period in *C. batrachus*. Statistically, the decrease in serum acid phosphatase level obtained after the 1st month of starvation was not significant ($p < 0.01$), but the values noted at the ends of the 2nd, 3rd, 4th and 5th months were significant ($p < 0.01$).

Discussion. The survival potential of fishes during the period of starvation differs from fish to fish. Metabolic cycles involve complex sequences of reactions mediated by enzymes. Love[10] noted that if in the 1st event, the arrival of metabolites in the blood stream from the gut is stopped, some reaction sequences will be arrested altogether and the enzyme will not be required. Thus, it is logical to expect that acid phosphatase, like other enzymes, will decline during a period of starvation. In the present study a linear and continuous decrease occurred in the serum acid phosphatase levels of the fish *C. batrachus*. Noda[6] studied various phosphatases of the rainbow trout, *Salmo gairdnerii*, during a period of starvation, and noted marked fluctuations in liver, intestine, pyloric caeca and spleen. He further noted that about 40% of the initial activity was lost in the course of 52 days of starvation. The trend of the decrease in the enzyme level of starved *C. batrachus* can be compared with the observations of Noda[4]. Vellas and Creach[5] studied ureogenesis in starving carp for 12 months and noted the essential role of the liver and the small participation of the kidney in it. Joshi[6] reported a fall of 49.3% and 47.3% in blood glucose and nonprotein nitrogen levels respectively by the end of the 150th day of starvation in *C. batrachus*. During the starvation experiments, the fish appeared to be gradually growing weak and sluggish, which can be correlated with a reduced metabolic activity. This feature has been noted also by Van Dam[11] and Smith[7] in eels and lungfish respectively. A regular decrease in blood urea in the catfish clearly revealed a declining metabolic rate with an increasing starvation period[2].

The gradual falling-off of serum acid phosphatase levels of *C. batrachus* with an increasing period of starvation clearly indicated that due to the sudden cut-off of its food-supply, the fish tried its best to fullfil its requirement for energy by metabolizing the reserve food stored in the liver and other parts of the body. However, with the gradual exhaustion of the reserve food, metabolic activities also ceased to function, and this resulted in a linear and continuous decrease in the level of acid phosphatase, which is known to take part in carbohydrate metabolism, transportation and absorption.

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Inhibitory effects of 1-iodo-3-aminomethyl-5,6,7,8-tetrahydro-2-naphthol (ONO-3122) and prostaglandin H_2 on vasopressin-induced osmotic water flow in toad bladder

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Summary. Both 1-iodo-3-aminomethyl-5,6,7,8-tetrahydro-2-naphthol (ONO-3122), which increases endogenous PGH_2, and PGH_2 itself, significantly depressed vasopressin-induced osmotic water flow in the toad bladder. These results suggest that ONO-3122 increases endogenous PGH_2 synthesis, and that PGH_2 and/or its metabolites inhibit vasopressin-induced water flow.

In many biological systems, prostaglandins activate adenylate cyclase and thus increase cellular levels of cyclic AMP. In some other systems, prostaglandins inhibit adenylate cyclase after having been activated by agonists such as vasoppdssin. We found that PGE_1 inhibited vasopressin-mediated adenylate cyclase activity of the hamster kidney[2], as reported previously. Beck et al.[3] also found inhibitory effects of PGE_1 on vasopressin action of the rat kidney.

In a physiological study, Orloff et al.[4] reported that PGE_1 inhibited vasopressin-induced osmotic water flow across the toad bladder membrane. They suggested that the inhibitory effect of PGE_1 was associated with the inhibition of adenylate cyclase activity.

In the present study, we examined the effect of an increased production of endogenous PGH_2 on the exogenous vasopressin-induced increment of the osmotic water flow across the toad bladder membrane.

Methods and materials. The urinary bladder of the toad, *Bufo bufo japonicus*, was used. After double pithing of the toads, the bladders were excised and immediately placed into Ringer's solution. The composition of the Ringer's solution was as follows: NaCl, 111 mM; KCl, 3.5 mM; $CaCl_2$, 0.9 mM; $MgCl_2$, 1.5 mM; NaH_2PO_4, 1.9 mM; Na_2HPO_4, 8.1 mM. The osmolality was 232 mOsm/l and the pH was 7.4. The osmotic water flow was measured as previously described[5].

1-Iodo-3-aminomethyl-5,6,7,8-tetrahydro-2-naphthol (ONO-3122) and prostaglandin H_2 (PGH_2), which were supplied by Ono Pharmaceutical Co. Ltd, and arginine vasopressin (Sigma Chem. Co., St. Louis) were used for the present study. ONO-3122 was dissolved in 0.5 N NaOH and then adjusted to pH 8.0 by using 0.1 N HCl. Acetone, a solvent of PGH_2, was evaporated by N_2 gas, and then PGH_2 was dissolved in the Ringer's solution. All procedures were performed on ice immediately before the addition to the chamber. Statistical analysis was performed by the paired t-test.

Results and discussion. Vasopressin and ONO-3122 were

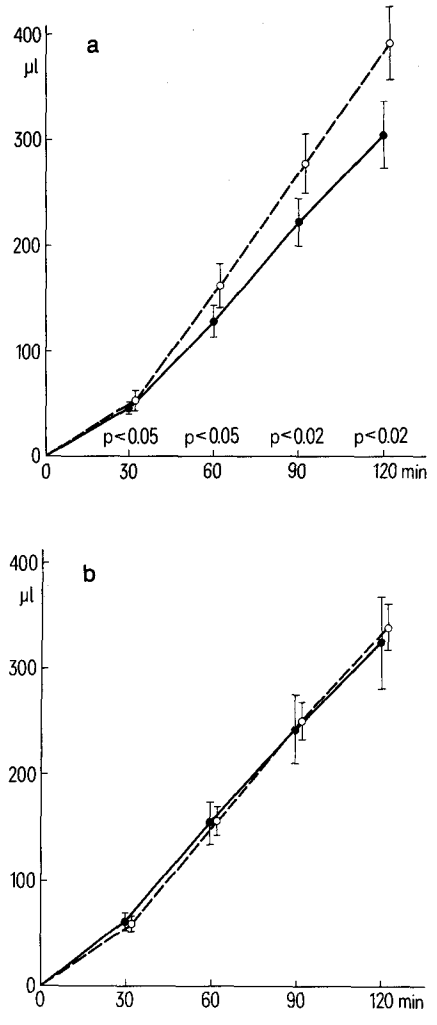

Figure 1. Effect of ONO-1322 on vasopressin-induced water flow. ONO-3122 and vasopressin were added in the serosal chamber of the experimental hemibladder (O——O) at concentrations of 1×10^{-4} M (a), 1×10^{-5} M (b), and 10 mU/ml, respectively. Only vasopressin was added to the control (O---O) (a: n = 17, b: n = 8). No direct effect of 1×10^{-4} M ONO-3122 alone on the water flow was found. Vertical axis indicates the cumulative water flow. Vertical line indicates 1 SEM.

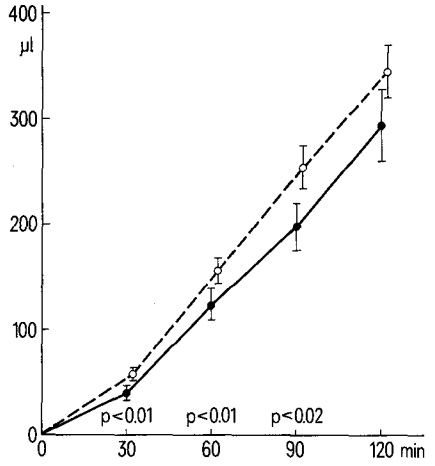

Figure 2. Effect of PGH_2 on vasopressin-induced water flow. PGH_2 and vasopressin were added to the experimental chamber of the hemibladder (O——O) at concentrations of 1×10^{-7} M and 10 mU/ml, respectively. Only vasopressin was added to the control (O---O) ($n=11$). No direct effect of PGH_2 alone on the water flow was found.

added to the serosal side of the experimental chamber at concentrations of 10 mU/ml and 1×10^{-4} M, respectively, and vasopressin only to the control chamber. As shown in figure 1a, ONO-3122 significantly depressed the vasopressin-induced osmotic water flow. At 120 min after addition of the agonists, the increment of water movement was 393 ± 35 µl in the control group, while it was 305 ± 30 in the experimental group ($n=17$, $p<0.02$). However, ONO-3122 did not show any effect on the vasopressin-induced water flow at a concentration of 1×10^{-5} M as shown in figure 1b. As shown in figure 2, exogenous PGH_2 significantly suppressed the vasopressin-induced water flow from 30 to 90 min after adding the agonist, at a concentration of 1×10^{-7} M. At 90 min, the increment of water movement was 254 ± 20 µl in the control group, while 197 ± 21 in the experimental ($n=11$, $p<0.02$). To examine the direct effect on the osmotic water flow, ONO-3122 was added to the chamber facing the serosal side of the membrane at a concentration of 1×10^{-4} M. However, no significant difference in the water flow was observed between the ONO-3122 added group and the control during 3 h of observation.

Prostaglandin hydroperoxidase catalyzes the reduction of the hydroperoxy group at position 15 of PGG_2 to produce PGH_2. This reaction requires hemoglobin, and is stimulated by tryptophan and other aromatic compounds such as indole, serotonin, melatonin, tyrosine, epinephrine, hydroquinone and benzoquinone. 2-Aminomethyl-4-*t*-butyl-6-iodophenol hydrochloride (MK-447) has a stimulating effect on the PGH_2 synthesis from PGG_2[6,7]. 1-Iodo-3-aminomethyl-5,6,7,8-tetrahydro-2-naphthol (ONO-3122) also has this stimulatory effect on PGH_2 and PGI_2 synthesis[8]. Therefore, the present study suggests that ONO-3122 inhibits the vasopressin-induced water flow by increasing endogenous PGH_2 synthesis in the bladder. The finding that exogenous PGH_2 inhibits vasopressin-induced water flow supports this assumption.

Orloff et al.[4] had reported that 1.7×10^{-9}–10^{-6} M of PGE_1 significantly diminished the response of the toad bladder to 1 mU/ml vasopressin. The half-life of PGH_2 is known to be 5 min at 37 °C. It is difficult to determine the role of the inhibitory effect of exogenously added PGH_2 on the vasopressin-induced water flow of the toad bladder. The present study suggests that ONO-3122 increases endogenous PGH_2 in the toad bladder, and that PGH_2 and/or its metabolites inhibit vasopressin-induced osmotic water flow of the toad bladder.

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