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

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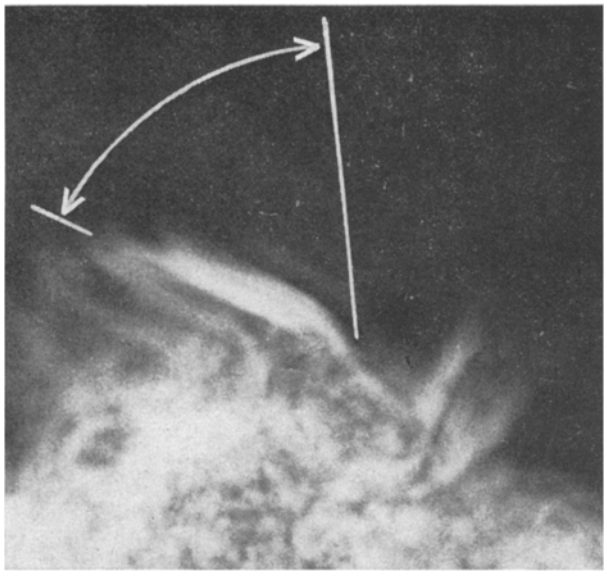
Summary. The role of Ca ions in the ciliary arrest response of *Mytilus* gill cilia was studied with Triton-extracted models. The cilia continue to beat when the Ca ion concentration is lower than about 10^{-7} M, but stop beating and incline in the direction of the recovery stroke as in the arrest response 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

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[2] The author wishes to thank Prof. K. TAKAHASHI of Tokyo University for the continuous guidance and encouragement during the course of this experiment and for critical reading of the manuscript. Thanks are also due to Prof. H. SUGI of Teikyo University for critical reading of the manuscript.

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20–30 min extraction at 1–4 °C, the washing solution (150 m*M* KCl, 10 m*M* $MgCl_2$, 5 m*M* EGTA, 10 m*M* *Tris* buffer, pH 8.0) was introduced at room temperature. Then, one of two types of the reactivating solution was applied at the room temperature; one was beat-reactivating solution (the standard composition; 150 m*M* KCl, 10 m*M* $MgCl_2$, 1 m*M* ATP, 5 m*M* EGTA, 10 m*M* *Tris* buffer, pH 8.0) and the other was the arrest-reactivating solution (the standard composition; 150 m*M* KCl, 10 m*M* $MgCl_2$, 0.05 m*M* $CaCl_2$, 1 m*M* ATP, 10 m*M* *Tris* buffer, pH 8.0). The Ca ion concentrations higher than 10^{-6} *M* in the arrest-reactivating solution were obtained by a simple addition of $CaCl_2$ to the solution without EGTA as shown in the standard composition. However, when Ca ion concentration of the arrest-reactivating solution was lowered than 3×10^{-7} *M*, 'Ca-buffer' was used; the solution contained 5 m*M* EGTA and appropriate concentration of $CaCl_2$ (below 5 m*M*).

Results. The lateral cilia almost immediately began to beat vigorously when the washing solution was replaced by the beat-reactivating solution with the beat frequency, reaching a steady value within a few minutes; full reactivation was obtained in the solution containing 2–6 m*M* ATP, 2–6 m*M* $MgCl_2$, pH 8.0. The frequency was dependent on ATP and Mg ion concentrations, as has been shown in other cilia and flagella by many authors.

When the arrest-reactivating solution was applied after the washing solution, it was observed that in Ca ion concentrations lower than 10^{-7} *M*, the lateral cilia continued to beat vigorously at high frequencies as in the beat-reactivating solution. In concentrations higher than 10^{-7}–10^{-6} *M*, however, the lateral cilia did not beat but exhibited the arrest response; the cilia inclined in the direction of the recovery stroke just as do the live cilia in response to an electrical stimulation. The amplitude of the arrest response could be estimated by measuring the change in the angle of inclination of the cilia, which was observed when the arrest-reactivating solution was introduced (Figure). The amplitude of the response became larger as the Ca ion concentration was increased. When the Ca ion concentration was again decreased to less than 10^{-7} *M*, the cilia which had exhibited an arrest response resumed beating. When the reactivating solution contained Ca ions and ATP without Mg ion, neither the beating nor the arrest response was observed. Similar results were obtained when the reactivating solution contained Ca and Mg ions without ATP. It may be concluded that Mg ions are indispensable not only for the ciliary beating but also for the arrest response, and that the arrest response cannot be induced by ATP and Ca ions only. The optimal pH for the initiation of the arrest response was 8.0, the same value as that for the beating. An appreciable arrest response was also induced by ADP but not by ITP, GTP, CTP, UTP and c-AMP.

Concerning the ciliary activity in *Paramecium*, it has been suggested that there are two separate motile systems: one which produces the cyclic beating, requiring ATP and Mg ions for activation, and the other which determines the orientation of ciliary movement, requiring ATP and Ca ions. A similar mechanism is also supposed to exist in *Crithidia*[7,8]. However, the present results, particularly the finding that the arrest response requires Mg ions for activation as does the beating, which is inhibited by Ca ions, may indicate that here the two types of ciliary activity depend on a single ATP-utilizing system. Detailed results of this work will be given elsewhere[10].

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Release of Renal Kallikrein to the Perfusate by Isolated Rat Kidney

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Summary. The addition of furosemide to the fluid used to perfuse isolated rat kidney increases the kallikrein activity found in the perfusion fluid. The experiments favour the concept that furosemide activates a kallikrein precursor or/and the synthesis and release of kallikrein in the kidneys.

Renal kallikrein seems to play an important role in the kidney as antagonist of the renin-angiotensin system[1,2]. Experiments from our laboratory[3] suggest that renal kallikrein is released into the blood circulation, and therefore may also exert a systemic action. In the present study, this possibility was explored in the isolated, perfused kidney using kallikrein-free perfusion medium and furosemide, a well-known diuretic and natriuretic agent, which produces a significant increase in the total amount of urinary kallikrein in the rat[4].

Methods. 15 male rats (Sprague-Dawley, 220–300 g) were anesthetized i.p. with Dial-Urethane (10 g of diallylbarbituric acid, 40 g of urethane and 40 g of monoethylurea in 100 ml of water), 0.12 ml/100 g body wt. 9 rats were used for control experiments and 6 for Furosemide experiments. The renal circulation was isolated by exposing the inferior vena cava, abdominal aorta and superior mesenteric artery; all other small blood vessels of the kidney area were tied off. The right renal artery was cannulated without ischemia by placing a PE-50 tubing initially in the superior mesenteric artery. Both kidneys were perfused with perfusion medium (after tying off the abdominal aorta) for 10 min to wash out all the renal blood. Then, the cannula was advanced into the right renal artery. Immediately, the left kidney was removed and frozen for subsequent assay. At the end of the experiment, the right kidney was also excized, frozen and assayed. The inferior vena cava was cannulated with PE-240 tubing so that the tip of the cannula was placed at the right renal vein in order to collect the renal venous outflow. The isolated right kidney was perfused and controlled by pulsatil perfusion pump (Sigmamotor T-8). Systolic perfusion pressure was held constant at 120 mm Hg and mean perfusion pressure varied between 90 and 115 mm Hg. Under these experimental conditions, renal flow was 8–10 ml/min. Perfusion pressure was measured by stain-gauge manometer (Statham P23). Renal outflow

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