EFFECTS OF CADMIUM ON THE ACTIVE TRANSPORT OF SODIUM BY THE ABDOMINAL SKIN OF A BULLFROG (*RANA CATESBEIANA*)

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Abstract Effects of Cd on the active transport of Na in SO_4 -Ringer's solution on the abdominal skin of a bullfrog (*Rana catesbeiana*) were investigated. The short circuit current (SCC), potential difference (PD), and Na flux were determined. The skin resistance (R_M), the electromotive force of active Na transport (E_{Na}), and the resistance to the active Na current (R_{Na}) were calculated from the measured data. The application of Cd to the epidermal side at 2 mm was more effective in increasing SCC than that to the dermal side at the same concentration. The time course of changes in SCC induced by Cd were classified into three types: (I) a simple increase in SCC during Cd application, (II) an early decrease followed by an increase in SCC, and (III) a decrease in SCC except for a small increase in the initial short period. Type I response was observed in the majority of cases (about two-thirds). Types II and III were observed in 7 and 32 percent of 28 cases, respectively. The Cd-induced changes in SCC were analyzed on the basis of changes in E_{Na} and R_{Na} . In Type I, the increase in SCC was due to a remarkable decrease in R_{Na} without an appreciable change in E_{Na} ($\because \text{SCC} = E_{\text{Na}}/R_{\text{Na}}$). In Types II and III, the changes in SCC in the early and the later stages could be explained by the differences in the extents to which E_{Na} and R_{Na} decreased. The experimental and calculated ratios of PD_{Cd} to $\text{PD}_{\text{control}}$ in these stages were in good agreement with each other.

Frog skin has long been regarded as an excellent material for the study of active transport of Na because of the easiness of flux measurements and electrical studies (USSING, 1949). The effects of various metal ions, such as Ca (CURRAN and GILL, 1962), Cu (ZADUNAISKY *et al.*, 1963; FERREIRA, 1970), La (MARTINEZ-PALOMO *et al.*, 1971) and Cd (STYMANS and BORGHGRAEF, 1969; HILLYARD and GONICK, 1976), have been studied in the frog skin to elucidate the mechanism of

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the active transport on Na. We are especially interested in the effect of Cd, because it has been known to be antagonistic to calcium which plays some important roles in regulation of normal physiological functions in various preparations such as contraction of cardiac muscle and neurotransmitter release from autonomic nerve endings (HAYASHI and HORIUCHI, 1971; HAYASHI and TAKAYAMA, 1977). The active transport by frog skin was decreased by Ca (CURRAN and GILL, 1962), therefore, we examined Cd effects on the frog skin in order to find out whether Cd might exert an effect opposite to Ca, *i.e.*, does it increase the Na active transport or not.

The effects of Cd on the frog skin have been tested by other investigators (STYMANS and BORGHGRAEF, 1969; HILLYARD and GONICK, 1976); however, their results do not seem to be consistent with respect to Cd-induced changes in the short circuit current (SCC) and the skin resistance (R_M). We examined Cd effects under simplified conditions, namely, all Cl ions in Ringer's solution were replaced by more impermeable anions, SO_4 and gluconate, so that we could exclude the probable effects on passive and the possible active transport of anions.

Preliminary accounts of this work were given elsewhere (HAYASHI *et al.*, 1974).

MATERIALS AND METHODS

The abdominal skin of a bullfrog (*Rana catesbeiana*) of both sexes were used between December 1973 and May 1974. The experimental animals, which were captured around the Chiba district, were supplied by the "Supply Center of Biological Materials in Japan." The frogs were kept at room temperature in a bath for a week or two without feeding. The water level was adjusted to just above the abdomen of the animals.

Fig. 1. Diagram of an apparatus (type A) for measuring the potential difference and the short circuit current of abdominal frog skin. The preparation was mounted on a tip of a vertical glass cannula. I: microammeter, V: millivoltmeter.

Fig. 2. Diagram of an apparatus (type B) for determining Na flux under the short circuited condition in frog skin. The preparation was mounted between Ussing-type lucite chambers. V_{com} : ground potential.

The frogs were anesthetized with intrathecal injection of 0.5 ml urethane solution (0.25 g/ml). The abdominal skin was removed by dissection and mounted either on the tip of a glass cannula (A-type apparatus, Fig. 1) or between the Ussing-type lucite chambers (B-type apparatus, Fig. 2). A-type apparatus was used for measuring the potential difference (PD) and the short circuit current (SCC) of frog skin, and B-type apparatus was used for determining Na flux in addition to measurements of PD and SCC.

The PD of the skin was measured (dermal side positive) at room temperature through a pair of salt bridge electrodes (3 M KCl, 2% agar—3 M KCl—Ag—AgCl plate), a DC amplifier (ARITA and HAYASHI, 1973), and a millivoltmeter (YEW 2051-05). The asymmetry potential between the tips of the electrodes was usually less than 1 mV.

The current was applied across the skin through a pair of silver electrodes. In the experiments with the A-type apparatus, SCC was determined by adjusting the PD to zero manually (USSING and ZERAHN, 1951). The current was supplied from a constant current device, *i.e.*, a high resistance, a microammeter (YEW 2011-6), an isolator and a pulse generator (Nihon Kohden MSE-JT and MSE-40). In the experiments with the B-type apparatus, SCC was regulated automatically by using a feedback amplifier (voltage clamp method). The gains of two DC amplifiers were both 40 dB. The command signal of the control amplifier (V_{com}) was maintained at zero (ground potential). The data of the SCC were expressed as $\mu\text{A}/\text{cm}^2$. The exposed skin areas were 0.79 cm^2 for A-type experiment, and

4.9 cm² for B-type experiment.

The skin resistance (R_M) was calculated by using the following equation: $R_M = PD/SCC$ (k Ω ·cm²). The change in PD was shown to be directly proportional to the applied current ranging from zero to SCC.

Both sides of the skin preparation were soaked in aerated Ringer's solution whose hydrostatic pressure difference upon epidermal and dermal surfaces was adjusted to zero.

Most of the experiments were carried out in SO₄-Ringer's solution (Na₂SO₄ 55 mM, K₂SO₄ 1 mM, Ca gluconate 1 mM, glucose 10 mM, sucrose 60 mM, and Tris-SO₄ buffer 10 mM, pH 7.2). In a few preliminary experiments, Cl-Ringer's solution (HAYASHI and HORIUCHI, 1971) was also used.

Unidirectional sodium flux was occasionally measured in the short-circuited skins using ²²Na. Radioactive tracer (20 μ Ci/0.8 ml) was added to the inside chamber when the outflux was measured (final concentration: 1.04 μ Ci/ml), while 2 μ Ci/0.08 ml was added to the outside solution for the influx measurement (final concentration: 0.10 μ Ci/ml). Samples (4 ml) were removed from the solution of the opposite side every 15 min for radioactivity determination. Immediately after the removal of 'hot' Ringer's solution, the same amount of 'cold' Ringer's solution was added to the chamber to keep the volume of solution (20 ml) constant. The radioactivity of the sample in a lucite test tube was counted in a well-type scintillation counter (Aloka Autowell Gamma System JDC-752). The fall in the activity due to dilution by sampling was arithmetically compensated.

RESULTS

1. *Effects of Cd concentration*

Effects of Cd at various concentrations on the potential difference (PD) of abdominal frog skin was preliminary examined in Cl-Ringer's solution by using the A-type apparatus. A small amount of concentrated Cd solution was applied to both sides of the skin. The change in osmolarity due to the addition of Cd was less than 1 percent. The higher the concentration of Cd, the larger the increase in the PD in the range from 2 mM to 0.1 mM Cd. An example of effects of Cd at two concentrations is represented in Fig. 3.

Change in PD was not remarkable in most cases when Cd was applied in Cl-Ringer's solution. In order to exclude the effect on passive and probable active transport of Cl in frog skin, we replaced Cl with more impermeable SO₄ in Ringer's solution in the following experiments.

2. *Effect of epidermal and dermal Cd*

The difference between effects of epidermal (outside) and dermal (inside) application of Cd was examined in SO₄-Ringer's solution. The solutions of both sides were renewed about one hour after the setup of the skin preparation. The

Fig. 3. Effects of concentration of Cd on the potential difference (PD) of frog skin. Two mM Cd was applied to both sides of the preparation.

skin under the open-circuited conditions was allowed to equilibrate for at least two hours before application of Cd. Both short circuit current (SCC) and PD of the skin were intermittently recorded. The concentration of Cd was fixed at 2 mM which exerted the maximum effect on the PD. Figure 4 represents a typical example of the effect of Cd given to either dermal or epidermal solution (A-type

Fig. 4. Effects of epidermal (outside) and dermal (inside) 2 mM Cd on the short circuit current (SCC) and the potential difference (PD) of frog skin.

experiment). Both SCC and PD were remarkably increased immediately after the application of Cd to the outside; SCC was increased to 136 and 135 percent of the control, and PD was also increased to 115 and 117 percent of the control in this particular case. On the contrary, application to the inside brought about no remarkable changes in SCC and PD (less than 2 percent in this case).

3. Classification of Cd effects

In most preparations, both SCC and PD were increased throughout the period of Cd application. However, more complicated time courses of changes in SCC and PD were sometimes observed. Figure 5 illustrates an example which shows

Fig. 5. Three kinds of patterns on the changes in the short circuit current (SCC) and the potential difference (PD) induced by successive applications of epidermal Cd (2 mM).

different time courses of the changes induced by successive epidermal application of Cd at 2 mM in a single skin preparation.

We classified the responses of SCC and PD to Cd into three types: (I) a simple increase in SCC and/or PD during the presence of Cd, (II) a decrease followed by an increase in SCC and/or PD except initial short period of small increase, and (III) a decrease in SCC and/or PD except initial short period of small increase.

4. Cd effect on Na flux

In order to elucidate mechanisms underlying above stated complicated responses of frog skins, Na flux was measured in following conditions; the concentration of Cd: 2 mM, side of application of Cd: epidermal side (outside), solu-

tion: SO_4 -Ringer's solution. Cd-induced changes in electromotive force of Na (E_{Na}), resistance to the active Na current (R_{Na}), and resistance to the passive ions (R_{Σ}) were compared with those in PD, SCC, and resistance of skin (R_M). E_{Na} , R_{Na} , and R_{Σ} were calculated by using the following equations: $E_{\text{Na}} = (RT/F) \ln(\text{Na influx/Na outflux})$, $R_{\text{Na}} = E_{\text{Na}}/\text{SCC}$, and $R_M = R_{\Sigma} \cdot R_{\text{Na}} / (R_{\Sigma} + R_{\text{Na}})$, respectively. As the unidirectional flux of Na was determined in the present experiment, the Na flux in the opposite direction was calculated from the equation: $\text{SCC} - (\text{unidirectional flux})$. Both influx and outflux were increased to similar extents by epidermal application of Cd in the preliminary experiment of Na flux determination. In order to minimize the error in calculating E_{Na} , influx was obtained from the difference between SCC and Na outflux. Na was considered to be a sole ionic species responsible for the SCC because transport of anion (SO_4) might be little affected by Cd because of its low permeability.

E_{Na} , R_{Na} , and R_{Σ} before and after application of Cd were calculated by determining Na flux in the cases of Fig. 6A, B and C which were the typical examples of Type I, II, and III, respectively. ^{22}Na was injected to the dermal side one hour before the application of Cd. Sampling was started after 30 min of ^{22}Na introduction in order to equilibrate radioactive tracer in the frog skin. The data of Na determination during initial 10 to 15 min of Cd application were also discarded because the equilibrium state in the skin might not be attained during this period.

Table 1 summarizes the effects of Cd on the characteristics of the frog skin. The data in the upper, middle, and lower sections of Table 1 were obtained from the experiments in Fig. 6A, B, and C, respectively. All the data in Table 1 are

A

Fig. 6. Three typical examples of the effects of Cd on the short circuit current (SCC), the potential difference (PD), and the skin resistance (R_M). Na fluxes were determined every 15 min in these preparations. A: (Type I) increase in SCC during the application of Cd (1 hr). B: (Type II) decrease in SCC at the early stage (30 min after Cd application) followed by increase at the later stage (1 hr). C: (Type III) decrease in SCC (1 hr).

expressed as the means of the respective data throughout the period mentioned in parentheses at the item of experimental conditions.

A. Analysis of Type I response. In the experiment of Fig. 6A (a typical

Table 1. Effects of Cd on the characteristics of the frog skin. M_i : Na influx, M_o : Na outflux, SCC: short circuit current, PD: potential difference of frog skin, R_M : skin resistance, E_{Na} : electromotive force of active Na (Ussing's model), R_{Na} : resistance to the active Na current (Ussing's model), R_S : resistance to the passive ions (Ussing's model).

Experimental condition		Characteristics of frog skin							
		M_i ($\mu A/cm^2$)	M_o ($\mu A/cm^2$)	SCC ($\mu A/cm^2$)	PD (mV)	R_M ($k\Omega \cdot cm^2$)	E_{Na} (mV)	R_{Na} ($k\Omega \cdot cm^2$)	R_S ($k\Omega \cdot cm^2$)
041874-II (23.4°C)	control (30 min)	13.3	1.89	11.4	40.1	3.20	50.0	4.39	11.8
	Cd (10–40 min)	21.0	3.13	17.9	44.3	2.34	48.8	2.72	16.7
	(Cd/control)	(1.58)	(1.66)	(1.57)	(1.10)	(0.731)	(0.976)	(0.620)	(1.42)
052374-II (27.0°C)	control (30 min)	17.0	0.404	16.6	83.6	5.06	95.7	5.79	39.8
	Cd (30–45 min)	26.9	11.8	15.1	18.9	1.25	21.1	1.40	11.7
	(Cd/control)	(1.58)	(29.2)	(0.910)	(0.226)	(0.247)	(0.220)	(0.242)	(0.294)
	Cd (75–105 min)	26.8	3.50	23.3	50.5	2.17	52.2	2.24	69.4
	(Cd/control)	(1.58)	(8.66)	(1.40)	(0.604)	(0.428)	(0.545)	(0.386)	(1.74)
050974-II (25.0°C)	control (30 min)	14.8	2.66	12.1	34.6	2.87	43.9	3.63	13.7
	Cd (15–30 min)	17.7	7.46	10.2	19.1	1.87	22.1	2.17	13.5
	(Cd/control)	(1.20)	(2.80)	(0.843)	(0.552)	(0.652)	(0.503)	(0.598)	(0.985)
	Cd (30–60 min)	15.6	5.10	10.5	23.7	2.25	28.6	2.72	13.0
	(Cd/control)	(1.06)	(1.92)	(0.868)	(0.685)	(0.784)	(0.651)	(0.749)	(0.949)
	reversal (25–55 min)	18.5	4.69	13.8	34.3	2.50	35.1	2.55	128
	(reversal/control)	(1.25)	(1.76)	(1.14)	(0.991)	(0.871)	(0.800)	(0.702)	(9.34)

example of Type I group), SCC was greatly increased immediately after Cd application, and reached maximum after about 20 min. Cd also induced a slight increase in PD which attained maximum about 20 min after Cd application. On the contrary, R_M was remarkably decreased during Cd application.

The Cd-induced increase in SCC in Fig. 6A seems to be explained by the remarkable decrease in R_{Na} (62.0% of the control) accompanied by little change in E_{Na} (97.6%). (Note that $SCC = E_{Na}/R_{Na}$.) Although the rates of decrease in R_M (73.1%) and R_{Na} (62.0%) were almost the same, R_y seemed to be increased (142%). According to Ussing's model for E_{Na} , R_{Na} , and R_y , PD is calculated by using the following equation: $PD = E_{Na} \cdot R_y / (R_{Na} + R_y)$. Therefore, PD increase in Fig. 6A might be explained by R_{Na} decrease, no change in E_{Na} , and an increase in R_y . Good coincidence between the experimental and calculated values of the ratios of $PD_{Cd}/PD_{control}$ was obtained, the former being 1.10 and the latter 1.14, respectively. Calculation of $PD_{Cd}/PD_{control}$ was made by using the following equation:

$$PD_{Cd}/PD_{control} = (E_{Na,Cd})/(E_{Na,control}) \cdot (R_{y,Cd})/(R_{y,control}) \cdot (R_{Na,control} + R_{y,control})/(R_{Na,Cd} + R_{y,Cd}).$$

It is noteworthy that the above equation for the calculation of PD ratio does not include the experimental PD values at all.

B. Analysis of Type II response. Figure 6B shows a typical example of Type II response in regard to the time course of SCC. Cd brought about a small initial increase in SCC (112% of the control) for a short period of 10 min, which was followed by a decrease at a relatively early stage (the minimum value, 83%, was attained at 30 min) and the second increase appeared in a later stage (90 min for the maximum value, 139%).

Cd induced a small initial increase in PD (110% of the control) which was followed by a remarkable decrease (30 min for the minimum value, 21%) and a slight slow recovery (80 min for the maximum value, 65%).

The remarkable decrease in R_M in the early stage (30 min for the minimum value, 22%) and the slight recovery (80 min for the maximum value, 42%) in the later stage were observed during Cd application.

The Cd-induced initial changes in the characteristics of the skin could not be analyzed by determining Na flux, because these initial changes disappeared within 10 min though new equilibrium of Na in the skin took at least 15 min after the application of Cd.

The Cd-induced decrease in SCC in the early stage can be explained by the difference in the extents of decreases in both E_{Na} (22.0%) and R_{Na} (24.2%).

The experimental and calculated ratios of PD_{Cd} to $PD_{control}$ in the early stage were in good agreement with each other: 22.6% and 22.5%, respectively.

As R_y was always much larger than R_{Na} , R_M was mainly determined by R_{Na} . Therefore, the remarkable decrease in R_M in the early stage is due to the remarkable decrease in R_{Na} .

The increase in SCC in the later stage of Cd effect can be explained by the difference in the extents of the recovery of E_{Na} (22.0→54.5%) and R_{Na} (24.2→38.6%), respectively.

The experimental and calculated ratios of PD_{Cd} to $PD_{control}$ in the later stage were in good agreement with each other: 60.4% and 60.3%, respectively.

Slight recovery of R_M (24.7→42.8%) is caused by that of R_{Na} (24.2→38.6%).

C. *Analysis of Type III response.* Figure 6C shows a typical example of Type III response. As in the case of Fig. 6B, Cd induced a small initial increase in SCC (105% of the control); however, SCC was decreased during the whole period of Cd application though very slight recovery was observed. The time courses of PD and R_M during the effect of Cd were similar to that of SCC. The good recovery was observed after washing with SO_4 -Ringer's solution.

The mechanism of Cd-induced changes in SCC, PD, and R_M in the early and the later stages in Fig. 6C seems to be essentially the same, with respect to E_{Na} and R_{Na} , as that in Fig. 6B except for the extent of recovery during Cd application which is very slight in Fig. 6C as compared with that in Fig. 6B. There is a good agreement in experimental and calculated values of the ratio ($PD_{Cd}/PD_{control}$): 55.2 and 54.8% in the early stage, 68.5 and 68.1% in the later stage, respectively.

5. *Analyses of SCC and PD in terms of E_{Na} and R_{Na}*

The results of 28 cases of Na flux determination are summarized in Figs. 7 and 8. Among the total experiments, 17 cases (61%) belong to Type I (an increase in SCC during Cd application), 2 cases (7%) to Type II (an early decrease followed by a later increase in SCC), and 9 cases (32%) to Type III (a decrease in SCC during Cd application). All data in these figures are standardized by control data ($datum_{Cd}/datum_{control}$).

Figure 7A shows the relationship between R_{Na} and SCC. The dots in the quadrant IV belong to Type I. There is a rough linear relationship between them; the larger the SCC, the smaller the R_{Na} . The arrows indicate the transition from the state of the early stage to that of the later stage during Cd application.

The dots in the quadrants II and III belong to Type III. The Cd induced SCC decrease was accompanied with R_{Na} decrease in the most cases of Type III group. SCC is roughly proportional to R_{Na} .

There are two dots which translocate from the quadrant III to the quadrant IV as indicated by arrows. These cases belong to Type II group.

Figure 7B represents the relation between E_{Na} and SCC. No appreciable change in E_{Na} is observed despite significant increases in SCC in the most cases of Type I group. There is a rough linear relationship between E_{Na} and SCC in the most cases of Type III group.

The relations of R_{Na} and E_{Na} with respect to PD are illustrated in Fig. 8A and B, respectively. No appreciable correlation between R_{Na} and PD is observed; however, clear correlation is demonstrated between E_{Na} and PD.

Fig. 7. Relationship between R_{Na} and SCC (A), and that between E_{Na} and SCC (B) after epidermal 2 mM Cd. All data are standardized. Arrows indicate the data obtained at the early stage and the later stage during Cd application.

DISCUSSION

The mechanism of active transport of Na was studied by applying Cd to frog skin in simplified system, *i.e.*, under a short-circuited condition in SO_4 -Ringer's solution. The reasons why the present experiments were carried out in SO_4 -

Fig. 8. Relationship between R_{Na} and PD (A), and that between E_{Na} and PD (B) after epidermal 2 mM Cd. All data are standardized. Arrows indicate the data obtained at the early stage and the later stage during Cd application.

Ringer's solution instead of Cl-Ringer's solution were as follows; 1) Our preliminary experiments on Cd effects on the frog skin during one year revealed that change in SCC and/or PD was not remarkable in most cases of Cl-Ringer's solution. This finding seemed to be in accord with those of other investigators'.

2) The Cl transport might not be passive only; there is a high possibility of the active Cl transport (ZADUNAISKY *et al.*, 1963; USSING and ZERAHN, 1951; MARTIN and CURRAN, 1966); therefore, we used less a permeable anion, SO_4 , (KOEFOED-JOHNSEN and USSING, 1958; HAGIWARA *et al.*, 1971) instead of Cl in Ringer's solution.

No consistent results were obtained for the direction of Cd-induced change in SCC by other investigators; in other words, both increase and decrease in SCC were reported by them. We have classified the time courses of SCC after Cd application into three types. As previously described, in approximately two-thirds of cases (Type I), SCC was sustained at an increased level, while in the rest of cases (Types II and III), SCC changed in a triphasic manner (an initial increase, a successive decrease in the early stage and an increase in the later stage). The above stated classification suggests that the time course of Cd-induced change in SCC was composed of at least three exponential curves whose time constants and latencies after application of Cd were different from each other. The first positive component increases immediately after the application of Cd, and reaches maximum within about 5 min. The second negative component begins to decrease about 5–10 min after Cd, and reaches minimum within 20–30 min. The third positive component starts to increase 20–30 min after Cd, and attains maximum within about 1 hr.

The three different "phenotypes" (Types I, II, and III) in regard to time course of SCC, therefore, seem to be consistently explained by a sequence of three components or processes. The appearance of each phenotype depends on the predominance of one of three processes over the others. Thus, if the first process prevails against the others throughout, Type I will appear. The early stages of Type II and III (the decrease in SCC) are due to the predominance of the second process over the first one. The large predominance of the third process will explain the later stage of Type II (the increase in SCC).

The possible mechanisms of these three processes will be tentatively assumed. The first process is due to decrease in the membrane resistance of the outer barrier within epidermis. This assumption is supported by the following experimental facts: 1) a decrease in calculated R_{Na} , 2) a decrease in total skin resistance, 3) a decrease in skin resistance of epidermal side (*cf.* our next paper), and 4) ineffectiveness of dermally applied Cd.

The second process is supposed to be an inhibitory effect of Cd which invades the epidermis and lowers the activity of Na, K-ATPase. This assumption is supported by the experimental facts: 1) a decrease in calculated E_{Na} , 2) histochemical verification of Cd in epidermis (no Cd existence in epidermis when Cd was applied dermally) (unpublished observation), and 3) a decrease in Na, K-ATPase activity over 10^{-6} M Cd (unpublished data).

The third process is phenomenologically due to the increase in E_{Na} or the recovery in the later stage preceded by E_{Na} decrease in the early stage. However,

the underlying mechanism is still unclear. A few assumptions may be considered as the responsible factors as 1) an increase in Na concentration in the epidermal cells, 2) disinhibition of Na,K-ATPase which is once inhibited by Cd, and 3) changes in the morphological or functional bindings between epidermal cells, *etc.*

Cd increases the total skin resistance (R_M) in Cl-Ringer's solution (BANKS, 1974; BORGHGRAEF *et al.*, 1971), whereas, R_M is clearly decreased by Cd in SO_4 -Ringer's solution in our present study. This result agrees with that of Banks. As Cl is considered to be a main ionic species for passive transport in Cl-Ringer's solution on frog skin, Cd is supposed to narrow the passive channel of the skin. This assumption is strongly supported by our observation that R_s (the resistance to the passive ions) was increased several times by Cd in some cases (*cf.* Table 1). The electronmicroscopic study on whether intercellular space in the epidermis would actually be reduced will be reported elsewhere. The Cd-induced decrease in R_M in spite of an increase in R_s in SO_4 -Ringer's solution is thus explained by the remarkable decrease in R_{Na} which is brought about as a result of the decrease in the resistance of epidermal cells.

We have succeeded in demonstrating the clear difference between the effects of epidermal and dermal Cd. As shown in Fig. 4, dermal application of Cd at the same concentration as that applied to the epidermal side and at the same period does not affect SCC at all. This difference might be explained by the histochemical observation that dermally applied Cd does not reach the epidermis (unpublished observation). The Cd blockade in dermis might be due to the capture of Cd by the large amount of Ca in dermis (especially in the sieve layer) (TAYLOR *et al.*, 1966; HAYASHI *et al.*, 1977).

Ca has been known as a competitive antagonist with Cd in the contractility of the cardiac muscle (HAYASHI and HORIUCHI, 1971). Ca has been also shown to counteract with Cd in the acetylcholine releasing mechanism from the peripheral end of vagal nerve (HAYASHI and TAKAYAMA, 1977). In the present study with frog skin, we observed approximately 35% increase in SCC, 10% decrease in R_M in Ca-free EDTA Ringer's solution, while application of 2 mM Ca brought about 20% decrease in SCC. These results closely resemble those of HILLYARD and GONICK (1976) and CURRAN and GILL (1962). From the above results, however, we could not conclude at the present moment that Cd and Ca counteract with each other though these two ionic species have an opposite effects on SCC and R_M .

The effects of Cd on the frog skin are interesting not only from the standpoint of general physiology, but also from that of pathophysiology. Some type of renal hypertension has been caused by Cd as the result of increased active Na transport (VANDER, 1962; SCHROEDER, 1964).

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