FIG. 1 Effect of combined stomachic on inhibition of the movement of rabbit stomach by sodium pentobarbitone.

Ordinate: percent changes in stomach motility, Abscissa: time (min) after application of sodium pentobarbitone. Control: with sodium pentobarbitone alone, Treated: with sodium pentobarbitone and the combined stomachic. Each result is presented as a mean $\pm$ S.E. of 5 experiments. P-value was determined by the paired *t*-test, since the values in "Control" and "Treated" were obtained in the same rabbit. (* $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$)

was stimulated by the combined stomachic, whose effective ingredients were fennel, gentian and 1-menthol, and that their action appears to be due to an increase in the cholinergic nerve activity but not to direct stimulation of the smooth muscle itself.

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THE UNCOUPLING ACTION OF URCHI-TOXIN F_3 AND F_4

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Fujiwara (1) reported that the spine of a toxic sea urchin induces dyspnea in man. The bronchospastic action of the toxic substance is apparently the main cause of dyspnea, but the toxic substance may also influence the function of cellular mitochondria. In this work, we investigated effects of the toxins on the mitochondrial respiration system, viz., effects of toxins on the reaction mechanism of oxidative phosphorylation were examined.

F_3 and F_4 fractions of so-called Urchi-toxin (UT), isolated by the Cephadex G-25 column

chromatographic method of Kimura et al. (2), were used as the experimental material. Rat liver mitochondria were obtained according to the method of Myers et al. (3) which is a modification of the Hogeboom method (4). The biuret method reported by Cleland and Slater (5) was applied for determinations of mitochondrial protein. The reaction mixture totalling 4.5 ml contained 25 mM tris buffer (pH 7.0), 50 mM sucrose, 5 mM magnesium chloride, 2 mM EDTA, 15 mM potassium chloride and other reagents. The mitochondrial respiration rate was measured by the method of Utsumi et al. (6). Effects of F_3 and F_4 fractions on the mitochondrial respiration were observed after blocking the electron transport from the NAD system by rotenone when succinate was used as substrate (A) and by blocking the oxidation of succinate by malonate when glutamate served as substrate (B).

Fig. 1 represents the relation between variation of the respiration rate and concentration of F_3 and F_4 fractions. The fractions were found to stimulate the mitochondrial respiration rate in proportion to increase in the concentration, but behavior of the stimulating activity varied slightly depending on the fractions and substrates. In the presence of succinate and glutamate, F_3 fraction, 10 mg, stimulated the respiration rate 2.7 and 1.9 times as high as the rate (n MO_2 /min/mg protein) of the State 4 ([UT]=0) respectively, while 10 mg of F_4 fraction under the same conditions stimulated the respiration rate 3.6 and 4.0 times as high as the rate of the State 4, respectively. Oxygen consumption, however, tended to decrease when the concentration of the fractions exceeded 10 mg. The stimulating activity of the fractions as seen in Fig. 2 was similar to that in Fig. 1. ADP and DNP, used as the control

Fig. 1 Influence of Urchi-toxin F_3 and F_4 on oxygen consumption of rat liver mitochondria using succinate (A) and glutamate (B) as substrate.

Oxygen consumption rates were measured with oxygen electrode (YSI, Model 54) in 4.5-ml closed chamber at 20°C. The concentrations added were 2 μ g rotenone (A), 10 mM malonate (B), 10 mM succinate (A), 10 mM glutamate and 5 mM L-malate (B). The concentrations of F_3 and F_4 fractions are shown in Fig. 1. Reactions were initiated by the addition of 5.6 mg mitochondrial protein.

○—○ Urchi-toxin F_3 ; △—△ Urchi-toxin F_4

Fig. 2 A comparison of the degree of oxygen consumption in rat liver mitochondria.

The reaction mixture, sampling and assay were the same as described in Fig. 1. The concentrations added, as shown by arrows, were 2 μ g rotenone (A_1 , A_2 and A_3), 5 mM malonate (B_1 , B_2 and B_3), 10 mM succinate (A_1 , A_2 and A_3), 10 mM glutamate (A_1 , A_2 and A_3), 5 mM L-malate (B_1 , B_2 and B_3), 200 μ M ADP (A_1), 300 μ M ADP (B_1), 50 μ M DNP (A_1 , B_1), 10 mg Urchi-toxin F_3 (A_2) and F_4 (A_3) and 5 mg (3 successive doses) Urchi-toxin F_3 and F_4 (B_2 , B_3).

1*, 10 mM malonate; 2*, 5 mM L-malate; UT, Urchi-toxin

agents, in the presence of succinate stimulated the respiration rate 5.9 and 6.4 times as high as the normal rate of the mitochondrial respiration respectively. ADP and DNP in the presence of glutamate stimulated the respiration rate 8 and 8 times as high as the normal rate respectively. The stimulating activity of F_3 and F_4 fractions was lower than that of ADP and DNP. ADP exerted a regulatory action on the mitochondrial respiration, while the fractions did not induce respiratory regulation. In addition, the reaction system of mitochondria was not affected by ADP in the presence of the fractions, viz., the fractions were found to exert an action similar to the respiration-releasing action of uncouplers. These findings suggest that F_3 and F_4 fractions have an uncoupling action. The reaction

patterns indicated that the fractions would additionally elicit inhibition of mitochondrial oxidation.

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