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Effect of Surface-active Agent on the Active Transport of Sodium in Frog Skin[1)]

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The effect of surface-active agent on the active transport of sodium in the frog skin was studied. It was assumed that alkyldimethylbenzylammonium chloride (alkyl-DBAC) in 10^{-3}M concentration inhibited the active transport of sodium accompanied with Na^+–K^+ adenosine triphosphatase (ATP ase), whereas it increased the influx and outflux of ^{24}Na through the frog skin probably due to the structural damage on the frog skin. This effect was observed above the critical micellar concentration of this surfactant. The increased influx of ^{24}Na was decreased by the addition of 1 μg Pi/ml of phospholipid to 10^{-3}M alkyl-DBAC, but it still remained at a higher level. The effect of phospholipase C on the active transport of sodium in the frog skin was different from that of alkyl-DBAC, and 0.5 mg/ml of phospholipase C also increased the influx of ^{24}Na, whereas the increased influx of ^{24}Na was depressed by 10^{-5}M ouabain.

It is well known that biomembrane consists of lipid and protein.[3)] The interaction between surfactant and the component of biomembrane was reported to cause the denaturation of protein structure,[4)] inhibition of enzyme activity,[5)] elimination of phospholipid from erythrocyte,[6)] and the destruction and solubilization of erythrocytes.[7)] Alkyldimethylbenzylammonium chloride (alkyl-DBAC), a cationic surface-active agent, is widely used as a broad spectrum antimicrobial agent[8)] and externally applied on the skin or mucosa. It is important, therefore, to know the nature of its effect on the function of the skin. The effect of alkyl-DBAC on the skin of mammals was examined only visually or histopathologically[9)] owing to the complexity of the structure and the function. As pointed out by Bangham[10)] and Iwasaki,[11)] the active transport of sodium and potasium performs an important role in inflaming the tissue. We preliminarily studied the effect of alkyl-DBAC on the active transport of sodium using the frog skin, the structure of which is known to be fairly simple to begin with.[12)] The concentration of the alkyl-DBAC in the experiments was selected to be close to the critical micellar concentration because many physical properties of the solution of the surfactants frequently changes abruptly at the critical micellar concentration. The influence on the frog skin of the addition of phospholipid to alkyl-DBAC solution was investigated. And the

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3) S.J. Singer and G.L. Nicolson, *Science*, **175**, 720 (1972).
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different effect between alkyl-DBAC and phospholipase C was also examined, because the elimination of lipids that was the characteristics of surfactant may have an important effect on the biomembrane.

Experimental

Reagents——Alkyl-DBAC: Dodecyl-DBAC was used and the purity of hydrocarbon chain was 99.0%.

Ouabain: Uabanin was obtained from Takeda Chemical Industries, Osaka.

Phospholipid: The total mixed phospholipid was extracted from rat liver by Hanahan's method[13] and phosphorus was determined by Chen's method[14] after wet combustion with $HClO_4$.

Phospholipase C: Phospholipase C was obtained from Sigma Chemical Co., USA.

Krebs-Ringer Bicarbonate Solution (Ringer solution): Consisted of 100 ml 0.9% NaCl, 4 ml 1.15% KCl, 3 ml 1.22% $CaCl_2$, 1 ml 2.11% KH_2PO_4, 1 ml 3.8% $MgSO_4 \cdot 7H_2O$, and 21 ml 1.3% $NaHCO_3$. pH was adjusted to 7.2—7.4 with CO_2 before use.

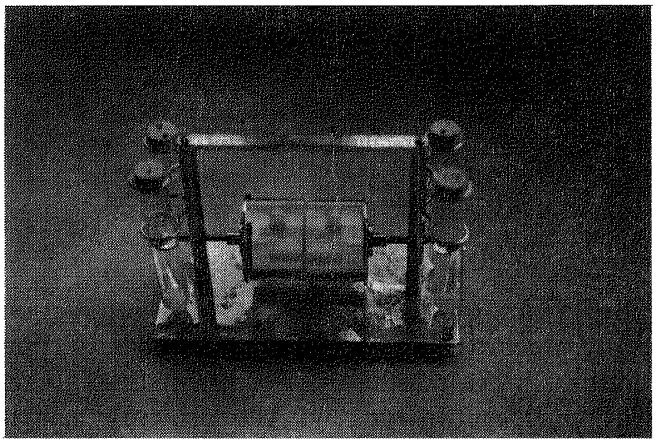

Photo. 1. Apparatus used in Measurement of the Flux of ^{24}Na

The inner diameter of the each chamber was 3 cm and maximum volume was 16 ml.

Apparatus——The apparatus shown in Photo. 1 is a modification of that used by Ussing.[15] The inner diameter of the chamber was 3 cm and maximum volume was 16 ml. This experiment was performed in the state of open-circuit.

Animals——Every experimental group consisted of 6 frogs (*Rana catesbiana* or *Rana nigromacurata*). Two pieces of abdominal skin, 5 cm in diameter, were obtained from each *Rana catesbiana*, but only one piece was obtained from each *Rana nigromacurata* because of its small size.

Treatment with Chemicals——One of the isolated skins was treated with chemicals dissolved in the Ringer solution for 30 min. The other was used as a control.

Measurement of the Flux of ^{24}Na——After the chemical treatment, the skin was rinsed with the Ringer solution and mounted on the chamber. Immediately after the mounting, the chamber was filled with 15 ml of the Ringer solution, and it was left to stand for 1 hr until the condition of the skin became stable. In the case of influx, approximately 10 μ Ci of ^{24}Na in the Ringer solution was added to the chamber fluid bathed on the outer mucosal side of the frog skin. After 0.5 and 1 hr, 0.5 ml of the inside solution and 0.1 ml of the outside solution were taken in duplicate, and placed in the counting dishes. After the samples were dried by an infrared lamp, the radioactivity was counted by the Aloka GP-101 type GM tube. In the case of outflux, *Rana nigromacurata* was used and the procedure was performed in reverse of that of influx. The temperature was kept at 20° and, to avoid the fatigue of the frog skin, the experiments were performed in winter, from December to March.[16]

Calculation of Influx and Outflux——The influx of ^{24}Na was calculated from the following equation.[12]

$$Mi = \frac{Ns.\ (Vi/Vs).\ mst.\ F}{Nst.\ 3600.\ A} \quad (\mu A.\ cm^{-2})$$

where Mi=influx of ^{24}Na ($\mu A.\ cm^{-2}$),
Ns=cpm increase of ^{24}Na/hr in inner chamber,
Vi=total volume of Ringer solution in inner chamber,
$vi°$=14.5 ml, Vs=sampling volume, Vs=0.5 ml,
Nst=cpm of ^{24}Na in standard, mst=Na eq in standard,
mst (0.1 ml)=11.7 μeq, F=Faraday's constant (coulomb/eq),
A=area of frog skin, A=7.07 cm^2, 3600=sec

Measurement of the Critical Micellar Concentration——The critical micellar concentration was determined by the drop weight method.

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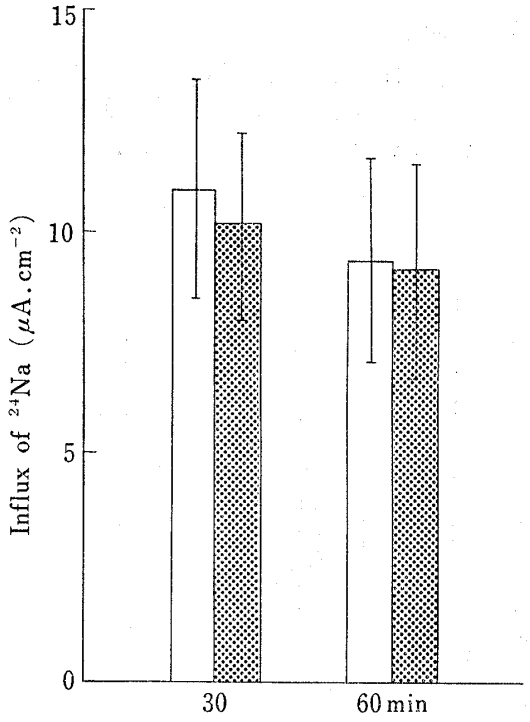

Fig. 1. Effect of 10^{-4}M Alkyl-DBAC on the Influx of ^{24}Na through Frog Skin

Two abdominal skin were isolated from Rana catesbiana. One of the skin was treated with 10^{-4}M alkyl-DBAC for 30 min and the other was used as a control. All the procedure was performed at 20°.

□: control
▨: 10^{-4}M alkyl-DBAC

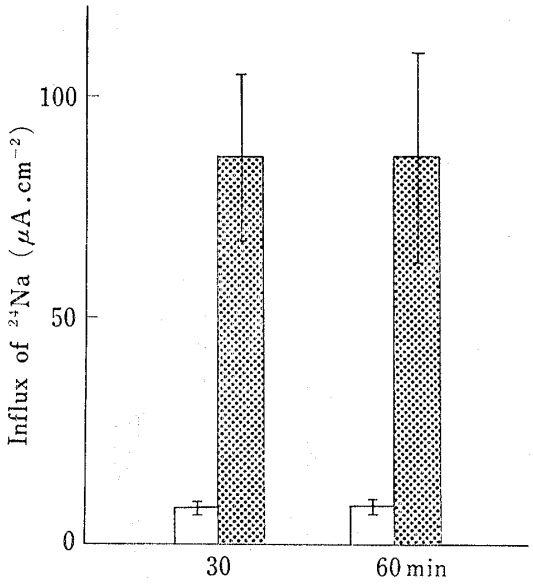

Fig. 2. Effect of 10^{-3}M Alkyl-DBAC on the Influx of ^{24}Na through Frog Skin

□: control
▨: 10^{-3}M alkyl-DBAC

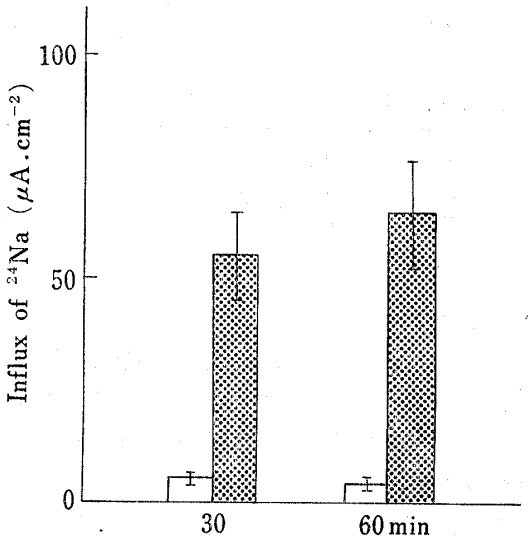

Fig. 3. Effect of 10^{-3}M Alkyl-DBAC on the Influx of ^{24}Na through Frog Skin at 0°

□: control
▨: 10^{-3}M alkyl-DBAC

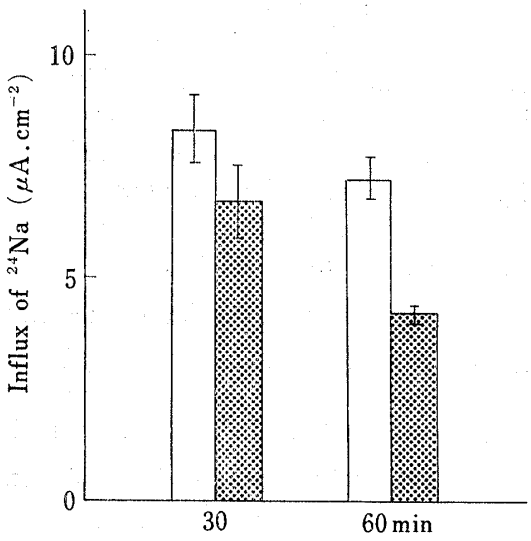

Fig. 4. Effect of 10^{-5}M Ouabain on the Influx of ^{24}Na through Frog Skin

□: control
▨: 10^{-5}M ouabain

Result

Effect of Alkyl-DBAC on the Influx of ^{24}Na through Frog Skin

The effect of alkyl-DBAC on the influx of ^{24}Na through the frog skin is presented in Figs. 1—3. The increased influx of ^{24}Na through the frog skin was observed at 10^{-3}M alkyl-DBAC and the same effect was observed when all the procedure was performed at 0°.

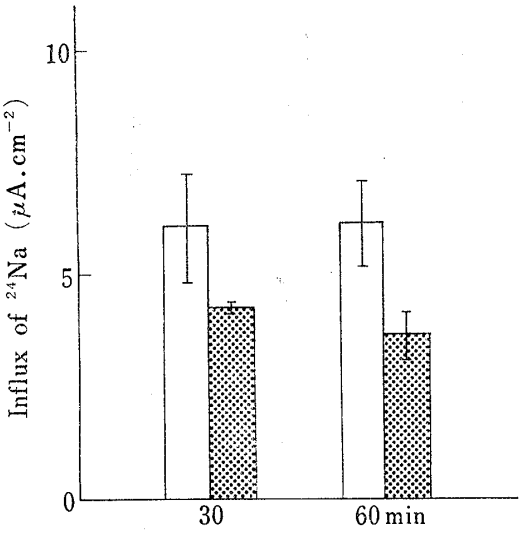

Fig. 5. Effect of 10^{-5}M Ouabain on the Influx of ^{24}Na through Frog Skin treated with 10^{-4}M Alkyl-DBAC

Frog skin was treated with the solution which contained 10^{-5}M ouabain and 10^{-4}M alkyl-DBAC.
□: 10^{-4}M alkyl-DBAC
▨: 10^{-4}M alkyl-DBAC+10^{-5}M ouabain

Effect of Ouabain on the Influx of ^{24}Na through Frog Skin

As shown in Figs. 4 and 5, 10^{-5}M ouabain decreased the active transport of ^{24}Na both in the normal frog skin and in the frog skin treated with 10^{-4}M alkyl-DBAC. However, as presented in Fig. 6, the effect of 10^{-5}M ouabain disappeared when the frog skin was treated with 10^{-3}M alkyl-DBAC.

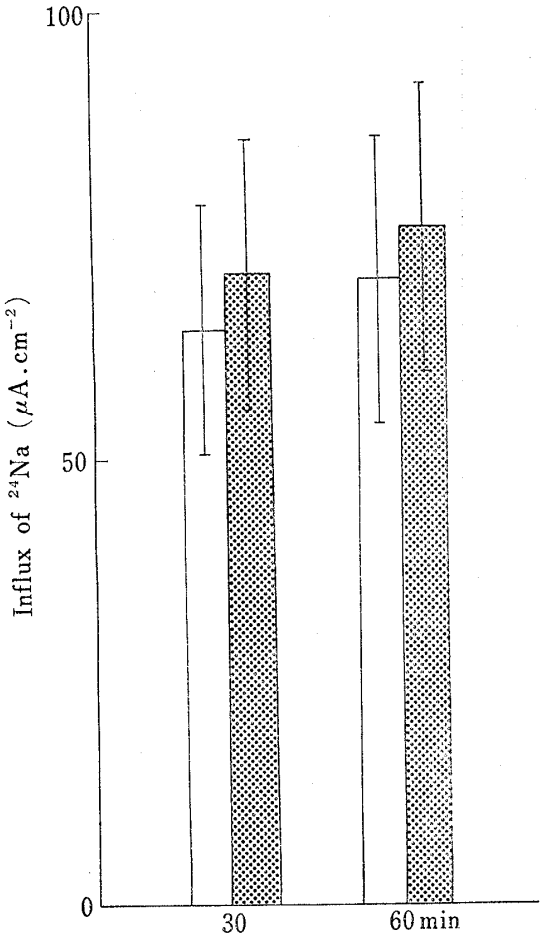

Fig. 6. Effect of 10^{-5}M Ouabain on the Influx of ^{24}Na through Frog Skin treated with 10^{-3}M Alkyl-DBAC

□: 10^{-3}M alkyl-DBAC
▨: 10^{-3}M alkyl-DBAC+10^{-5}M ouabain

Effect of Phospholipid on the Influx of ^{24}Na through Frog Skin

As described before, the increased influx of ^{24}Na through the frog skin treated with 10^{-3}M alkyl-DBAC was not depressed by 10^{-5}M ouabain but, as presented in Fig. 7, it was decreased by the addition of 1 μg Pi/ml of phospholipid from rat liver to 10^{-3}M alkyl-DBAC solution.

Effect of Alkyl-DBAC on the Outflux of ^{24}Na through Frog Skin

The effect of alkyl-DBAC on the outflux of ^{24}Na is presented in Fig. 8. These data suggest that 10^{-3}M alkyl-DBAC elevated the outflux of ^{24}Na through the frog skin, although the comparative method was not taken owing to the insufficient size of *Rana nigromacurata*.

Effect of Phospholipase C on the Influx of ^{24}Na through Frog Skin

The effect of 0.5 mg/ml of phospholipase C on the influx of ^{24}Na is presented in Figs. 9 and 10. It will be seen from Fig. 9 that 0.5 mg/ml of phospholipase C increased the influx of ^{24}Na

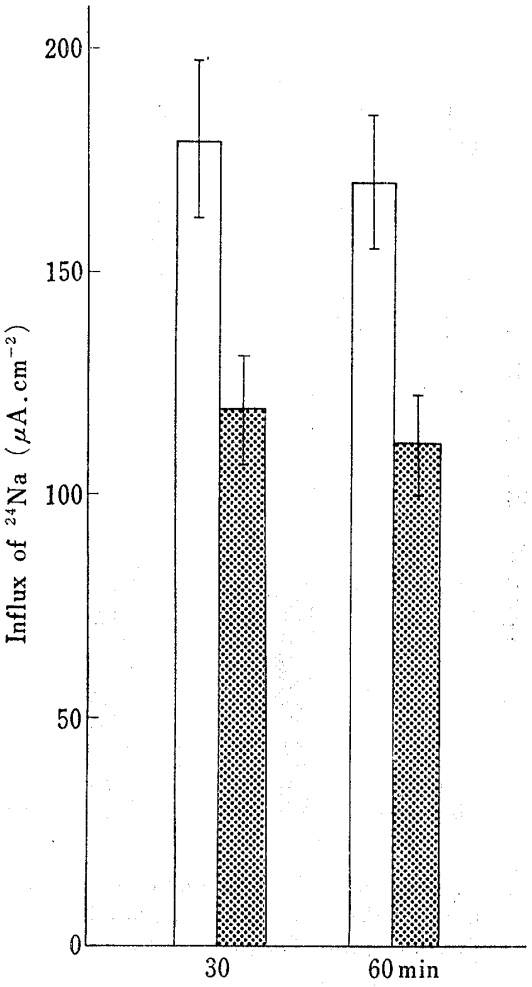

Fig. 7. Effect of phospholipid on the Influx of ^{24}Na through Frog Skin treated with 10^{-3}M Alkyl-DBAC

1 μg.Pi/ml phospholipid was added to 10^{-3}M alkyl-DBAC solution and this state was dispersive.
□: 10^{-3}M alkyl-DBAC
▨: 10^{-3}M alkyl-DBAC+1 μg.Pi/ml phospholipid

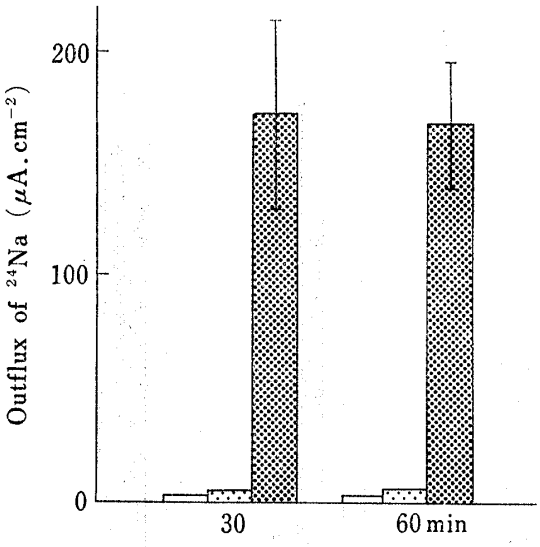

Fig. 8. Effect of Alkyl-DBAC on the Outflux of ^{24}Na through Frog Skin

One abdominal skin was solated from Rana nigromacurata and the procedure was performed reversibly that of the influx.
□: control
⊡: 10^{-4}M alkyl-DBAC
▨: 10^{-3}M alkyl-DBAC

through the frog skin but, as presented in Fig. 10, the increase was depressed by 10^{-5}M ouabain which was different from the effect of 10^{-3}M alkyl-DBAC.

The critical micellar concentration of this surfactant in Ringer solution was 8.2×10^{-4}M.

Discussion

According to Takenaka[17] and Keynes,[18] the short circuit current in frog skin was inhibited at 0°. However, as shown in Fig. 3, the increased influx of ^{24}Na through the skin treated with 10^{-3}M alkyl-DBAC was not depressed at 0°. It has been known that ouabain inhibits the active transport of sodium accompanied with Na^+-K^+ adenosine triphosphatase (ATP ase)[19] but, as shown in Fig. 6, the increased influx of ^{24}Na treated with 10^{-3}M alkyl-DBAC was not depressed by 10^{-5}M ouabain. It is shown in Fig. 8 that the outflux of ^{24}Na was also elevated by 10^{-3}M alkyl-DBAC. These findings suggest that the influx and outflux of ^{24}Na would be increased by diffusion, probably due to the structural damages on frog skin, although the active transport of sodium accompanied with Na^+-K^+ ATP ase would be inhibited by 10^{-3}M alkyl-DBAC. Furthermore, it is very interesting that the increased influx of ^{24}Na through the frog skin treated with alkyl-DBAC was observed above the critical micellar concentration of this surfactant (8.2×10^{-4}M in the Ringer solution). This suggest that the effect of alkyl-DBAC on the frog skin may be associated with the micell formation of this surfactant. As shown in Fig. 7, the influx of ^{24}Na increased by 10^{-3}M alkyl-DBAC was decreased by the addition

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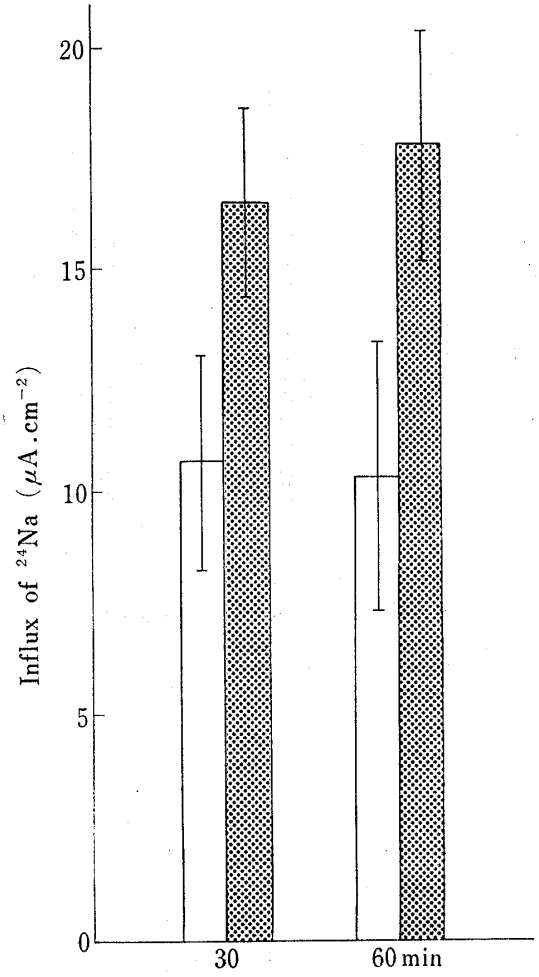

Fig. 9. Effect of 0.5 mg/ml Phospholipase C on the Influx of ^{24}Na through Frog Skin

□: control
▨: 0.5 mg/ml phospholipase C

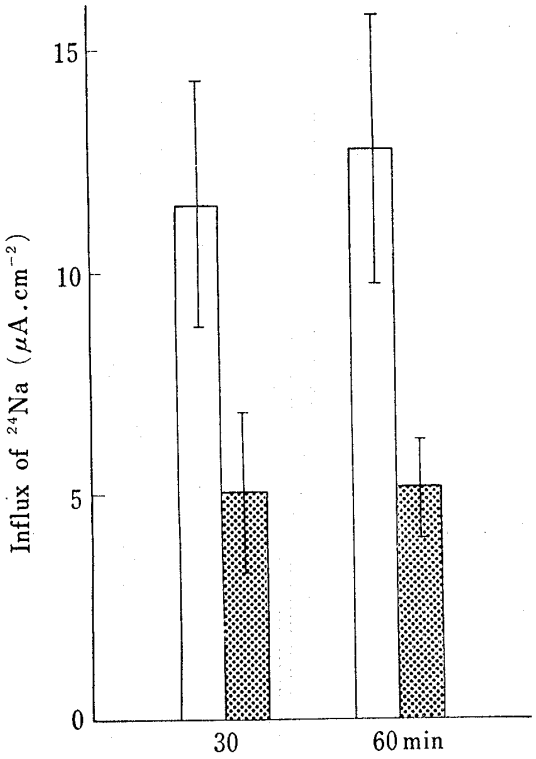

Fig. 10. Effect of 10^{-5}M Ouabain on the Influx of ^{24}Na through Frog Skin treated with 0.5 mg/ml Phospholipase C

□: 0.5 mg/ml phospholipase C
▨: 0.5 mg/ml phospholipase C+10^{-5}M ouabain

of 1μg Pi/ml of phospholipid from the rat liver to 10^{-3}M alkyl-DBAC solution as dispersion, but the recovery did not reach to the normal level. It appears that the damages on the frog skin caused by 10^{-3}M alkyl-DBAC was not only due to the elimination of phospholipid from the frog skin but also to the denaturation of protein or to other actions. The decreased influx of ^{24}Na caused by the addition of phospholipid to 10^{-3}M alkyl-DBAC may be attributed to the functions as follows: The effect of 10^{-3}M alkyl-DBAC which extracts phospholipid from the frog skin may be decreased by the addition of phospholipid from the rat liver. Because the amounts of alkyl-DBAC which adsorbed on the frog skin may decrease due to the distribution of alkyl-DBAC molecules among the dispersed phospholipid and phospholipid in the frog skin. There remained another possibility that the added phospholipid may be an obstacle to the influx of ^{24}Na owing to the adsorption on the damaged frog skin.

On the other hand, as shown in Figs. 9 and 10, 0.5 mg/ml of phospholipase C increased the influx of ^{24}Na through the frog skin, whereas this effect was depressed by 10^{-5}M ouabain. This suggests that the influx of ^{24}Na through the frog skin may be increased by phospholipase C owing to the degradation of phospholipid in the frog skin, but Na^+-K^+ ATPase activity still remained and the activity was not inhibited directly by phospholipase C.

From these findings, we speculate that the effect of alkyl-DBAC on the flux of ^{24}Na in the frog skin is similar to that of phospholipase C, but the mode of action of the two chemicals is different, and the effect of two chemicals on Na^+-K^+ ATPase is different.

As described above, it was assumed that alkyl-DBAC eliminates phospholipids from the frog skin, denaturates protein structure by unfolding and finally destruct cell membrane in the frog skin. These actions may contribute to the increase of the flux of ^{24}Na due to the structural damage on the frog skin and cause the disturbance of active transport of sodium in the frog skin.