(1) the enzyme acting on the receptor of the frog rectus muscle is pseudo ChE (see Table II and [8]), therefore ButCh is a good substrate of the enzyme pseudo ChE and also a good receptor-stimulator;

(2) tertACh has little or no biological effect on the receptor, because its hydrolysis in stage A produces dimethylaminoethanol (tertCh) which probably has a low affinity for the stage B receptor-component (compare with Table III, where the acyl moiety is kept constant, while the choline moiety changes). Thus tertACh's behaviour is analogous to that of ASCh towards the muscarinic receptor, ASCh being a very good substrate for ChE-s, even though it has no affinity for the muscarinic receptor.

M. WURZEL

The Hebrew University, Hadassah Medical School, Department of Physiology, Jerusalem (Israel), June 5, 1959.

Résumé

Le mécanisme d'action des esters carboxyliques de la choline commence, probablement, par une hydrolyse enzymatique provoquée par une des cholinestérases. Les produits de l'hydrolyse réagissent avec un facteur inconnu, dont la spécificité est cependant définissable par la structure du résidu cholinique.

[8] J. DECHAVASSINE, Exper. *12*, 434 (1956).

Observations on Spontaneous Hemolysis in Shed Blood

The existence of a relation between the content of reduced glutathione of erythrocytes and their rate of spontaneous hemolysis has been suggested by various workers (LEMBERG and LEGGE[1]).

FEGLER[2] concluded from a study of horse blood that the rate of hemolysis bears a certain relation to the extent of oxidation of the reduced glutathione. KEILIN and HARTREE[3] found that the treatment of horse erythrocytes with sodium nitrite, though it produced complete oxidation of the reduced glutathione and converted the hemoglobin to methemoglobin, did not result in hemolysis of the erythrocytes until after 12 hours of storage in the cold. Since these two views appear to be mutually exclusive, a study of the relationship between the rate of hemolysis of nitrite treated and untreated erythrocytes and their glutathione content was attempted.

Blood samples from 10 Bali oxen (*Bos banteng*), 3 monkeys (*Macaca cynomolgus*), and 1 horse were collected in flasks containing potassium oxalate crystals, while those from 4 normal human subjects were collected in flasks containing heparin. The erythrocytes were washed with a 1% NaCl aqueous solution, 3–4 times with centrifuging. Aliquots were treated with equal volumes of an isotonic solution of $NaNO_2$ (1·06% in water) for 1–2 min and the nitrite was removed using the saline, washing 3–4 times, and centrifuging. The treated and untreated erythrocytes were washed twice with isotonic saline which contained: Streptomycin, 1 mg/ml, and 1000 units of penicillin, and then measured volumes of these cells were suspended, separately, in equal amounts of the isotonic saline. These cells were contained in centrifuge tubes, plugged with cotton wool and kept in a water bath at 37°C. The total hemoglobin and hemoglobin in the supernatant fluid were measured by a modification of the methode for plasma hemoglobin (DACIE[4]). Reduced glutathione was measured by the nitroprusside colour reaction of THOMPSON and WATSON[5]. The type of hemoglobin contained in the erythrocytes was determined by a paper electrophoretic technique (VELLA[6]). Determinations of reduced glutathione in the erythrocyte suspension and hemoglobin in the supernatant fluid were made at intervals of 0, 3, 6, 10, and 24 h.

The treatment of the erythrocytes with $NaNO_2$ oxidised the glutathione completely. In the untreated erythrocytes the content of reduced glutathione fell gradually to 8–40% (average: 20%) in ox, to 15–50% (average: 36%) in monkey, to 53% in the horse, and to 10–16% (average: 26%) in the human samples. The amount of hemoglobin in the supernatant fluid after 24 h at 37°C amounted to between 0·5 and 2·5% of the total hemoglobin at zero time. No significant differences were detected in the rates of spontaneous hemolysis in the nitrite treated and untreated erythrocytes from the same blood sample, though the rate of hemolysis appeared to be specific for each blood sample. The type of hemoglobin present (A, B, C, AB, or AC) in the samples from oxen did not appear to be related to the rate of spontaneous oxidation of the glutathione in the untreated cells or the rate of hemolysis of either the treated or the untreated samples. The hemoglobin in the horse, the monkey, and the human samples was electrophoretically homogeneous and normal in type for each species.

Spontaneous oxidation of reduced glutathione of shed blood is only one of many chemical changes taking place in red blood cells kept under conditions similar to those employed in these experiments. Loss of bicarbonate, conversion of glucose to lactic acid, loss of inorganic phosphate from breakdown of ester phosphate in the cells, loss of K^+ and gain in Na^+ (VARLEY[7]), loss of 'labile' iron and bilirubin (BARKAN and WALKER[8]) and of pyruvate (LONG[9]) are well known occurrences in the human shed blood.

The treatment of erythrocytes with $NaNO_2$ produces complete oxidation of reduced glutathione and converts oxyhemoglobin to methemoglobin but does not affect the catalase and the carbonic anhydrase activity (KEILIN and HARTREE[3]); it also denatures the globin of hemoglobin producing 'nitrite cat-hemoglobin' (BARNARD[10]). Methemoglobin formation *per se* does not appear to damage the erythrocytes (LEMBERG and LEGGE[1]).

From the present experiments, it appears that the rate of spontaneous hemolysis of erythrocytes from several species is independent of their content of reduced glutathione and is not affected adversely by the marked changes produced by treatment with nitrite.

Acknowledgment. I am grateful to Dr. LEE LIANG HIN of the Department of Bacteriology, Faculty of Medicine, University of

[1] R. LEMBERG and J. W. LEGGE, *Hematin Compounds and Bile Pigments* (Interscience Publishers, Inc., New York 1949), p. 517 and 523.

[2] G. FEGLER, Nature *170*, 624 (1952).

[3] D. KEILIN and E. G. HARTREE, Nature *157*, 210 (1946).

[4] J. V. DACIE, *Practical Haematology* (J. & A. Churchill Ltd., London 1956), p. 139.

[5] R. H. S. THOMPSON and D. WATSON, J. clin. Pathol. *5*, 25 (1952).

[6] F. VELLA, Nature *181*, 564 (1958).

[7] H. VARLEY, *Practical Clinical Biochemistry* (William Heinmann Medical Books, Ltd., London 1958), p. 7.

[8] G. BARKAN and B. S. WALKER, J. biol. Chem. *131*, 447 (1939).

[9] C. LONG, Biochem. J. *38*, 447 (1944).

[10] R. D. BARNARD, J. biol. Chem. *120*, 177 (1937).

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:

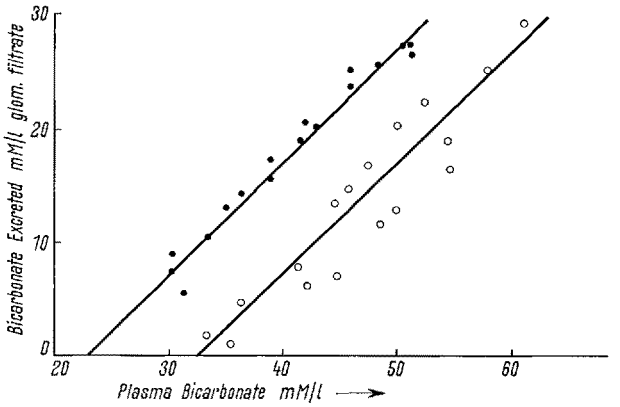

Fig. 1

• 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).