Each point on the graph is a result from a single session. Above the graph are pairs of circular phase histograms which are arranged in the same order as points on the graph. That is, the first pair of histograms shows the phase of the driving cycle at which muscles L2b and D1a were recruited in each cycle during the session from which the first point on the graph was derived. Histograms 3, 4, 5, 7 and 8, relating to sessions in which the driven frequency was equal to the driving frequency or was half the driving frequency, show that the muscle recruitment cycle became phase locked to the driving current during these sessions. During entrainment the normal L2b–D1a phase angle of about 80° was maintained.

During sessions 1, 2 and 5, absolute coordination of the sort described for the other sessions was not observed, but the neural motor exhibited a tendency to maintain a preferred phase relationship with the driving signal, slipping rapidly through regions of unfavourable phase relationship in the manner described by VON HOLST[5] as relative coordination.

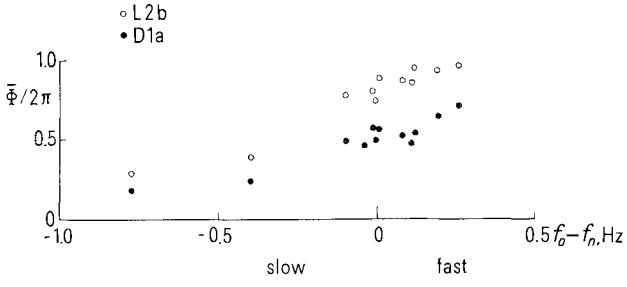

Fig. 3. Regression of coupling phase angle on frequency difference. Ordinate: phase of muscle recruitment in driving signal cycle (positive going zero voltage crossing = zero phase). Abscissa: difference between driving frequency (f_o) and mean free-running frequency (f_n). Abbreviation: ∅, mean recruitment phase angle during an entraining session (radians). Other abbreviations as in Figure 2.

In Figure 3 results from a different set of experiments are presented. The phase in the driving cycle at which muscles were recruited is shown on the ordinate. Each point is obtained from a single session during which forward beating was absolutely entrained to the driving signal for a minimum of 10 cycles, and represents the mean recruitment phase angle during that session[6]. The difference between the driving frequency and the mean free running frequency for each session is plotted along the abscissa. The neural motor evidently becomes advanced in the driving cycle when it is forced to run slowly, and retarded when it is speeded up.

Four features of these results indicate that the driving signal entrains the neural motor rather than that it drives the muscles independently of the neural motor. These are, 1. the maintenance of intermuscular phase angles during entrainment; 2. the restricted range of driving frequencies over which absolute coupling is observed, 3. the relative coordination observed outside the absolute coupling frequency bands, and 4. the phase retardation observed when the system is made to run fast[7].

It is not possible to decide whether the neural oscillator is entrained directly to the applied current, or whether that current modulates activity in a set of neurones to which the neural oscillator can become entrained. Nevertheless now that experimental entrainment has been demonstrated, it should be possible using a small electrode to map the regions of the ganglion having the greatest sensitivity to the driving signal. In this way one could hope to locate the neural oscillator, control its period, and even obtain an indication of its size and structure.

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The Oxygen-Linked Hydrogen Ion Binding (the Haldane Coefficient) of Bovine Hemoglobin

N. TAKANO[1] and K. NISHIKURA

Department of Physiology, School of Medicine, Kanazawa University, Kanazawa 920 (Japan), 25 March 1976.

Summary. The Haldane coefficient (the amount of the oxygen-linked hydrogen ion binding of hemoglobin)was determined in bovine erythrolysate (Hb concentration = 13.5 m*M*) by means of the differential titration method with varying PCO_2 from 0 to 74 mm Hg and pH from 6.0 to 8.5 at 37 °C. The maximum value of the coefficient was found to be 0.49 m*M* per m*M* Hb at PCO_2 = 0 and pH 7.20. With increasing of PCO_2, the coefficient became smaller in all ranges of pH studied. The coefficient under the conditions of pH 7.20 and PCO_2 = 45 mm Hg that are normally prevailing in the interior of bovine erythrocytes was 0.31.

At a physiological pH hemoglobin releases protons as O_2 binds (the alkaline Bohr effect). The magnitude of this effect is represented by the Haldane coefficient expressed as $-\delta Hb\text{–}H^+/\delta Hb\text{–}O_2$. The Haldane coefficient of human hemoglobin has been reported to be 0.47 under the conditions of pH 7.20, PCO_2 = 34 mm Hg, DPG/Hb_4 = 0.84 and Hb concentration (on a monomer basis) = 12 m*M*[2]. DPG stands for 2,3-diphosphoglycerate. With oxygenation of blood, an increase in the negative charges of the hemoglobin due to the alkaline Bohr effect leads to a decrease in the pH of the interior of erythrocytes at a given plasma pH, the magnitude being proportional to the Haldane coefficient[3]. Recently, we had an opportunity to measure the erythrocyte pH for bovine blood[4]. It was 7.24 on oxygenation of the blood and 7.252 on deoxygenation at plasma pH of 7.4, the difference between the two values being not significant. This finding for the bovine blood made us wonder whether the Haldane coefficient of bovine hemoglobin is very small at physiological pH and PCO_2. Results of studies on the Haldane coefficient for bovine hemoglobin and blood are not

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readily available. In the present work, we attempted to determine the Haldane coefficient for the lysate of bovine erythrocytes with varying pH and PCO_2 at 37 °C. The lysate of the erythrocytes was chosen in order to make the determination under the conditions as close to the interior of the erythrocytes as possible. Since bovine blood contains only trace amounts of DPG[5], and it slightly affects the affinities of O_2 and CO_2 to bovine hemoglobin[6,7], the effect of DPG on the Haldane coefficient was not taken into account.

Methods. The Haldane coefficient was determined from the difference between titration curves of oxygenated and deoxygenated erythrolysates (differential titration curve). The principle of the method was based on SIGGAARD-ANDERSEN[2]. Bovine blood was sampled during venesection at a slaughterhouse. The blood to which heparin was added, was centrifuged to obtain packed erythrocytes. The packed erythrocytes washed with saline were rapidly frozen in the liquid nitrogen and then thawed at room temperature. To aliquots of 1.0 ml of erythrolysate thus obtained, 0.6 ml of titrant of acid or base were added. The titrant was made by mixing 0.15 *M* KCl and 0.15 *N* HCl or 0.15 *N* $KHCO_3$ in varying proportions. The titrated solutions of erythrolysate were then equilibrated at 37 °C in a microtonometer (Radiometer, Blood microsystem BMS-2) with gas mixtures containing various CO_2 contents (0 to 10%) in O_2 or in N_2. Following the equilibration, the pH of the solutions of erythrolysate was measured at 37 °C (Radiometer glass electrode, BMS-2 and PHM71Mk2), calibrated with Radiometer precision buffers. The Hb concentration of the solutions of erythrolysate were measured by the cyanomethemoglobin method, the molecular weight of bovine hemoglobin (monomer) being estimated to be 16,250[8]. The fraction of methemoglobin in deoxygenated solutions of erythrolysate was determined by EVELYN and MALLOY's method[9].

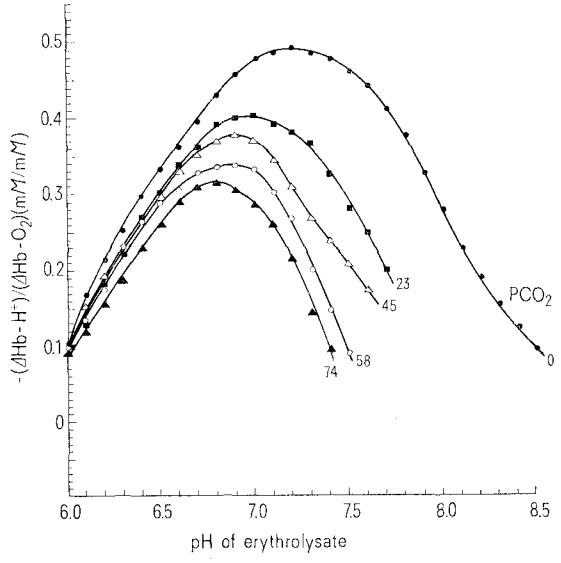

Fig. 1. The Haldane coefficient of bovine erythrolysate at varying pH and PCO_2 (in mm Hg) at 37 °C.

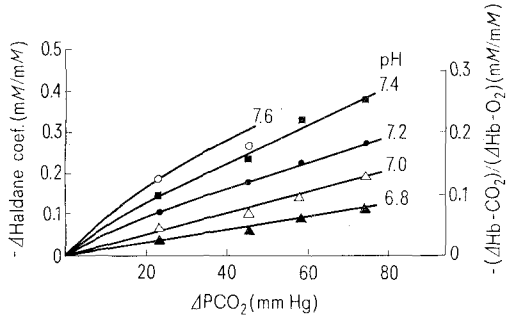

Fig. 2. The decrease in the Haldane coefficient (-δHaldane coef.) of erythrolysate against the increase in PCO_2 (δPCO_2) at varying pH. The relationships shown in this figure were obtained based on the results shown in Figure 1. The ordinate on the right hand of the figure indicates the amount of oxygen-linked carbamate. For the computation of this amount, see text.

Results and discussion. The Hb concentration of the solutions of erythrolysate after the titration and equilibration was approximately 13.5 m*M*/l. Figure 1 shows the Haldane coefficient of bovine erythrolysate with varying pH and PCO_2. Each point represents a mean value of the coefficients obtained from 4 to 7 titration experiments, the SD of all the means being $\pm$ 0.015. The Haldane coefficient had a maximum value at a certain pH in any PCO_2 levels. At PCO_2 = 0, the maximum value was seen at pH 7.2, the value being 0.49. This value is slightly smaller than that of human DPG-depleted erythrolysate, 0.51, determined by SIGGAARD-ANDERSEN[2].

Under the conditions of pH 7.20, PCO_2 = 45 mm Hg, $DPG/Hb_4 \simeq 0$ and Hb concentration = 13.5 m*M* that are supposed to prevail normally in the interior of bovine erythrocytes, the Haldane coefficient of bovine erythrolysate was found to be 0.31. This value is considerably smaller than that for the human erythrolysate under similar conditions obtained by SIGGAARD-ANDERSEN[2]. The small value of the Haldane coefficient of bovine erythrolysate may explain in part the non-appreciable difference in erythrocyte pH between oxygenated and deoxygenated blood of oxen at a given plasma pH, a finding that stimulated us to make the present study. Detailed discussion in this context is in progress[10].

As seen in Figure 1, the Haldane coefficient of bovine erythrolysate decreased with increasing of PCO_2. Figure 2 shows the decrease in the coefficient against the increase in PCO_2 at varying pH. The effect of CO_2 in decreasing the coefficient was greater at higher pH. The effect of CO_2 on the Haldane coefficient is due to the formation of carbamino-hemoglobin. At physiological pH (7.0–7.6), deoxyhemoglobin has a higher affinity to CO_2 than oxyhemoglobin, i.e., the oxygen-linked carbamate formation[11]. The binding of CO_2 to hemoglobin causes a re-

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lease of protons, i.e., the carbamino-linked proton formation. The higher extent of carbamate formation in deoxyhemoglobin causes the increase in the proton release from the deoxyhemoglobin, and as the result of this the Haldane coefficient is reduced. If the carbamino-linked proton formation is assumed to be approximately 1.5 mM per mM of carbamate at physiological pH[12], one can compute the amount of oxygen-linked carbamate formation of bovine hemoglobin from the value of $-\delta$Haldane coefficient in Figure 2. The ordinate shown on the right side of Figure 2 indicates the mM of oxygen-linked carbamate per mM of hemoglobin. The figure shows that the oxygen-linked carbamate formation of bovine hemoglobin is estimated to be 0.11 mM at PCO_2 = 40 mm Hg and pH 7.2. This value is slightly higher than that obtained by VAN KEMPEN et al.[6]. Figure 2 also shows that the oxygen-linked carbamate formation is increased with PCO_2 and pH, as has been demonstrated by VAN KEMPEN et al.[6]

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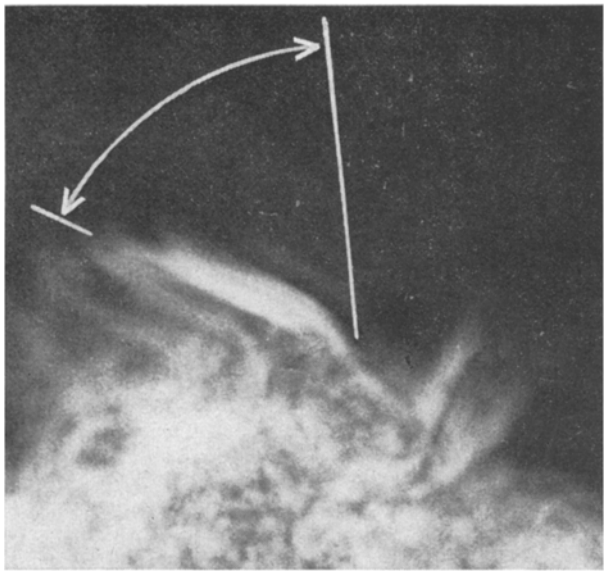
Ca-Induced Arrest Response in Triton-Extracted Lateral Cilia of *Mytilus* Gill

T. TSUCHIYA[1,2]

Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113 (Japan), 18 May 1976.

Summary. The role of Ca ions in the ciliary arrest response of *Mytilus* gill cilia was studied with Triton-extracted models. The cilia continue to beat when the Ca ion concentration is lower than about 10^{-7} M, but stop beating and incline in the direction of the recovery stroke as in the arrest response observed in the living lateral cilia when Ca ion concentration is raised above 10^{-7}–10^{-6} M. ATP, Mg and Ca ions are all indispensable for the arrest response in the model system.

The ciliary arrest response, i.e. an abrupt stoppage of ciliary beating, has been observed in many animal phyla. Recently, much attention has been directed to the arrest response of the lateral cilia in the gill of the common mussel, *Mytilus edulis*. These cilia stop beating when the branchial nerve or the visceral ganglion is stimulated, temporarily assuming a posture inclined in the direction of the recovery stroke. This response can also be induced by mechanical or chemical stimulation given to the cilia[3–5]. Recently, it has been reported that the response is accompanied by a membrane depolarization of the ciliated cell[6]. These results indicate certain similarities between the arrest response and the ciliary reversal response of the protozoans such as *Paramecium*.



Photomicrograph of Triton-extracted gill cilia of *Mytilus* during the arrest response. The long white line indicates the position of the cilia in the washing solution and the circular arc indicates the change in angle of inclination of the cilia following the application of the reactivating solution inducing the arrest response (1 mM ATP, 10 mM $MgCl_2$, 0.05 mM $CaCl_2$, 150 mM KCl, 10 mM *Tris* buffer pH 8.0).

Studies made with Triton-extracted models have shown that both the reversal of ciliary beating in *Paramecium* and the reversal in the direction of flagellar wave propagation in *Crithidia* are controlled by Ca ions[7,8]. In the ciliary arrest response in *Mytilus*, it has already been suggested that Ca ions are also necessary[9]. In view of the general importance of Ca ions in the modification of ciliary or flagellar movement, it is of special interest to study the effect of Ca ions in the ciliary arrest response of *Mytilus* gill.

Material and methods. A single gill filament isolated from the gill of *Mytilus* was placed in the experimental chamber filled with artificial sea water (434 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 53 mM $MgCl_2$, pH adjusted to 8.0 with $NaHCO_3$) and the movement of the lateral cilia was observed in profile. Then the gill filament was soaked in the extracting solution (0.010–0.012% Triton X-100, 150 mM KCl, 20 mM $MgCl_2$, 5 mM EGTA; ethylene glycol *bis*(β-aminoethylether)-N,N'tetraacetic acid, 10 mM *Tris* buffer, pH 7.0) precooled to 1–4 °C. After

[1] Present address: Department of Physiology, School of Medicine, Teikyo University, Kaga, Itabashi-ku, Tokyo 173, Japan.

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