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Co-operative Effects of an Inhibitor NH_4^+ and of an Activator K^+ on the Acid Secretory Rate in Frog Gastric Mucosa *in Vitro* ——— A Multi-Enzyme System

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Effects of concentrations of ammonium ion (NH_4^+) and potassium ion (K^+) in the serosal solution on the acid secretory rate by bullfrog gastric mucosa were studied at near saturating substrates concentrations *in vitro*. The hydrogen chloride production system was treated as a multi-enzyme system. From kinetic analyses, the system was found to have co-operativities with regard to the activator K^+ and the inhibitor NH_4^+. The co-operativity of the inhibitor increased as the K^+ concentration increased. The co-operativity of K^+ increased as the NH_4^+ concentration increased. Considering the fact that chloride ion (Cl^-) and thiocyanate ion (SCN^-) do not have co-operativities, it was suggested that SCN^- inhibits the Cl^- transport chain reaction and that NH_4^+ inhibits the hydrogen (H^+) transport chain reaction. It was also discussed that NH_4^+ and SCN^- were not uncoupler-type inhibitors like 2,4-dinitrophenol.

Current models for metabolism-secretion coupling in gastric mucosa have been divided into two general categolies, redox mechanism and adenosine triphosphatase (ATPase) mechanism, although recent models involve a combination of redox and ATPase mechanisms.

The essential feature of the ATPase model is that the immediate energy source for H^+ translocation is derived from the hydrolysis of ATP to adenosine diphosphate (ADP) and P_i. In this simple version the H^+ which is secreted is derived from the general celluar pool. The products of ATP hydrolysis are converted to ATP *via* oxidative phosphorylation in the mitochondria.

In its simplest scheme for the redox model, a substrate is oxidized by a dehydrogenase to yield hydrogen atoms. The hydrogen atoms are split into hydrogen ions, which are secreted, and electrons by a redox component. The electrons are then transferred by a redox component. The electrons are then transferred to an oxidase where they reduce molecular oxygen.

There is another problem that how the H^+ can be transported from the actual site of H^+ production to the lumen of the gastric pits without intermixing with the cytoplasmic contents. From the point of the question, recent morphological studies[2–7] seem to support the ATPase model, although they are not decisive. These studies have drawn attention to changes in the organization of tubules in association with a resting to secreting transition in gastric mucosa.

In our recent paper,[8] we showed that K^+ uptake by gastric mucosa from the serosal side was very sensitive to the K^+ concentration in the serosal solution. The K^+ content was found to be expressed as in m Eq/kg wet weight

1) Location: *3190, Gofuku, Toyama.*
2) A.W. Sedar and V. Wiebelhaus, *Amer. J. Physiol.*, **223**, 1088 (1972).
3) A.W. Sedar, *Federation Proc.*, **24**, 1360 ((1965).
4) D.K. Kasbekar, T.M. Forte, and J.G. Forte, *Biochim. Biophys. Acta*, **163**, 1 (1968).
5) T.M. Forte and J.G. Forte, *J. Ultrastructure Res.*, **37**, 322 (1971).
6) L. Limlomwonge and J.G. Forte, *Amer. J. Physiol.*, **219**, 1717 (1970).
7) J.G. Forte, T.M. Forte, and T.K. Ray, "Gastric Secretion" ed. by G. Sachs, E. Heintz, and K.J. Ullrich, Academic Press Inc., New York and London, 1972, p. 37.
8) N. Takeguchi, I. Horikoshi, and M. Hattori, *Amer. J. Physiol.*, submitted.

$$(K^+)_{content} = 58.4 + 38.5(K^+)_s/[9.52+(K^+)_s] \quad (1)$$

where $(K^+)_s$ is the serosal K^+ concentration. A slope of 40 mV for a ten-fold K^+ concentration change in the serosal solution in a region of 5—80 mM was explained satisfactorily from the K^+ content change in the mucosa, which indicated that the serosal membrane of the mucosa has a large K^+ selectivity.

The acid secretory rate was shown to be dependent on the K^+ concentration in certain regions of oxyntic cells in which a part of or whole acid secretory mechanisms exist. The K^+ concentration in certain regions of the cells was found to be regulated by a fraction of the K^+ content. The fraction represents the K^+ content which can move reversibly across the serosal membrane from outside to inside or *vice versa* when the $(K^+)_s$ increases or decreases, respectively. That is, the acid secretory rate is dependent on the second term on the right hand in the equation (1). This shows implicitly that the actual site of acid production is the labile compartment in the cells accessible to the external solution. The site is not sequestered in organelles which have constant K^+ contents when the bathing K^+ concentration changes.

We consider that many enzymes engage in HCl production in certain regions of the cells. Their enzymes are arranged in the *loci* in a specific manner to enable the chain reactions for HCl production to have a steady and large velocity. Dixon proposed to call this kind of enzymatic system "multi-enzyme system."[9] He suggested that enzymes are closely located one another in multi-enzyme systems so that unstable intermediates can be taken into the chain reactions effectively. The total velocity of the chain reaction is greatly large because the velocity depends on the distance between two adjacent enzymes which is rate-determining.

In this paper, we report some co-operative properties of the HCl production system as a multi-enzyme system. We selected NH_4^+ as one of the inhibitors, K^+ as one of the activators and the acid secretory rate as the activity of the multi-enzyme system. Co-operative effects of these effectors on the acid secretory rate will be explained from a model for the multi-enzyme system, in which NH_4^+ site and K^+ site are located closely in space on a H^+ transport chain reaction. It will be shown that NH_4^+ inhibits the H^+ transport chain reaction and that SCN^- inhibits a Cl^- transport chain reaction, although NH^{4+} and SCN^- have been suggested to have the same inhibitory mechanism.[10]

Experimental

Materials——1) Gastric Mucosa: Gastric mucosa of bullfrog (*Rana catesbeiana*) were used. Bullfrogs were kept in about 1 cm depth of water at 7° until used. The mucosa was separated from the muscle coats of stomach, and mounted between two lucite chambers.

Apparatus and Procedure——1) Chamber: The chamber was made of a rod of transparent metaacryl resin. The diameter of the rod was 5 cm. Fig. 1 shows the chamber design. The mucosa was placed between two square plates (A and B in Fig. 1). The plate had a hole of diameter of 1.52 cm (*i.e.*, the free area for transport was 1.814 cm^2). One small o-ring was half-buried around the hole on the surface of the plate B. Other two large o-rings were half-buried on the side surfaces of chambers which were to be in contact with the surfaces of plates A and B. The two plates A and B with the mucosa were placed between two lucite chambers and mounted tightly by screws. These o-rings eliminated the mechanical destruction of the mucosa and also water leak which could have occurred if two chambers with two plates had been assembled together tightly without o-rings. The volume of bathing solutions was 7 cm^3.

2) Potential Difference (PD): The PD was measured with agar bridges containing 1M NaCl and connected *via* calomel electrodes to a potentiometer. We did not use KCl bridges in order to avoid K^+ leakage from KCl bridges into bathing solutions.

3) Acid Secretory Rate: The amount of HCl secreted into the mucosal solution was determined continuously by a pH stat method. A Radiometer pH stat meter (Copenhagen, Denmark) was used. The mucosal solution was constantly maintained at pH 4.6 by the addition of standardized 10 mM NaOH.

9) M. Dixon, "Multi-Enzyme System," Cambridge Univ. Press, Cambridge, 1949.
10) M.E. LeFevre, E.J. Gohmann Jr. and W.S. Rehm, *Amer. J. Physiol.*, **207**, 613 (1964).

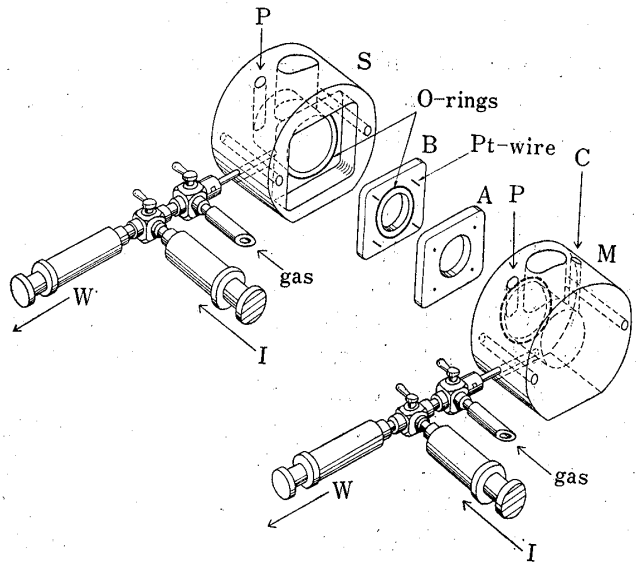

Fig. 1. Chamber Design for Measuring the Acid Secretory Rate and the Potential Difference

The mucosa separated from the muscle coats of stomach was sandwiched between two plates A and B with holes. The two plates A and B with the mucosa were placed between two lucite chambers S and M, and mounted tightly by screws. The M is the mucosal chamber and S the serosal chamber. Changing old solution with new solution was done through the pore located bottom of the chambers. The W is the withdrawal of solution and I the injection of solution. The 95% O_2—5% CO_2 gas was supplied through the same pore. The P is the hole for a salt bridge and C the hole for a combination electrode.

4) Compositions of Bathing Solutions: The standard mucosal solution had 120 mM NaCl only. The standard serosal solution had the following composition in mM: 105Na^+, 5K^+, 1Mg^{2+}, 2Ca^{2+}, 97Cl^-, 18HCO_3^{-1}, 1 $H_2PO_4^{-1}$, 11 glucose and 0.05 histamin base. Solutions containing different K^+ and/or NH_4^+ levels from the standard solutions were prepared by substituting K^+ and/or NH_4^+ for Na^+ (the sum of K^+, NH_4^+, and Na^+ concentrations was constant). Both serosal and mucosal solutions were gassed continuously with 95% O_2—5% CO_2 gas during experiments and stirred vigorously by the gas. All experiments were done at 20°—23°. In the standard bathing solutions, the mucosa secreted at maximum speed (3—5 μEq. HCl/cm²/hr) for about 8 hours, then the acid secretory rate decreased slowly owing to the tissue deterioration and finally deceased after about 16—20 hrs.

5) Procedure: Every experiments were done in the following manner: A control experiment in the standard solutions→the first test experiment→the other control experiment→the second test experiment in which the K^+ and/or NH_4^+ levels were different from the first test experiment. When the K^+ and/or NH_4^+ levels were changed to another, including the control experiments, we washed the mucosa with new solutions during a period of 80 min. Predetermined numbers and times of the washing were done. The second washing was done 5 min after the initial washing. The third, fourth and fifth washings were done 15 min, 35 min and 80 min after the initial washing, respectively. Usually, the acid secretory rate and the PD were measured 80—90 min after the initial washing.

Results

Fig. 2 shows the effect of NH_4^+ on the acid secretory rate in the absence of K^+ in both bathing solutions. The relative acid secretory rate, H^+ rate (%), is defined as % of the control value in the standard serosal and mucosal solutions. A solid line in Fig. 2 shows the effect of NH_4^+ concentration on the serosal side, $(NH_4^+)_s$, and a broken line shows the effect of NH_4^+ concentration on the mucosal side, $(NH_4^+)_m$. Table I shows the numerical data. Our finding is in agreement with the finding by LeFevre, *et al.*[10] They showed that the addition of 9 mM NH_4^+ to the mucosal solution was much less effective in inhibiting secretion than when it was added to the serosal solution. The addition of 10 mM NH_4^+ on the serosal side abolished perfectly the acid secretion within a period of 10—15 min. In the K^+-free and NH_4^+-free serosal solution and the standard mucosal solution, the acid secretory rate which was measured 80—90 min after the initial washing has been reported to be 52.6%.[8] Therefore, the addition of NH_4^+ on the serosal side in the absence of K^+ reduces greatly the acid secretion, and the addition on the mucosal side reduces the acid secretion slightly.

In the following (Fig. 3—8), the K^+ and NH_4^+ were added only to the serosal side and the mucosal solution had 120 mM NaCl.

Fig. 3 shows the relative acid rate as a function of the K^+ concentration in the serosal solution, $(K^+)_s$, with zero (—●—),[11] 2 (—×—) and 5 mM NH_4^+ (—○—). Numerical data are listed in Table II. In the lowest K^+ concentration, the acid secretion did not go to zero,

11) Data with zero $(NH_4^+)_s$ were taken from the paper cited in present reference 8.

because we measured the acid secretory rate 80—90 min after the initial washing with the test solution. As reported elsewhere,[8] the acid secretory rate was zero in 0.1 mM and zero mM K^+ solutions when we measured the steady state value of the acid secretion. The steady state values were able to be measured 120—180 min after the initial washing.

Fig. 4 shows the effect of NH_4^+ on the acid secretion with various concentrations of zero (—●—), 2 (—×—) and 5 mM K^+ (—○—) when NH_4^+ is added on the serosal side.

Fig. 5 shows the double reciprocal plots of $1/(H^+$ rate) *versus* $1/(K^+)_s$ with various concentrations of zero (—●—), 2 (—×—) and 5 mM NH_4^+ (—○—). The Lineweaver-Burk plots were found to be not linear but parabolic.

Fig. 6 shows the Hill type plot.[12] From the gradients of the plots, Hill coefficients n for the activator K^+ were found to be 2.29 at zero mM NH_4^+, 2.64 at 2 mM NH_4^+ and 7.61 at 5 mM NH_4^+.

Fig. 7 shows the Hofstee type plot.[13] They shows the typical convex curves which have maxima.

Fig. 8 shows the PD as a function of NH_4^+ concentration with various concentrations of zero (—●—), 2 (—×—) and 5 mM K^+ (—○—). It was known that when NH_4^+ was added on serosal the side, the PD was roughly independent of the NH_4^+ concentration and the PD was dependent on the K^+ concentration. In the K^+-free solution, the PD at zero mM NH_4^+ had a discontinuous value from others.

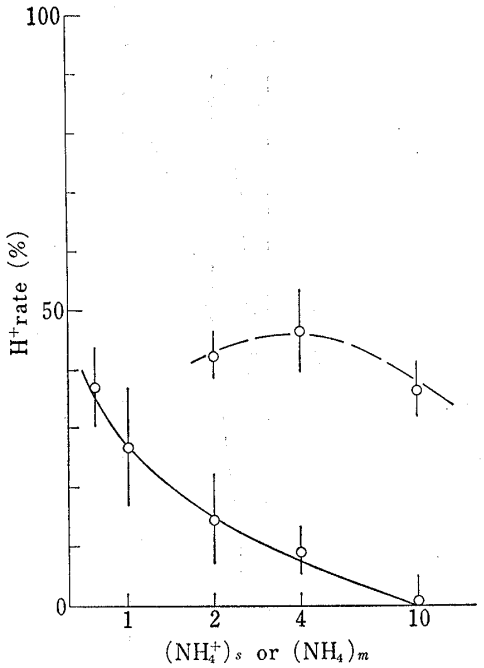

Fig. 2. Effects of NH_4^+ on the Acid Secretory Rate in the Absence of K^+ in both Bathing Solutions

A solid line shows the effect of NH_4^+ on the serosal side and a broken line on the mucosal side. The acid secretory rate was expressed as % of control in the standard solutions. The $(NH_4^+)_s$ is the NH_4^+ concentration in the serosal solution and $(NH_4^+)_m$ the NH_4^+ concentration in the mucosal solution.

Table I. NH_4^+ Effects on the Acid Secretion and the Potential Difference in K^+-free Solutions

NH_4^+ concentration (mM)		Acid secretory rate as % of control[a]	PD (mV)[a,b]
Serosal	Mucosal		
0.8	0	37.2± 7.1(4)	42.1±1.5(4)
1	0	26.7±10.9(3)	37.2±3.2(3)
2	0	14.6± 8.2(4)	44.8±1.2(2)
4	0	9.1± 4.6(3)	43.8±1.3(3)
0	2	42.0± 4.4(4)	37.6±2.0(4)
0	4	46.3± 7.0(8)	38.9±1.7(8)
0	10	36.1± 4.8(8)	37.6±1.4(8)

a) All values are mean ± standard error (no. of observations).
b) Serosal side is positive.

12) A.V. Hill, *Physiol. J.* (London), **40**, 4 (1910).
13) B.H.J. Hofstee, *Nature*, **184**, 1296 (1959).

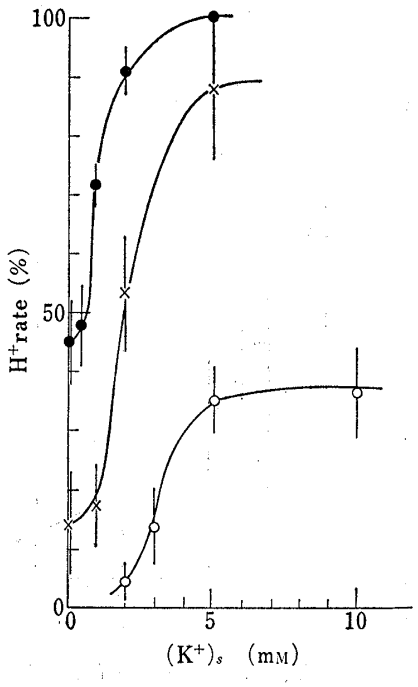

Fig. 3. Effects of Activator K^+ upon the Acid Secretory Rate

The $(K^+)_s$ is the K^+ concentration in the serosal solution. The mucosal solution was the standard solution. —●—: zero mM, —×—: 2 mM and —○—: 5 mM $(NH_4^+)_s$ where $(NH_4^+)_s$ is the NH_4^+ concentration in the serosal solution.

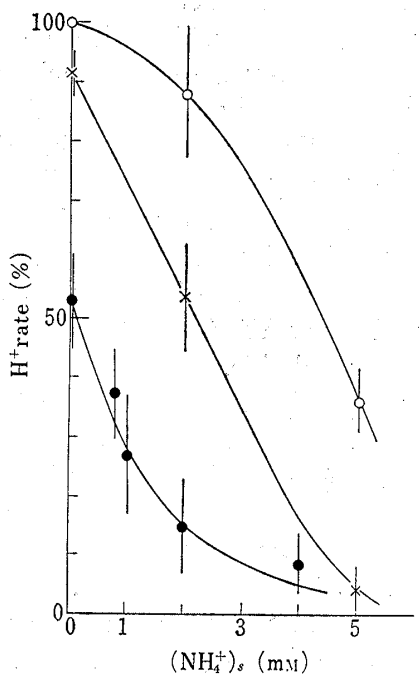

Fig. 4. Effects of the Inhibitor NH_4^+ upon the Acid Secretory Rate

—●—: zero mM, —×—: 2 mM —○—: 5 mM $(K^+)_s$
The addition of the activator K^+ reveal the highly co-operative effects of the inhibitor NH_4^+.

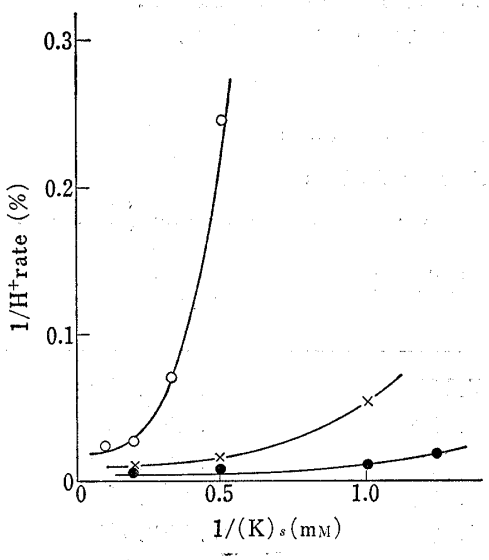

Fig. 5. A Double Reciprocal Plot between the Acid Secretory Rate and the Activator K^+ Concentration

—●—: zero mM, —×—: 2mM —○—: 5mM $(NH_4^+)_s$
The co-operativity of the activator K^+ increased as the inhibitor NH_4^+ concentration increased.

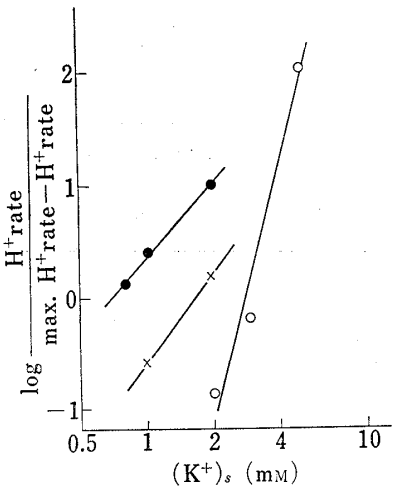

Fig. 6. Hill Type Plot

—●—: zero mM, —×—: 2mM —○—: 5mM $(NH_4^+)_s$
Hill coefficients n (gradient of straight line) were 2.29 for zero mM, 2.64 for 2 mM and 7.61 for 5 mM $(NH_4^+)_s$.

Table II Coexisting Effects of NH_4^+ and K^+ in the Serosal Solution on the Acid Secretion and the Potential Difference

Concentrations (mM) $(K^+)_s$	Concentrations (mM) $(NH_4^+)_s$	Acid secretory rate as % of control[a]	PD[a,b]
2	5	4.1± 4.1(7)	33.9±4.2(4)
3	5	13.7± 6.7(8)	40.2±1.0(9)
5	5	36.1± 5.3(8)	29.8±2.3(6)
10	5	36.4± 7.6(8)	26.0±1.1(6)
1	2	17.7± 7.0(8)	33.8±2.7(7)
2	2	53.8± 9.4(8)	37.3±1.3(5)
5	2	88.1±11.5(8)	29.9±2.4(7)

a) All values are mean ± standard error (no. of observations).
b) Serosal side is positive.

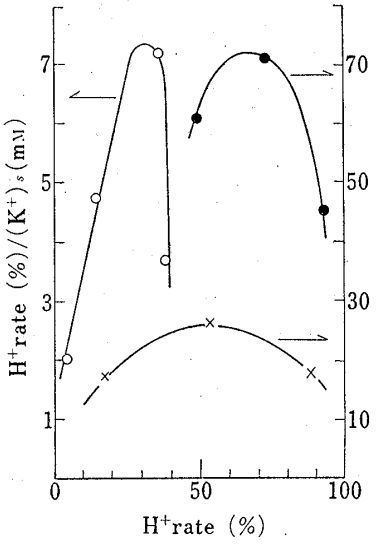

Fig. 7. Hofstee Type Plot
—●—: zero mM, —×—: 2 mM
—○—: 5 mM $(NH_4^+)_s$

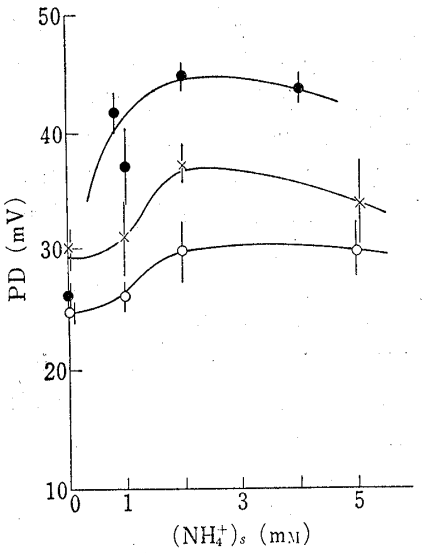

Fig. 8. Effects of $(NH_4^+)_s$ on the Potential Difference
—●—: zero mM, —×—: 2 mM
—○—: 5 mM $(K^+)_s$

Discussion

It is considered that under present experimental conditions, there are constant and near saturatory concentrations of substrates of H^+ and Cl^- in the multi-enzyme system.

It is well known that in allosteric enzymes co-operativity arises from following facts. Effector sites and active sites of an allosteric enzyme are not located on the same place but located on the different places in one of subunit or in different subunits of which the enzyme is composed. Interactions among effectors and substrate are mediated through conformational changes of the quaternal structure of the protein molecule.

If the both effectors K^+ and NH_4^+ in the present system worked on an allosteric enzyme, it would be expected that the effects of these effectors on the acid secretion was co-operative. However, it has not been known of the existence of such allosteric enzymes until now.

It is known that there are two different K^+-dependent enzymes in gastric mucosa, that is, *p*-nitrophenylphosphatase (PNPPase)[14] and K^+-dependent ATPase.[15] A double reciprocal

14) J.G. Forte, T.M. Forte, and P. Saltmann, *J. Cell Physiol.*, **69**, 293 (1967).
15) A.L. Ganser and J.G. Forte, *Biochim. Biophys. Acta*, **307**, 169 (1973).

plot of 1/(PNPP hydrolysis rate) against 1/(substrate concentration) of the isolated PNPPase gave a linearity and another plot of 1/(PNPP hydrolysis rate) against 1/(K^+ concentration) at constant PNPP concentration also gave a linearity. This means that there is no co-operative interactions between the substrate and the activator K^+. The NH_4^+ was reported to be also an activator of the isolated PNPPase. Therefore, there is no possibility that the PNPPase works as an allosteric enzyme in the system in which NH_4^+ works inhibitorily.

One of another possibilities which can induce the co-operative effect is that if two sites of the activator and the inhibitor were located nearly each other in space in the system, there would be a co-operative interaction between them through a chain reaction for the H^+ transport (It will be discussed forward that there are at least two different chain reactions in the system. One is for H^+ and the other for Cl^-, although these two chain reactions are metabolically coupled with each other in order to secrete the equal amount of H^+ and Cl^-). In multi-enzyme systems, interactions between substrates and effectors are mediated through fluxes of matter and/or energy. Generally speaking, the co-operativity is not restricted to allosteric enzymes. Changeux, *et al.*[16] suggested that the highly co-operative structure of cell membranes have important roles in, for example, the initiation and propagation of nerve impulses, the specific killing of some bacterial cells by a single molecule of colicine[17] and several all-or-none processes related to phage infection, bacterial conjugation, and fertilization. The co-operativity was formulated by Monod, *et al.* (MWC model)[18] and Koshland, *et al.* (KNF model)[19] for allosteric enzymes and Changeux, *et al.*[16] and Hill[20] for the biological membranes.

To know whether the interaction between the effectors in the present system have co-operative properties or not, we will compare our results with that of typical allosteric enzymes.

Fig. 3 would correspond with Fig. 7 in the paper by Monod, *et al.*,[18] in which the activity of deoxycytidylate aminohydrolase is plotted against the concentration of the activator of deoxycytidine triphosphate (dCTP) in the presence of substrate of deoxycytidine monophosphate (dCMP) at near saturating concentration and at various concentrations of inhibitor of deoxythymidine triphosphate (dTTP). It is noted that from Fig. 3 our system was found to have a property of a V-system.[18] In which system, the maximum activity depended on the inhibitor concentration when the activator concentration was changed with constant inhibitor concentrations. In Fig. 4, it can be seen that the addition of the activator K^+ reveals significantly the co-operative effect of the inhibitor NH_4^+. This corresponds with, for example, the effect of the inhibitor of *l*-isoleucine upon the activity of *l*-threonine deaminase (Fig. 4 in the paper[18]). In the K^+-free solution, the NH_4^+ inhibition in the present system as well as allosteric enzymes is not highly co-operative.

In order to know whether an enzyme has a co-operativity or not, a relationship between the reaction velocity and the substrate concentration is usually plotted in three different ways *i.e.*, Lineweaver-Burk plot, Hill plot and Hofstee plot. It is easily understandable that the substrate concentration in these three plots can be substituted with the activator concentration when the substrate concentration is constant.[21] In Fig. 5, 6 and 7, the relationship between

16) J.-P. Changeux, J. Thiery, Y. Tung, and C. Kittel, *Proc. Natl. Acad. Sci. U. S.*, **57**, 335 (1967).
17) M. Nomura, *Proc. Natl. Acad. Sci. U. S.*, **52**, 1514 (1964).
18) J. Monod, J. Wyman, and J.-P. Changeux, *J. Mol. Biol.*, **12**, 88 (1965).
19) D.E. Koshland Jr., G. Nemethy, and D. Filmer, *Biochemistry*, **5**, 365 (1966).
21) From the MWC model, the reaction velocity (Y_s) in the K-system is given as following[18]

$$Y_s = \frac{\alpha(1+\alpha)^{n-1}}{L\frac{(1+\beta)^n}{(1+\gamma)^n}+(1+\alpha)^n} = \frac{(1+\gamma)^n}{L\frac{(1+\beta)^n}{\alpha(1+\alpha)^{n-1}}+(1+1/\alpha)(1+\gamma)^n}$$

where α is the substrate concentration, β the inhibitor concentration, γ the activator concentration and L the allosteric constant. Because present system is the V-system with regard to K^+ and NH_4^+, the equation can not be applied directly to present system. But, qualitative aspects of V-system would be speculated from the equation.

the reaction velocity and the activator concentration of the multi-enzyme system was plotted in three different ways at near saturating levels of substrates. These figures have similar nature of curves which are seen in typical allosteric enzymes. Therefore, they show qualitatively that the multi-enzyme system has co-operativities with regard to K^+ and NH_4^+.

Hereafter, we will discuss mechanisms of the activator K^+ and the inhibitor NH_4^+ on the acid secretion.

It is considered that K^+ is necessary for a reaction or reactions essential for the acid secretory mechanism.[22–25] In a separate paper,[34] the activity of microsomal PNPPase in certain regions of the cells was hinted to be rate-determining for the acid secretion. The relationship between the PNPPaseacti vity and the K^+ concentration in certain regions was not co-operative. This is consistent with the results of the isolated PNPPase *in vitro* by Forte, *et al.*[14] The K^+ concentration in certain regions is regulated by the K^+ concentration in the serosal solution, as described shortly in this introduction. Therefore, a transformation of the K^+ concentration from that in the serosal solution into that in certain regions of the cells by metabolic processes in the mucosa is partly speculated to originate the co-operativity with regard to K^+ in NH_4^+-free solutions (Hill constant n for K^+ in NH_4^+-free solutions is 2.29).

The existence of 2—5 mM NH_4^+ in the serosal solution elevated the K^+ co-operativity greatly. The Hill constant n for K^+ were 2.64 at 2 mM NH_4^+ and 7.61 at 5 mM NH_4^+. There have been controversial evidences and discussions about the inhibitory mechanism of NH_4^+ on the acid secretion. LeFevre, *et al.* have demonstrated that SCN^-, OCN^-, NO_2^- or NH_4^+ decreased the acid secretion and produced characteristically similar alterations in the electrical properties of gastric mucosa.[10] They pointed out that these compounds shared the common feature of a nitrogen atom with a pair of unshared electrons and proposed that this common feature was the basis for the inhibition of the acid secretion. Kidder, *et al.*[26] and Kidder[27] suggested these inhibitors work on cytochrome c which was probably located external to the mitochondria, presumably on the plasma membrane. Hersey showed negative indications for above possibility.[28] Sachs, *et al.* suggested that a microsomal ATPase was inhibited by SCN^- and NH_4^+.[29] But recently, Ganser, *et al.* showed that purified K^+-stimulated component of the microsomal ATPase did not be inhibited by SCN^- and that mitochondrial ATPase was inhibited by SCN^-.[15] Hersey suggested that SCN^- type inhibitors act as uncouplers rather than a true inhibitor from his finding that SCN^- inhibited the acid secretion without inhibiting respiration.[28] Addition of typical uncouplers of 2,4-dinitrophenol[30] and salicylate[31] in the serosal solution have been reported to abolish the acid secretion and the PD in due time. However, addition of SCN^- [32] and NH_4^+ Fig. (8) did not abolish the PD, although they work inhibitorily on the acid secretion. Addition of 2,4-dinitrophenol in a bathing solution was reported to induce a large decrease in the K^+ content in the mucosa.[33] The value of the K^+ content in the presence of K^+ and the uncoupler in the bathing solution, became far below the value which was measured in the K^+-free solution without the uncoupler. Our finding about the effect of NH_4^+ addition on the K^+ content in the mucosa showed that there was

22) H.W. Davenport, *Amer. J. Physiol.*, 204, 213 (1963).
23) T.L. Davis, J.R. Rutledge, and R.S. Rehm, *Amer. J. Physiol.*, **205**, 873 (1963).
24) J.B. Harris and I.S. Edelman, *Amer. J. Physiol.*, **206**, 769 (1964).
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no such large decrease in the K^+ content.[34] From the results on the PD and the K^+ content, it is unlikely that NH_4^+ is the uncoupler like 2,4-dinitrophenol and salicylate. Considering these facts with the co-operative inhibition by NH_4^+, we speculate NH_4^+ works as a true inhibitor, although the mechanism is to be studied further.

Finally, we will discuss an effect of the change in the Cl^- concentration on the acid secretion. Durbin showed that there is a linearity in a Lineweaver-Burk plot between the acid secretory rate and the serosal Cl^- concentration (the Cl^- concentration was altered by substituting glucuronate for Cl^-).[35] From the data given by Forte, *et al.*,[36] it can be calculated that the same linearity do exist in the same plot (isethionate was used for substituting Cl^-). These facts mean that Cl^- as the substrate in the multi-enzyme system do not have the co-operativity. Furthermore, effects of SCN^- on Cl^- flux were studied by these authors.[35,37,38] They suggested that SCN^- appears to act by competing with Cl^- in a reaction leading to the formation of acid by the mucosa. Forte suggested that SCN^- is only partially effective in the reduction of passive Cl^- flux, whereas it is a considerably more potent inhibitor of the acid secretion and the active component of Cl^- flux from the serosal side to mucosal side ΔJ_{Cl}^{sm}.[37] Furthermore, he suggested that the complex of SCN^- with a binding site or carrier substance within the membrane, does not interfere with the binding of the microsomal ATPase substrate to the enzyme involved with proton deposition but only with the velocity constant. Forte showed in another paper that the amount of the active component of Cl^- flux ΔJ_{Cl}^{sm} is the equal to the amount of the acid secretion.[38] From the data given by Forte (Table IV in the paper[37]), it is able to calculate the effect of SCN^- on ΔJ_{Cl}^{sm} or on the acid secretory rate. From a plot of ΔJ_{Cl}^{sm} or the acid secretory rate against the NaSCN concentration as presented in Fig. 4 in this paper, it is found that the inhibition of the active component ΔJ_{Cl}^{sm} or the acid secretion by NaSCN is not co-operative. In Forte's experiment described above,[37] the serosal solution included 4.3 mM K^+. It is noted that the existence of 4.3 mM K^+ on the serosal side does not induce the co-operativity in the inhibition by NaSCN, whereas the existence of 5 mM K^+ on the serosal side induces the strong co-operativity in the inhibition by NH_4^+ (Fig. 4). Therefore, present study on the co-operativities would suggest that NH_4^+ inhibits the H^+ transport chain reaction and that SCN^- inhibits the Cl^- transport chain reaction.

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