Ionic Control of the Reversal Response of Cilia in *Paramecium caudatum*

A calcium hypothesis

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ABSTRACT The duration of ciliary reversal of *Paramecium caudatum* in response to changes in external ionic factors was determined with various ionic compositions of both equilibration and stimulation media. The reversal response was found to occur when calcium ions bound by an inferred cellular cation exchange system were liberated in exchange for externally applied cations other than calcium. Factors which affect the duration of the response were (*a*) initial amount of calcium bound by the cation exchange system, (*b*) final amount of calcium bound by the system after equilibration with the stimulation medium, and (*c*) concentration of calcium ions in the stimulation medium. An empirical equation is presented which relates the duration of the response to these three factors. On the basis of these and previously published data, the following hypothesis is proposed for the mechanism underlying ciliary reversal in response to cationic stimulation: Ca^{++} liberated from the cellular cation exchange system activates a contractile system which is energized by ATP. Contraction of this component results in the reversal of effective beat direction of cilia by a mechanism not yet understood. The duration of reversal in live paramecia is related to the time course of bound calcium release.

INTRODUCTION

When the ciliate *Paramecium caudatum* is transferred into a medium high in potassium concentration, it temporarily swims backward because of a transient reversal in the direction of effective beat of the cilia (1–5). Shortly after the transfer of the organism into a high K solution, the direction of ciliary beat is fully reversed so that the predominant orientation of the cilia is toward the anterior. This results in a reversal of the swimming direction, so that the organism swims backward (Fig. 1 A). The cilia gradually resume their original orientation toward the posterior, so that the backward swimming organism slows, temporarily halts, and gradually regains forward momentum

as the cilia return to their original orientation (Fig. 1 A–C). This sequence is termed "ciliary reversal."

Ciliary reversal is generally associated with a depolarization of the membrane (6–9), and shows a number of similarities to the stimulus-response relationships found in muscle and nerve (10, 11). Studies on the response have frequently been made from the standpoint of cell excitation, as summarized by Jahn (12, 13) and by Kinosita and Murakami (14). Fundamental mechanisms underlying the response, however, still remain unclear.

Several decades ago, Kamada (15, 16) emphasized the importance of intracellular calcium to ciliary reversal in *Paramecium*. On the other hand,

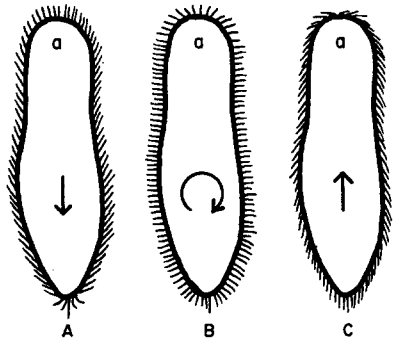

FIGURE 1. Change in the general orientation of beating cilia on *P. caudatum* in response to potassium stimulation. A, immediately after the specimen is transferred into the stimulation medium cilia are fully reversed causing the specimen to swim backward. B, just before cessation of backward swimming the cilia beat with an orientation largely perpendicular to the surface. The specimen sometimes shows rotation about the posterior end of the body due to the beat of cilia surrounding the peristome. C, the normal orientation of cilia is finally resumed, and the specimen swims forward. Arrows indicate swimming directions. The diagram omits such details as ciliary shape and metachrony. Anterior end is marked *a*.

Jahn (13) recently emphasized the important role of bound calcium on the cell surface, and suggested the applicability of the Gibbs-Donnan principle to the equilibrium established between the surface of *Paramecium* and the surrounding ions.

Using radiocalcium, Naitoh and Yasumasu (17) demonstrated a consistent Ca binding by *Paramecium* that was inhibited by the presence of certain other cations in a manner that agrees with the law of mass action (or Gibbs-Donnan principle). Furthermore, they found that the calcium-displacing effectiveness of these cations is quantitatively correlated with their effectiveness in inducing ciliary reversal. These findings suggest that the liberation of calcium from the anionic sites of *Paramecium* by an exchange reaction with other ions is somehow concerned with the reversal response.

Moreover, the fact that the reversal response of cilia in glycerol-extracted models of paramecia depends on the presence of calcium ions as well as ATP[1] strongly suggests that the liberated Ca ions activate, directly or indirectly, the mechanism concerned with ciliary reversal.

The present study, which examines the quantitative relationships between ionic exchanges and the reversal response, supports the hypothesis that liberation of calcium ions from anionic sites on the cell surface is the first step in the initiation of reversal.

MATERIALS AND METHODS

Specimens of *P. caudatum* reared in hay infusion were thoroughly washed in running solution and were then equilibrated for 30 min or more in the same solution (equilibration medium) prior to stimulation. Paramecia treated in this manner will be referred to as equilibrated specimens.

An equilibrated specimen along with a minute amount of the equilibration medium was pipetted under 10 times magnification into a large vessel of stimulation medium, and the duration of the characteristic reversed swimming which followed was measured with a stopwatch. This was usually repeated under the same conditions on 10 different specimens, from which a mean and a standard error were calculated. Fluctuations of measured values were so small that the standard errors did not exceed the diameter of circles indicating the mean values. Hereafter, this duration will be called the "duration of ciliary reversal."

The pH of all the experimental solutions was adjusted to 7.2 by Tris-HCl buffer of 1 mM in final Tris concentration, and chloride was the counterion for all the cations employed. Experiments were all performed at room temperatures of 19–21°C.

RESULTS

A. *Ionic Factors in the Equilibration Medium Affecting the Duration of Ciliary Reversal*

1. CONCENTRATION OF Ca^{++} The organisms were washed and equilibrated in various running[2] calcium solutions having different concentrations (0.16-10 mM) for 30 min. The organisms were then transferred into a stimulation medium which consisted of 20 mM K and 1 mM Ca in final concentration, and the duration of ciliary reversal exhibited by each organism in the stimulation medium was determined. The results shown in Fig. 2 indicate that organisms equilibrated in media having calcium concentrations higher

[1] Y. Naitoh. Control of the orientation of cilia in glycerol-extracted model of *Paramecium* by Ca^{++}, Zn^{++}, and ATP. Manuscript in preparation.

[2] Equilibration of the specimens in flowing solutions was necessary to determine the concentration effect of calcium. The specimens equilibrated in unstirred solutions with calcium concentration below 0.6 mM exhibited maximal ciliary reversal in response to K stimulation. Since the specimens tended to aggregate in the unstirred solution, the calcium concentration near the specimens might be above that in the bulk of the solution due to diffusion of calcium from the cytoplasm (18).

than about 0.6 mM exhibited a reversal of maximal duration. At calcium concentrations below 0.6 mM the duration decreased with a decrease in the calcium concentration.

2. $[K^+]$ AND $[Ba^{++}]$ CHANGES IN CONSTANT $[Ca^{++}]$ MEDIUM The organisms were equilibrated in two series of media, one of which consisted of

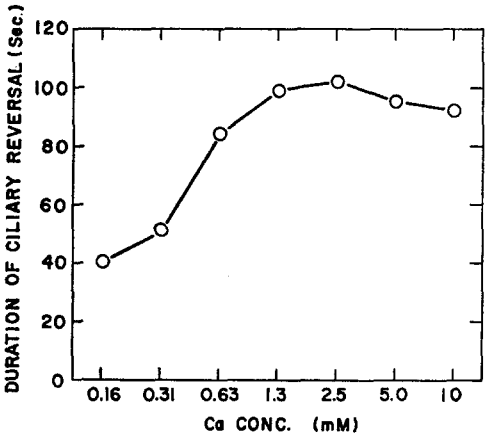

FIGURE 2. The influence of $[Ca^{++}]$ in the equilibration medium on the duration of ciliary reversal. Specimens equilibrated in media having different calcium concentrations were transferred into stimulation medium of 20 mM KCl + 1.0 mM $CaCl_2$.

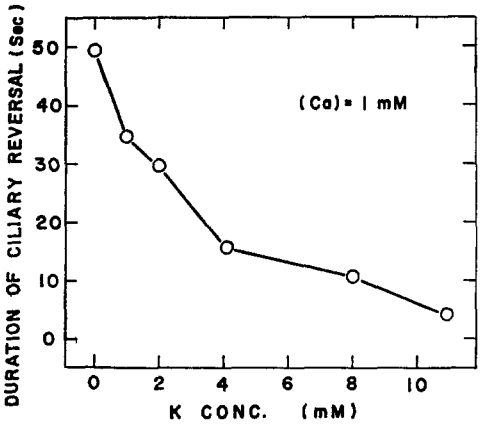

FIGURE 3. The influence of $[K^+]$ in the equilibration medium on the duration of ciliary reversal. Specimens equilibrated in media having different potassium concentrations but a constant calcium concentration (1 mM) were transferred into a stimulation medium having 20 mM KCl + 1 mM $CaCl_2$.

1 mM Ca plus K in several concentrations (0-11 mM), and the other consisted of 1 mM Ca plus Ba in several concentrations (0-8 mM). They were then transferred into a stimulation medium (20 mM K + 1 mM Ca) and reversal durations determined.

Fig. 3 (the potassium-equilibrated series) and Fig. 4 (the barium-equilibrated series) both clearly show that ciliary reversal was shorter in duration when equilibration occurred in the media with the higher K or Ba concen-

trations, and that ciliary reversal of maximal duration was obtained in organisms equilibrated in K- or Ba-free, calcium solution.

3. [ca^{++}] CHANGES IN CONSTANT [K^+] MEDIUM The organisms were equilibrated in a series of media, which consisted of 2 mM K plus Ca in several concentrations (0.06-16 mM), and were transferred into a stimulation medium (20 mM K + 1 mM Ca). The durations of reversal were determined and

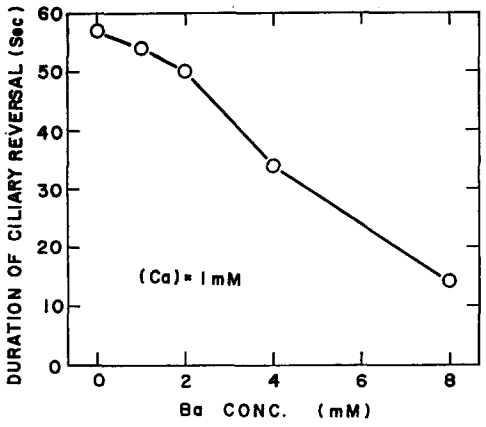

FIGURE 4. The influence of [Ba^{++}] in the equilibration medium on the duration of ciliary reversal. Specimens equilibrated in media of differing barium concentrations but a constant concentration of calcium (1 mM) were transferred into a stimulation medium having 20 mM KCl + 1 mM $CaCl_2$.

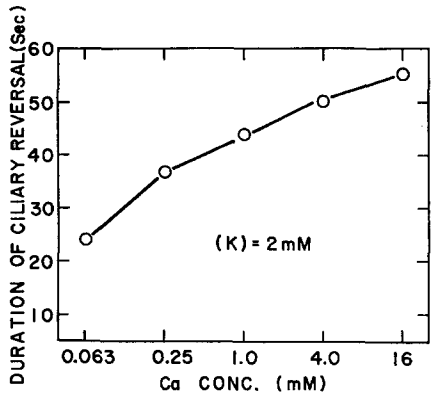

FIGURE 5. The influence of [Ca^{++}] in the equilibration medium on the duration of ciliary reversal. Specimens equilibrated in media having different calcium concentrations but a constant potassium concentration (2 mM) were transferred into a stimulation medium having 20 mM KCl + 1 mM $CaCl_2$.

plotted as shown in Fig. 5. A decrease in the concentration of Ca in the equilibration medium coincided with a decrease in the duration of ciliary reversal in response to a K stimulation. The effect of decreasing the calcium concentration was the same as that obtained by increasing the potassium concentration in the equilibration at a constant Ca level.

4. ANTAGONISM BETWEEN Ca^{++} AND K^+ OR Ba^{++} It is clear from the results of preceding sections that Ca and K (or Ba) in the equilibration medium antagonize each other with respect to the responsiveness of the reversal sys-

tem to K stimulation. In order to study the antagonism quantitatively, a determination was made of the amount of calcium ions required to just balance the effect of an increase in K or Ba concentration on the response to K stimulation (20 mM K + 1 mM Ca).

When the concentration of calcium was increased along with that of potassium so as to keep constant the ratio of the potassium concentration to the square root of the calcium concentration ($[K]/\sqrt{[Ca]}$), the duration of reversal in response to a subsequent K stimulation remained constant as is shown in Fig. 6. On the other hand, a constant calcium barium ratio

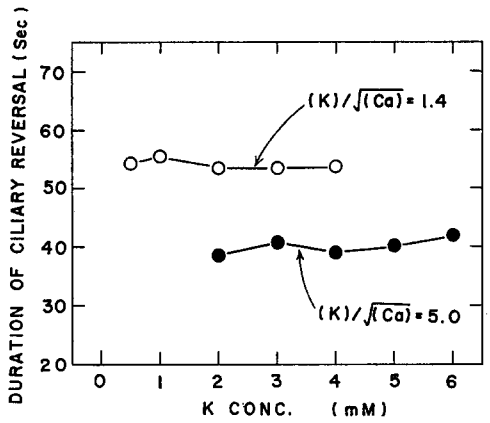

FIGURE 6. Antagonism between Ca^{++} and K^{+} in the equilibration medium with regard to the duration of ciliary reversal. The stimulation medium consisted of 20 mM KCl + 1 mM $CaCl_2$. The calcium concentration of the equilibration medium was increased in accordance with the increase in potassium concentration so as to keep the $[K]/\sqrt{[Ca]}$ ratio constant (1.4 in the case of open circles; 5.0 in the case of solid circles).

([Ba]/[Ca]) must be maintained in order to keep the duration of reversal response to K stimulation constant (Fig. 7).

These results demonstrated that the duration of ciliary reversal elicited by K stimulation is dependent on the ratio of cation concentrations ($[K]/\sqrt{[Ca]}$ or [Ba]/[Ca]) in the equilibration medium and not on their absolute concentrations. Organisms equilibrated at higher relative calcium concentrations show reversal times of longer duration.

B. *Ionic Factors in the Stimulation Medium Affecting the Duration of Ciliary Reversal*

1. $[K^+]$ CHANGES IN CONSTANT $[Ca^{++}]$ MEDIUM In order to determine the effect on the duration of reversal of the potassium concentration in the stimulation medium, paramecia equilibrated in a medium containing 1 mM Ca

and 1 mM K were transferred into stimulation media which contained 0.3 mM Ca and K of several concentrations (2.5-80 mM).

Fig. 8 indicates that the duration of the reversal was longer in stimulation media of higher potassium concentration. The duration, however, showed a tendency to decrease slightly with potassium concentrations higher than 40

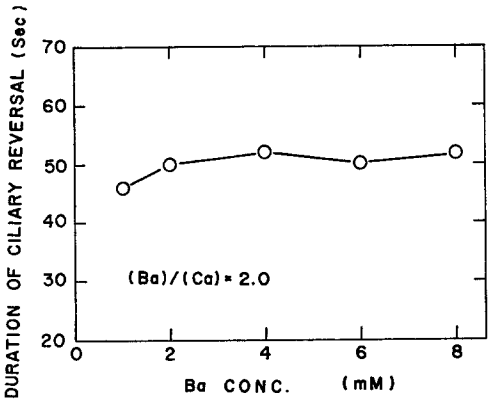

FIGURE 7. Antagonism between Ca^{++} and Ba^{++} in the equilibration medium with regard to the duration of ciliary reversal. The stimulation medium consisted of 20 mM KCl + 1 mM $CaCl_2$. The calcium concentration was increased in accordance with the increase in barium concentration to keep the [Ba]/[Ca] ratio constant (2).

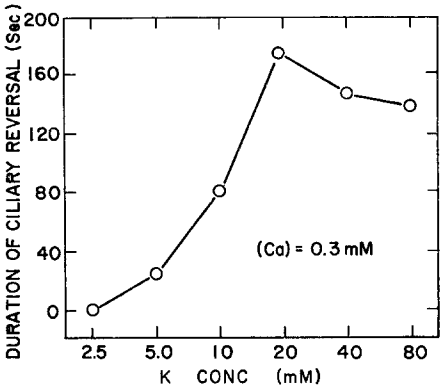

FIGURE 8. The influence of $[K^+]$ in the stimulation medium on the duration of ciliary reversal. The calcium concentration was kept at 0.3 mM. The specimens were first equilibrated in a medium having 1 mM KCl + 1 mM $CaCl_2$.

mM. The paramecia were found to be markedly flattened by exosmosis in these concentrations. Therefore, the slight decrease in the duration may be attributable, at least in part, to complicating factors associated with osmotic shrinkage.

2. $[Ca^{++}]$ CHANGES IN CONSTANT $[K^+]$ MEDIUM In order to determine the effect of calcium concentration in the stimulation medium on the duration of ciliary reversal, durations were measured in stimulation media con-

taining 20 mM K and Ca in several concentrations (0.01-9 mM). The organisms were first equilibrated in a medium having 1 mM Ca and 1 mM K.

As shown in Fig. 9, the reversal durations were longest in the intermediate range of calcium concentration (about 0.3 mM) falling off sharply below 0.1 mM and gradually above 1.0 mM.

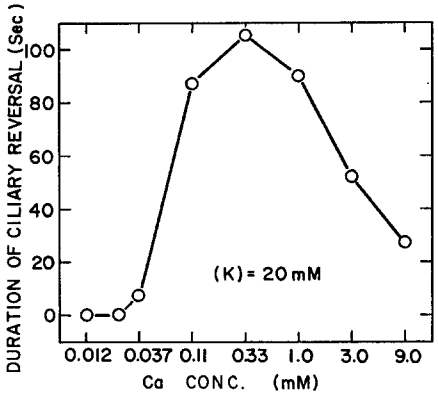

FIGURE 9. The influence of $[Ca^{++}]$ in the stimulation medium on the duration of ciliary reversal. The potassium concentration was kept at 20 mM. The specimens were equilibrated in a medium having 1 mM KCl + 1 mM $CaCl_2$.

TABLE I

DURATION OF CILIARY REVERSAL IN *Paramecium caudatum* ELICITED BY RAISING THE EXTERNAL $[K^+]/\sqrt{[Ca^{++}]}$ RATIO

Equilibration media			Stimulation media			
$[K^+]$			2.0	20	2.0	20
	$[Ca^{++}]$		0.01	1.0	0.001	0.1
		$[K^+]/\sqrt{[Ca^{++}]}$	20	20	63	63
			sec	*sec*	*sec*	*sec*
2.0	0.1	6.3	12.1 ± 0.4*	14.3 ± 0.3	22.8 ± 0.6*	40.2 ± 1.1
20	10	6.3	10.0 ± 0.9‡	12.4 ± 0.1*	17.8 ± 0.6‡	34.1 ± 0.8*

$[K^+]$ and $[Ca^{++}]$ are represented in mM.

* Durations in response to no change in external potassium concentration.

‡ Durations in response to a decrease in external potassium concentration (20 mM to 2.0 mM). Durations without any symbols are cases in which the external potassium concentration was raised (2.0 mM to 20 mM).

These results indicate that an antagonism between K^+ and Ca^{++} similar in nature to that found in equilibration media may hold true for stimulation media; that is, the important factor for initiation of ciliary reversal may be the ratio of $[K]/\sqrt{[Ca]}$ rather than the absolute concentration of K^+. This interpretation finds support in the data of Table I which show that specimens exhibit consistent reversal times in response to increases in the $[K]/\sqrt{[Ca]}$ ratio of the medium without requiring increases in the potassium concentration. Furthermore, ciliary reversal occurred in spite of a decrease in potassium

concentration (from 20 to 2 mM) providing the calcium concentration was adjusted so as to increase the ratio $[K]/\sqrt{[Ca]}$ in the stimulation medium.

3. $[ca^{++}]$ CHANGES IN CONSTANT $[K^+]/\sqrt{[ca^{++}]}$ MEDIUM Fig. 9 indicates that the duration of reversal was inhibited in media with low calcium concentration. In order to determine quantitatively the effect of calcium concentration on the duration, determinations of the reversal times in a series of stimulation media having the same $[K]/\sqrt{[Ca]}$ ratio (20) and different calcium concentrations ranging from 0.016 to 4 mM were made on specimens equilibrated in a medium with a $[K]/\sqrt{[Ca]}$ ratio of 1.4. As shown in Fig.

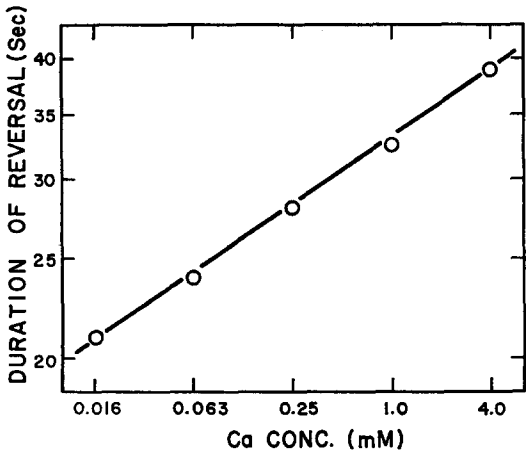

FIGURE 10. The influence of $[Ca^{++}]$ in the stimulation medium on the duration of ciliary reversal. The $[K]/\sqrt{[Ca]}$ ratio of each stimulation medium was kept at 20. The specimens were first equilibrated in a medium having 2 mM KCl + 2 mM $CaCl_2$.

10, the logarithm of the duration decreased linearly with the logarithm of calcium concentration. Therefore, the duration (T) can be represented as

$$T = T_1 [Ca^{++}]_s^a \qquad (1)$$

where $[Ca^{++}]_s$ represents the calcium concentration (mM) of stimulation medium; T_1 represents the duration of reversal at a $[Ca^{++}]_s$ of 1 mM; and a is the slope of the logarithmic plot. Although a differed slightly among the different groups of specimens obtained from different cultures, it appeared to be independent of the $[K]/\sqrt{[Ca]}$ ratios of both equilibration and stimulation media as is shown in Table II.

4. VARIOUS ALKALINE AND ALKALINE EARTH METAL IONS 20 mM solutions of various kinds of alkaline and alkaline earth metal salts (LiCl, NaCl, KCl, RbCl, CsCl, $MgCl_2$, $SrCl_2$, and $BaCl_2$; plus 1 mM Ca in one series and 0.1 mM Ca in another series) were tested to determine to what extent

they induce ciliary reversal. Specimens were first equilibrated in 1 mM K plus 1 mM Ca. The results (Table III) indicate that ciliary reversal can be induced by the external application of certain monovalent ions (Li^+, Na^+, Rb^+) other than K^+, and even by the bivalent barium ions; however, the effectiveness of maintaining reversal differed with the ion species. This is in agreement

TABLE II

FLUCTUATION IN VALUE OF a WITH REFERENCE TO $[K^+]/\sqrt{[Ca^{++}]}$ RATIOS

No. of groups* of paramecia	$[K^+]/\sqrt{[Ca^{++}]}$ of equilibration medium	$[K^+]/\sqrt{[Ca^{++}]}$ of stimulation medium	a
1	1.4	20	0.11‡
2	1.4	20	0.15
3	0.5	8.0	0.10
3	0.5	16	0.17
3	2.0	8.0	0.16
3	2.0	16	0.17
Mean			0.15 ± 0.01

* The groups of *Paramecium* are numbered according to the cultures from which they were obtained.

‡ Calculated from the data presented in Fig. 10.

TABLE III

DURATION OF CILIARY REVERSAL IN *Paramecium caudatum* ELICTED BY APPLICATION OF 20 mM ALKALINE AND ALKALINE EARTH METAL IONS

Coexisting Ca^{++}	Li^+	Na^+	K^+	Rb^+	Cs^+	Mg^{++}	Sr^{++}	Ba^{++}
mM	*sec*	*sec*	*sec*	*sec*	*sec*	*sec*	*sec*	*sec*
1.0	2.8 ± 0.2	2.0 ± 0.1	77.4 ± 1.9	72.4 ± 2.0	0	0	0	159.6 ± 1.7
0.1	4.8 ± 0.2	9.6 ± 0.3	129.8 ± 2.7	108.6 ± 4.5	0	0	0	Killed quickly

with previous findings regarding the effects of metallic ions on the ciliary beat of *Paramecium* (2–5).

5. EFFECTS OF EDTA The organisms equilibrated in a medium having 1 mM Ca and 1 mM K were transferred into 1 mM ethylenediaminetetraacetic acid (EDTA: 4 K salt). The organisms exhibited no ciliary reversal upon transfer to the EDTA solution.[3]

[3] Grębecki (20) observed that ciliary reversal occurred when EDTA-Na of relatively low concentration was injected into a $CaCl_2$ medium. At higher concentrations he observed no reversal. Reversal in his experiment was most probably due to a high ratio of Na^+ to residual Ca^{++} at low concentrations of EDTA-Na (see Table III).

C. *Quantitative Relations between the Duration of Reversal and* $[K^+]/\sqrt{[Ca^{++}]}$ *Ratios of Both Equilibration and Stimulation Media*

As demonstrated above, the external ionic factors affecting the duration of reversal are $[K]/\sqrt{[Ca]}$ ratios of both equilibration and stimulation media, if calcium concentration in the stimulation medium is kept constant.

In order to determine the quantitative relationships of the reversal duration with these ratios, five groups of specimens obtained from the same culture were equilibrated in five different media having different $[K]/\sqrt{[Ca]}$ ratios (0.50, 1.0, 2.0, 4.0, and 8.0) and were then each stimulated by five different media having different $[K]/\sqrt{[Ca]}$ ratios (7.1, 10, 14, 20, and 28) but the same calcium concentration (1 mM). The results are shown in Table

TABLE IV

DURATION OF CILIARY REVERSAL IN *Paramecium caudatum* WITH REFERENCE TO $[K^+]/\sqrt{[Ca^{++}]}$ RATIOS OF BOTH EQUILIBRATION AND STIMULATION MEDIA

$[K^+]/\sqrt{[Ca^{++}]}$ of stimulation medium	$[K^+]/\sqrt{[Ca^{++}]}$ of equilibration medium				
	0.5	1.0	2.0	4.0	8.0
	sec	*sec*	*sec*	*sec*	*sec*
7.1	6.2 ± 0.5	7.7 ± 0.4	7.6 ± 0.3	3.7 ± 0.2	—
10	11.7 ± 0.6	11.3 ± 0.3	11.7 ± 0.4	9.8 ± 0.5	3.5 ± 0.1
14	32.0 ± 0.5	27.7 ± 0.5	23.4 ± 0.5	25.6 ± 0.7	8.9 ± 0.7
20	56.0 ± 2.0	51.8 ± 0.6	45.6 ± 1.1	42.0 ± 0.0	25.6 ± 0.3
28	81.5 ± 1.4	68.5 ± 0.9	69.2 ± 1.0	57.5 ± 0.2	37.5 ± 0.7

IV. In the following section these data provide the basis for an empirical equation which describes these relationships.

DISCUSSION

Kamada and Kinosita (4) carried out a detailed series of experiments on the effect of the [K]/[Ca] ratios of both equilibration and stimulation media on the duration of ciliary reversal. However, they did not find any precise quantitative relationship between the ratio and the duration of reversal. Jahn (13) analyzed their data more recently and found that the maximal duration of ciliary reversal (in specimens equilibrated in a given medium) occurred in stimulation media having a given $[K]/\sqrt{[Ca]}$ ratio, and was largely independent of the absolute concentrations of these ions. From this finding Jahn proposed that a Gibbs-Donnan equilibrium was established between *Paramecium*, K and Ca ions. Furthermore, he suggested that ciliary reversal might be caused by the removal of calcium ions from the cell surface.

Grębecki (19, 20) and Kuznicki (21) also applied the Gibbs-Donnan principle to their data on ciliary reversal of *Paramecium* and emphasized an intimate relationship between the amount of surface calcium and the direction in which the beating cilia point.

Jahn's prediction of the applicability of the Gibbs-Donnan principle to the ion binding by *Paramecium* was confirmed by Naitoh and Yasumasu (17) with the use of ^{45}Ca. They described a cation exchange phenomenon in the binding of K^+, Na^+, Rb^+, Mg^{++}, and Ba^{++} by live *Paramecium*. All the anionic sites of the cation exchange system are saturated with these cations, at least under the ionic conditions employed.

The amount of calcium bound by the system after equilibration with a given medium which consisted of calcium and other cations can be represented as:

$$[P\mathrm{Ca}_{\frac{1}{2}}] = \frac{P_t}{kJ_a + 1} \qquad (2)$$

in which $[P\mathrm{Ca}_{\frac{1}{2}}]$ represents the amount of calcium bound by the cation exchange system; P_t represents the total binding capacity of the system; k represents the equilibrium constant;[4] J_a represents the ratio $\frac{[\text{Monovalent cation}]}{\sqrt{[Ca^{++}]}}$ or $\frac{\sqrt{[\text{Bivalent cation}]}}{\sqrt{[Ca^{++}]}}$ in the medium.[5]

It is clear from the equation that the amount of calcium bound by *Paramecium* is correlated with the J_a value in the medium, an increase in J_a bringing about a decrease in the bound calcium.

As is clearly shown in Fig. 2, there was no further increase in reversal time with equilibration concentrations of calcium higher than about 0.6 mM, whereas the duration decreased in accordance with the decrease in calcium

[4] A binding equilibrium established between the cation exchange system, Ca^{++} and mono (M^+)- or bivalent (B^{++}) cations can be formulated according to the law of mass action as:

$$P\mathrm{Ca}_{\frac{1}{2}} + M^+ \rightleftarrows PM + \tfrac{1}{2}Ca^{++} \qquad (a)$$

$$P\mathrm{Ca}_{\frac{1}{2}} + \tfrac{1}{2}B^{++} \rightleftarrows PB_{\frac{1}{2}} + \tfrac{1}{2}Ca^{++} \qquad (a')$$

$$\frac{[PM]}{[P\mathrm{Ca}_{\frac{1}{2}}]} = k\frac{[M^+]}{\sqrt{[Ca^{++}]}} \qquad (b)$$

$$\frac{[PB_{\frac{1}{2}}]}{[P\mathrm{Ca}_{\frac{1}{2}}]} = k'\frac{\sqrt{[B^{++}]}}{\sqrt{[Ca^{++}]}} \qquad (b')$$

[5] The activity coefficients were neglected in the present studies because of low ionic concentrations of the media employed.

concentration below 0.6 mM. This may be interpreted as the result of saturation of anionic sites on *Paramecium* by calcium ions when the external calcium concentration is raised to a critical value.

Organisms equilibrated in various mixtures of calcium and potassium (or barium) exhibited reversal times of almost the same duration in response to K stimulation when the J_a values of these mixtures were kept constant, irrespective of the absolute ionic concentrations (Figs. 6 and 7), whereas the duration of reversal was increased by lowering the J_a value, and decreased by raising it (Figs. 3–6). These observations strongly suggest that the calcium ions concerned with ciliary reversal are those bound by anionic sites of the proposed cation exchange system of *Paramecium*.

An increase of the J_a value in the external medium causes a binding of potassium (or other ions) to the anionic sites in exchange for liberated calcium so as to establish a new equilibrium dependent on the final J_a value. A vital question therefore arises: Is it liberation of calcium ions or the binding of potassium to the anionic sites of *Paramecium* which is effective in initiating and sustaining reversal? As shown in Table III, applications of certain cations other than potassium, such as lithium, sodium, rubidium, and barium, also induce ciliary reversal, although the durations vary. According to Naitoh and Yasumasu (17), these cations compete with calcium for the same anionic sites on *Paramecium*. Moreover, the affinities of these ions to the anionic sites are correlated with their effectiveness in inducing ciliary reversal. That is, the duration of reversal induced by an external application of the ions having high affinities, such as K^+, Rb^+, and Ba^{++}, was significantly longer than that induced by the ions having relatively low affinities, such as Na^+. Magnesium, which has a very low affinity, did not elicit the reversal response.

The lack of specificity in inducing ciliary reversal and the common effect of these ions in displacing calcium from the anionic sites strongly suggest that their effect is mediated by the liberation of calcium rather than by the binding of these ions.

As demonstrated in Fig. 4, addition of barium to a calcium-containing equilibration medium significantly shortened the duration of reversal in response to a subsequent K stimulation. This suggests that the liberation of bound barium from the sites in exchange for potassium does not cause ciliary reversal. Magnesium cannot substitute for calcium either, for as demonstrated by Naitoh and Yasumasu (17), that ion has a very low affinity for the cation exchange system of *Paramecium*.

The failure of EDTA (which would be expected to remove calcium from the anionic sites) to induce ciliary reversal suggests that calcium ions liberated from the cation exchange system must play an important role in inducing reversal. The liberated calcium ions may, for example, interact with other sites in the cell so as to cause the reversal response. The ineffectiveness of

EDTA also argues against a change in the configuration of the anionic binding site correlated with the release of calcium as the underlying cause of the reversal response. It should also be noted in this respect that ciliary reorientation induced in glycerol-extracted models of paramecia is dependent on the presence of calcium ions as well as ATP.[1]

On the basis of these findings the following hypothesis is proposed for the mechanism underlying the reversal of cilia in response to a cationic stimulus: Externally applied cations bind to the anionic sites on *Paramecium* in exchange for bound calcium in a manner consistent with the law of mass action. The calcium ions which are thus liberated are effective in activating, directly or indirectly, a contractile system which is energized by ATP. Contraction of the hypothetical reversal mechanism in turn results in the reversal of the effective beat direction of cilia or a reorientation of nonbeating cilia in Ni-poisoned (9) or glycerol-extracted cells.[1]

Ciliary reversal is graded not only in duration but also in the degree of change in the orientation of cilia (Fig. 1). In this connection, it is interesting to note that the angle of reorientation of cilia in glycerol-extracted paramecia increases with the concentration of externally applied calcium.[1] The local concentration of liberated calcium near the cation-binding sites of live specimens is assumed to be dependent in part on the rate of release of calcium from the sites; this led to the proposal that the degree of ciliary reversal is a function of the rate of release of calcium. The duration of reversal is therefore thought to be the time between the beginning of calcium release (when J_a value is experimentally raised) and the time when the rate of calcium release (and hence the local concentration of free Ca^{++}) drops below a threshold level as the binding system approaches equilibrium with the medium.

The effect of $[Ca^{++}]$ on the duration of reversal may be due to an influence on the rate of diffusion of the liberated calcium into the external medium, perhaps by its influence on the permeability of the plasma membrane.[6] The inhibition of ciliary reversal associated with low calcium concentration (Fig. 9) can be explained in this manner as due to a decrease in the amount of liberated calcium influencing the reversal system because of increased leakage of liberated calcium ions into the calcium-poor external medium. The lack of ciliary reversal in response to EDTA may be explained similarly.

As is well-known, the external application of calcium ions to live *Paramecium* never elicits ciliary reversal (1–5, 7). It is not known why only those calcium ions liberated from the cellular binding sites activate the reversal system. The reason may be a difference in the effective diameter of liberated and free calcium ions resulting from a difference in the degree of hydration. It should be

[6] Treatment of *Paramecium* with EDTA results in a marked increase in membrane conductance, while increasing $[Ca^{++}]$ increases the resistance of the membrane (Y. Naitoh and R. Eckert. 1967. Unpublished data).

noted in this respect that specimens subjected to treatment with concentrated EDTA solution[6] (20), or to repeated treatment with hypertonic solution[7] or glycerination[1] exhibit ciliary reversal in response to external applications of calcium.

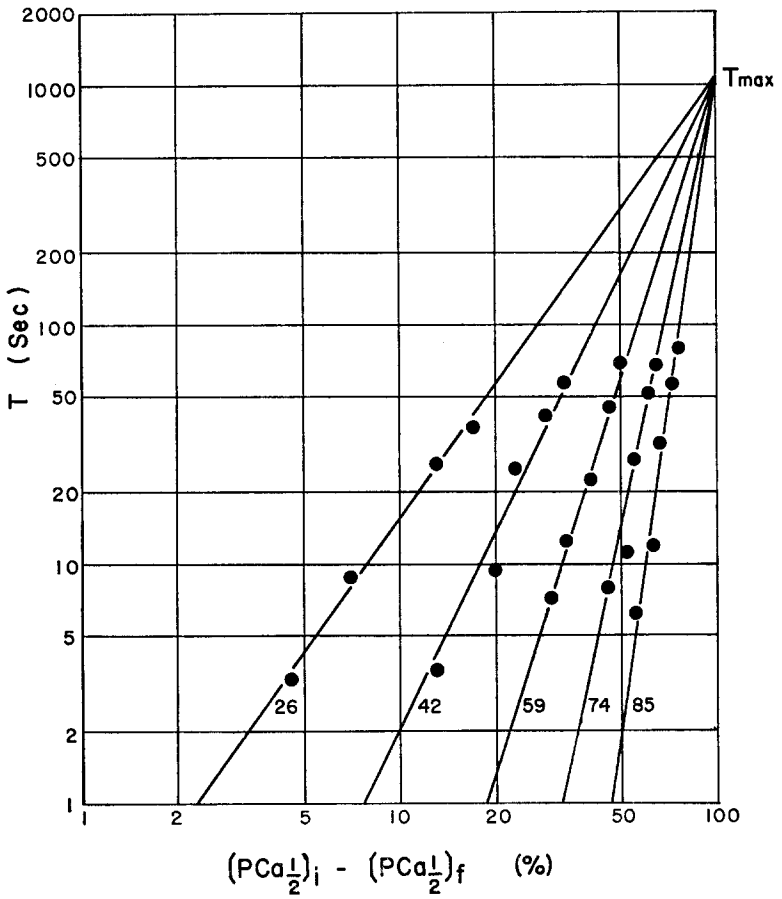

FIGURE 11. Relation between the duration of ciliary reversal (T in sec) and the difference between the initial ($[PCa\frac{1}{2}]_i$) and final ($[PCa\frac{1}{2}]_f$) amounts of bound calcium (as % of total binding capacity, P_t). Each straight line on the figure corresponds to a different initial amount of bound calcium, $[PCa\frac{1}{2}]_i$, as indicated in % of P_t[4] by the numerals beside each line.

In order to examine the empirical relationships among durations of reversal (T), initial amount of calcium bound by the cation exchange system ($[PCa\frac{1}{2}]_i$), and final amount of calcium bound by the system after equilibration with the stimulation medium ($[PCa\frac{1}{2}]_f$), the results shown in Table IV

[7] Y. Naitoh. 1967. Unpublished data.

were plotted with reversal duration as a function of ($[PCa\frac{1}{2}]_i - [PCa\frac{1}{2}]_f$) at five values of $[PCa\frac{1}{2}]_i$ (Fig. 11). The values of both $[PCa\frac{1}{2}]_i$ and $[PCa\frac{1}{2}]_f$ were calculated from Equation 2 by introducing the values of k (0.35) (17) and J_a of both equilibration and stimulation media, and were given as % of P_t.

Fig. 12 shows that the logarithm of T is proportional to the logarithm of ($[PCa\frac{1}{2}]_i - [PCa\frac{1}{2}]_f$) and that the slope of this proportionality depends on $[PCa\frac{1}{2}]_i$ being steeper at higher values of $[PCa\frac{1}{2}]_i$. Each line was extrapolated to the abscissa of the figure and the values of the intercepts were plotted

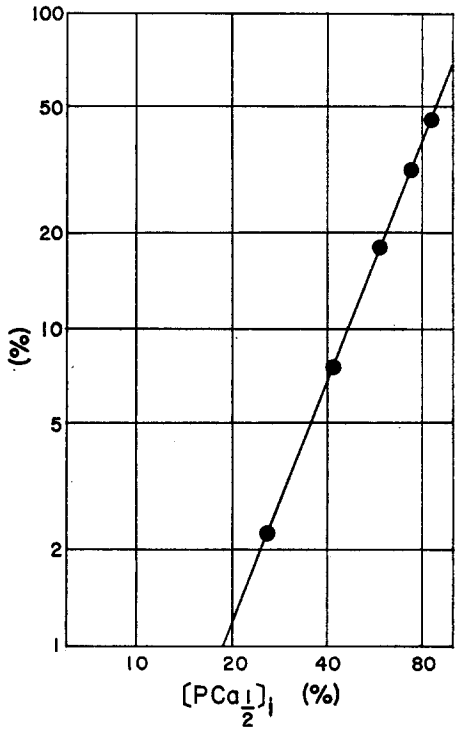

FIGURE 12. Relationship between value of $[PCa\frac{1}{2}]_i$ (abscissa) and the value of the intercept (ordinate) of each line with abscissa on Fig. 11.

against $[PCa\frac{1}{2}]_i$ (Fig. 12). It is seen that the logarithm of the value of the intercept is related linearly to the logarithm of $[PCa\frac{1}{2}]_i$, and the slope of this logarithmic plot (b) is about 2.5 (Table V).

From these findings, together with the fact that the logarithm of T is related linearly to the logarithm of the calcium concentration in the stimulation medium ($[Ca^{++}]_s$) (see Fig. 10 and Equation 1), the relation of T to $[PCa\frac{1}{2}]_i$, $[PCa\frac{1}{2}]_f$, and $[Ca^{++}]_s$ is seen to be

$$T = [Ca^{++}]_s^a \left(\frac{[PCa\frac{1}{2}]_i - [PCa\frac{1}{2}]_f}{c.[PCa\frac{1}{2}]_i^b} \right)^{\log \frac{\log T_{max}}{P_t}} \qquad (3)$$

in which T_{max} is a constant representing the maximum duration of reversal (sec) corresponding to the maximum difference between $[P\mathrm{Ca}\frac{1}{2}]_i$ and $[P\mathrm{Ca}\frac{1}{2}]_f$ (P_t; see Fig. 12); c is a constant representing a value of ($[P\mathrm{Ca}\frac{1}{2}]_i - [P\mathrm{Ca}\frac{1}{2}]_f$) (%) corresponding to a unit duration of reversal (1 sec) in the specimens having a unit amount of bound calcium (1% of P_t); both constants are, of course, for a $[Ca^{++}]_e$ of 1 mM.

The values of both T_{max} and c were found to fluctuate, apparently according to the condition and age of the culture (see Table V). Therefore, the duration of ciliary reversal in response to a given condition differed among groups of the specimens obtained from different cultures. On the other hand, the value of b was almost identical among the different groups of specimens (Table V). Data of Kamada and Kinosita (4) and of Grębecki (19) on the duration of reversal fit Equation 3 if different values of T_{max} and c are used.

Although the physicochemical meaning of this equation is presently unclear, it will be explored elsewhere in terms of the present hypothesis after determination of the time course of bound calcium release in response to ionic stimulation.

TABLE V

FLUCTUATIONS IN T_{max}, b AND c AMONG DIFFERENT GROUPS OF *Paramecium caudatum*

No. of groups	T_{max}	b	c
	sec		(10^{-4}) %
1	1120	2.5	6.2
2	3160	2.2	30.1
3	794	2.5	5.1
4	631	2.5	5.1
5	794	2.6	4.6

It is important to our understanding of the mechanism of ciliary reversal to determine the site of the cation exchange system. Presently, however, we have no precise data from which we can deduce its location; nevertheless, it is likely that the anionic sites are at or near the surface, because binding equilibrium is achieved relatively rapidly (17), and because the cilia with their associated cortical apparatus are, of course, located close to the cell surface. Radioautographic studies on calcium binding by *Paramecium*[8] demonstrated a consistent accumulation of ^{45}Ca at the surface of the organism.

The site of the proposed contractile component concerned with ciliary reversal also remains to be identified. It is of interest, in this regard that Ca-activated ATPase activity is associated with the cellular fraction of *Tetrahymena* which contains the remnants of the ciliary apparatus (22).

[8] T. Yamaguchi. 1967. Personal communication.

The involvement of calcium in the control of ciliary reversal is reminiscent of its role in muscle in which Ca^{++} is thought to be released from intracellular sequestering sites to activate the muscle ATPase (23–26). There is also evidence in ciliated protozoa that the reversal of local ciliary beat is correlated with local release of Ca^{++} (13, 16, 27, 28). The control of ciliary reversal appears, therefore, to exhibit some close analogies with the control of muscle contraction.

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