1957).