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THE EFFECTS OF CARBON DIOXIDE AND BICARBONATE ON CHLORIDE FLUXES ACROSS FROG GASTRIC MUCOSA

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SUMMARY

1. The effects of CO_2 and HCO_3^- upon the unidirectional Cl^- fluxes across isolated frog gastric mucosa were examined under normal conditions and during SCN^- inhibition.

2. When the nutrient solution was aerated with pure O_2 and the partial pressure of CO_2 on the secretory side ($p^s_{CO_2}$) was raised, the backward Cl^- flux from the secretory to nutrient side ($J^{Cl^-}_{sn}$) decreased, whilst the forward Cl^- flux from nutrient to the secretory side ($J^{Cl^-}_{ns}$) increased. The extent of the inhibition of $J^{Cl^-}_{sn}$ by SCN^- increased with the increase of $p^s_{CO_2}$ in a similar manner to the inhibition of $J^{Cl^-}_{ns}$.

3. Nutrient CO_2 increased both the H^+ secretion rate (Q_{H^+}) and $J^{Cl^-}_{ns}$ and SCN^- inhibition more effectively than did secretory CO_2. Nutrient HCO_3^- reduced this effect, whereas secretory HCO_3^- affected the system only slightly.

4. In order to explain the results consistently, a model for the SCN^- inhibition of gastric mucosal anion exchange was constructed, in which SCN^- inhibition was assumed as a dead-end one.

5. The secretion process is interpreted as follows: H^+ is produced by the electron transfer system on the secretory side and the resultant concentration of OH^- on the opposite side, facilitated by the action of CO_2, promotes the circulation of the anion-exchange carrier.

INTRODUCTION

Although gastric acid formation has been studied for a long time, its precise mechanism is still unknown. However, the importance of the CO_2–HCO_3^- system in acid production is fully recognized[1–5]. A certain amount of CO_2, customarily added to the O_2 used to aerate the physiological solutions bathing the isolated gastric mucosa of frog, is known to stimulate acid secretion. HOGBEN[3] showed that CO_2 also affected the unidirectional Cl^- fluxes but his work was confined to the range of CO_2 partial pressure (p_{CO_2}) from 0 to 5 %. In the previous study[6] by the author, the p_{CO_2} range was extended to 30 % and it was found that both the Cl^- flux from nutrient to secretory side and its inhibition by SCN^- increased upon raising p_{CO_2} on the secretory side. On the contrary, the reverse Cl^- flux from secretory to nutrient side seemed to be neither stimulated by secretory CO_2 nor inhibited by SCN^-. However, the experiment on the reverse flux was carried out only when p_{CO_2} on the nutrient side was 5 %.

In the present investigation, therefore, the effects of secretory CO_2 and SCN^- on the reverse Cl^- flux were examined, when the nutrient solution was aerated with pure O_2 instead. The results indicated that the backward flux in this instance was also affected by these stimuli in a similar, if not the same, manner as the forward flux. Accordingly, both the forward and backward Cl^- fluxes were supposed to be mediated by a carrier. It was also found that nutrient CO_2 had the same effect as secretory CO_2, whereas nutrient HCO_3^- reduced the CO_2 effect. In order to explain the present and previous results consistently, a model for the SCN^- inhibition of gastric mucosal anion exchange is constructed and discussed.

METHODS

Isolated gastric mucosa of the Japanese frog (*Rana nigromacurata*) was mounted between two lucite chambers. The mucosal area was 0.785 cm^2. Unless otherwise specified, both the nutrient and secretory sides were bathed with 20 ml of the same Ringer solution having the following composition: 102.3 mM NaCl, 4.0 mM KCl, 1.8 mM $CaCl_2$ and 0.8 mM $MgSO_4$. The nutrient solution also contained 0.04 mM histamine phosphate and 10.0 mM glucose. The partial pressure of CO_2 on the secretory side ($p^s_{CO_2}$) varied between 0 and 20 %. Unless otherwise specified the nutrient solution was aerated by 100 % O_2 and its pH was maintained near to 7.3 by addition of HCl. Acid secretion rate was measured by the use of a TOA HS-1B pH-stat device. The pH of the secretory solution was kept constant at 4.5 throughout the experiments. The temperature of the solutions was maintained at 25 $\pm$ 0.5°.

The Cl^- fluxes across the mucosae were measured by using $^{36}Cl^-$ as tracer. At 1-h intervals, 1-ml samples were withdrawn from the solutions and counted with a Packard liquid scintillation spectrometer 3375. The toluene–ethanol system[7] was generally used as the scintillator solution for the aqueous samples, but sometimes BRAY's[8] solution was also used. The automatic external standardization ratio for a series of the samples was so constant that a quenching correction was not usually necessary. After a control period of 1 h, NaSCN was added to the nutrient solution to give a final concentration of 10 mM. The mucosae were short-circuited during the experiments by manual adjustment of an external current.

RESULTS

As shown in Fig. 1, when the nutrient solution was aerated with pure O_2 and the partial pressure of CO_2 on the secretory side ($p^s_{CO_2}$) was raised, the Cl^- reverse flux from the secretory to nutrient side ($J^{Cl^-}_{sn}$) decreased. Each value represents the mean $\pm$ S.E. for five experiments. This effect of secretory CO_2 on $J^{Cl^-}_{sn}$ is in the opposite direction to that upon the forward flux ($J^{Cl^-}_{ns}$), which was previously observed under the same conditions[6]. These results agree with those obtained by HOGBEN[3]. On the other hand, the inhibition of $J^{Cl^-}_{sn}$ by SCN^- was increased with the increase of $p^s_{CO_2}$, in a similar manner to the inhibition of $J^{Cl^-}_{ns}$, though less steeply.

In previous work SCN^- inhibition of $J^{Cl^-}_{ns}$ was found to be weakened upon raising the p_{CO_2} on the nutrient side, but this reduction was probably due to the increased concentration of nutrient HCO_3^-, which was substituted for a part of Cl^-

so as to maintain the pH at 7.3 against the increase of $p^n_{CO_2}$. In order to avoid the ambiguity produced by simultaneous administration, the effects of nutrient CO_2 and HCO_3^- were examined separately.

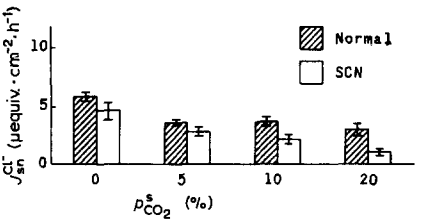

Fig. 1. The effect of secretory CO_2 partial pressure ($p^s_{CO_2}$)on SCN^- inhibition of the unidirectional Cl^- flux from secretory to nutrient side ($J^{Cl^-}_{sn}$), when nutrient CO_2 partial pressure ($p^n_{CO_2}$) was 0%; $\bar{X} \pm$ S.E. The shaded column represents the average value during the first 1-h normal period and the blank that during the subsequent 1-h SCN^- period.

In Fig. 2 nutrient CO_2 (center) increases both the H^+ secretion rate (Q_{H^+}) and $J^{Cl^-}_{ns}$ and SCN^- inhibition more effectively than does secretory CO_2 (left). Nutrient HCO_3^- reduces this effect (right). The left and right bar graphs were obtained from data supplied by IMAMURA[6]. A new finding should be noted here namely, the failure of low nutrient pH to inhibit acid secretion. Indeed, in the absence of the buffering HCO_3^-, nutrient CO_2 alone reduced the pH to 4.7–5.4, yet acid secretion was not prevented and was even increased.

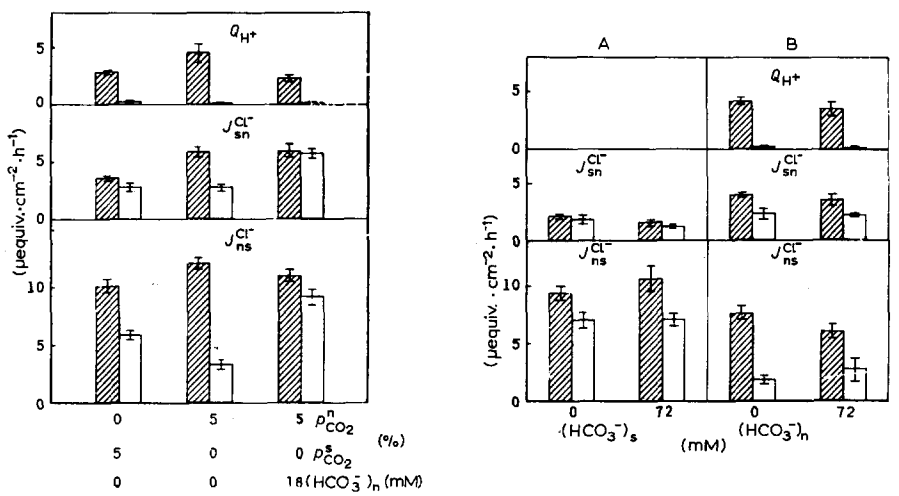

Fig. 2. The effect of p_{CO_2} on SCN^- inhibition of the H^+ secretion rate (Q_{H^+}), Cl^- flux from secretory to nutrient side ($J^{Cl^-}_{sn}$) and that from nutrient to secretory side ($J^{Cl^-}_{ns}$); $\bar{X} \pm$ S.E. The left and right bar graphs were obtained from data supplied by IMAMURA[6].

Fig. 3. The effect of HCO_3^- on the SCN^- inhibition of Q_{H^+}, $J^{Cl^-}_{sn}$ and $J^{Cl^-}_{ns}$; $\bar{X} \pm$ S.E. The HCO_3^- concentration on the side in question was varied by the equivalent replacement with sodium isethionate, Cl^- concentration being kept constant at 37 mM and this side was aerated with 100% O_2. The *trans* solutions were the ordinary nutrient ($[HCO_3^-]_n$ = 18 mM) and secretory ($[HCO_3^-]_s$ = 0 mM) solutions, respectively, and were aerated with O_2–CO_2 (95:5, v/v).

Fig. 3A shows that when the concentration of HCO_3^- on the secretory side was increased to 72 mM, $J_{sn}^{Cl^-}$ seemed to be reduced, $J_{ns}^{Cl^-}$ increased and SCN^- inhibition of both fluxes increased, although the effect was very small as compared with the CO_2 effect. With 72 mM $NaHCO_3$ in the secretory solution, however, the pH was initially approx. 8.5 and rose gradually to 9.0, whereas on replacement with an equivalent concentration of sodium isethionate, the pH was initially approx. 9.3 and fell gradually to 7.7. Since, in both cases, the pH never reached 4.5, Q_{H^+} was not measured by the pH-stat method. Fig. 3B shows that when the nutrient concentration of HCO_3^- was increased, $J_{ns}^{Cl^-}$ and Q_{H^+} were reduced and the inhibition of $J_{ns}^{Cl^-}$ by SCN^- was also reduced the effect being the reverse of that caused by CO_2.

THEORETICAL

A mobile carrier model for gastric mucosal anion transport is constructed after the manner of the models used for the sugar transport by ROSENBERG AND WILBRANDT[9], WILBRANDT AND KOTYK[10] and KOTYK[11] (Fig. 4). Some simplified assumptions are made about the mobilities of the carrier, since no qualitatively different conclusions are obtained from using more complicated assumptions. The carrier (C)

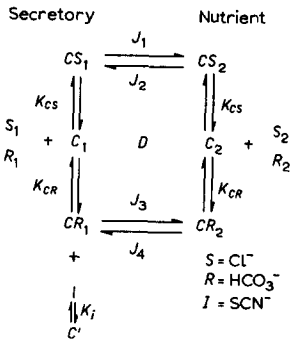

Fig. 4. A mobile carrier model for gastric mucosal anion transport. C, mobile carrier; D, mobility of the loaded carrier; K_{CS} and K_{CR}, dissociation constants. The carrier is assumed to traverse the membrane only in the combined state and the inhibition by SCN^- is assumed to be of the dead-end type.

is assumed to traverse the membrane only in the combined state (CS, CR) with the substrates Cl^- (S) and HCO_3^- (R). The mobilities through the membrane of both ions in the compound form are assumed to be the same, and also slow, compared with dissociation from and association with the carrier. In this instance, the concentrations of each of the carrier forms on both sides of the membrane will be in the steady state. Thus, instead of many rate constants, only two dissociation constants, K_{CS} and K_{CR}, and the mobility of the loaded carrier, D, are used as in ref. 11.

$$\frac{[S]\,[C]}{[CS]} = K_{CS} \tag{1}$$

$$\frac{[R]\,[C]}{[CR]} = K_{CR} \tag{2}$$

Since secretory CO_2 stimulates the SCN^- inhibition effectively, SCN^- is assumed to attack only the carrier form, CR_1, combined with HCO_3^- on the secretory side.

$$\frac{[CR_1]\,[SCN^-]}{[C']} = K_i \tag{3}$$

This inhibition is perhaps of the dead-end type as is classified in enzyme kinetics[12]. Thus SCN^- combines with the carrier and takes it out of the closed loop, reducing the total amount of carrier in the loop and consequently the available concentration of carrier for HCO_3^- and Cl^- transport. The inhibition, however, does not change the circuit constants K_{CS}, K_{CR} and D.

Thus the unidirectional fluxes of the Cl^- and HCO_3^- can be calculated from the equations

$$J_1 = [CS_1]D \quad etc. \tag{4}$$

where the abbreviations J_1, J_2, J_3 and J_4 are used for $J_{sn}^{Cl^-}$, $J_{ns}^{Cl^-}$, $J_{sn}^{HCO_3^-}$ and $J_{ns}^{HCO_3^-}$, respectively. Finally they are described by the following (for calculation, see APPENDIX):

$$J_1 = \frac{DC_T(S_2' + R_2')S_1'}{(S_1' + R_1')\,(1 + S_2' + R_2') + (S_2' + R_2')\,(1 + S_1' + (1 + \beta)R_1')}$$

$$J_2 = \frac{DC_T(S_1' + R_1')S_2'}{(S_1' + R_1')\,(1 + S_2' + R_2') + (S_2' + R_2')\,(1 + S_1' + (1 + \beta)R_1')}$$

$$J_3 = \frac{DC_T(S_2' + R_2')R_1'}{(S_1' + R_1')\,(1 + S_2' + R_2') + (S_2' + R_2')\,(1 + S_1' + (1 + \beta)R_1')} \tag{5}$$

$$J_4 = \frac{DC_T(S_1' + R_1')R_2'}{(S_1' + R_1')\,(1 + S_2' + R_2') + (S_2' + R_2')\,(1 + S_1' + (1 + \beta)R_1')}$$

where,

$$S' = [S]/K_{CS},\ R' = [R]/K_{CR} \text{ and } \beta = [I]/K_i \tag{6}$$

From Eqn. 5, the correlations are predicted between the four fluxes and the substrate concentrations on both sides as shown in Table I, where + denotes positive and − negative correlations, respectively. Thus, in the normal period, when the

TABLE I

CORRELATIONS BETWEEN THE FOUR UNIDIRECTIONAL FLUXES AND THE SUBSTRATE CONCENTRATIONS ON BOTH SIDES

The signs ++, + and − mean very positive, positive and negative correlations, respectively.

	Normal ($\beta = 0$)				SCN^- ($\beta \gg 1$)			
	J_1	J_2	J_3	J_4	J_1	J_2	J_3	J_4
S_1	++	+	−	+	+	+	−	+
S_2	+	++	+	−	+	+	+	−
R_1	−	+	++	+	−	−	+	−
R_2	+	−	+	++	+	−	+	+

concentration of a substrate on one (*cis*) side increases, its forward flux increases steeply (++) (*cf.* Eqn. 17), its backward flux from the *trans* side also increases (+) and the flux of the competitive substrate from the *cis* side decreases (−). In the SCN^- period, a different correlation from that in the normal period is predicted only between R_1 and J_2 or R_1 and J_4.

Specifically, when R_1 or $p^s_{CO_2}$ increases, J_1 decreases and J_2 increases in the normal period, while both J_1 and J_2 decrease in the SCN^- period, in good agreement with the present and previous observations. Furthermore, when the secretory Cl^- concentration S_1 increases, not only J_1 but also J_2 increases; the well-known *trans*-concentration effect found by HEINZ AND DURBIN[13].

Although J_3 and J_4 were not measured directly, the difference J_3-J_4 would correspond to the acid secretion rate Q_{H^+} if J_3 were derived from CO_2 and OH^-, the partner of the secreted H^+. Therefore, so far as Q_{H^+} is concerned, R_1 in Table I should be regarded as $p^s_{CO_2}$. The model also predicts that nutrient Cl^- favors Q_{H^+}, whilst secretory Cl^- reduces it. The former effect was well confirmed[15] but the latter was not. With HCO_3^- the reverse is the case: R_1 or $p^s_{CO_2}$ favors Q_{H^+}, whilst R_2 or nutrient HCO_3^- reduces it. Both effects were observed in the previous and present experiments.

There remains, however, a sharp contradiction that nutrient HCO_3^- actually reduced the SCN^- inhibition whereas the model predicts the opposite. This will be discussed later.

DISCUSSION

The results show that when p_{CO_2} was raised, Q_{H^+} and $J^{Cl^-}_{ns}$ increased, while $J^{Cl^-}_{sn}$ decreased and simultaneously SCN^- inhibition increased. The effect of secretory HCO_3^- was small whilst nutrient HCO_3^- reduced the CO_2 effect. FORTE[14] explained the mutually opposite directions of the changes in the unidirectional Cl^- fluxes caused by metabolic inhibitors, on the basis of the accompanying decrease of bulk flow. The observations on the changes of $J^{Cl^-}_{sn}$ and $J^{Cl^-}_{ns}$ produced with the increase of $p^s_{CO_2}$, might also be interpreted as being due to the increase of bulk flow. However, the simultaneous increase of SCN^- inhibition of $J^{Cl^-}_{sn}$ and $J^{Cl^-}_{ns}$, when p_{CO_2} was increased, would be better explained by the dead-end type inhibition of the anion carrier. It is true, but nutrient HCO_3^- reduced the SCN^- inhibition. Therefore, the inhibition might not be a perfectly dead-end one, but there might be further conversion of the ternary complex C' ($CR_1 + I \rightleftharpoons C' \rightleftharpoons CI + R$) and nutrient HCO_3^- might push back the equilibrium to the left, consequently reducing the inhibition.

Although the model predicts that low secretory $[Cl^-]$ itself does not reduce but favors Q_{H^+}, no such observation was found. In the present experiment replacement of secretory NaCl with sodium isethionate raised the pH and reduced Q_{H^+} to a minimum, but FORTE[16] showed that Q_{H^+} was not affected by variation in $[Cl^-]_s$. The difference observed was probably due to the different secretory gas used; O_2–CO_2 (95:5, v/v) in the experiment of FORTE[16] and 100 % O_2 in the present one. Therefore, the presence of secretory CO_2 may be a necessary condition for low secretory $[Cl^-]$ to enhance Q_{H^+}.

The inhibition of acid production by 72 mM HCO_3^- in the secretory solution may need some explanation. As stated in the theoretical discussion, it is not HCO_3^-,

but CO_2, which enhances the transfer of OH^- or H^+ production. Secretory HCO_3^- will tend to compete with HCO_3^- derived from OH^- and CO_2, thus blocking Q_{H^+}.

Since secretory HCO_3^- affected the system more weakly than did CO_2, on a molar basis, it appeared that the site of HCO_3^- transfer was located on the inside of the secretory membrane and that the permeability favored CO_2. That is, the overall diagram for the secretion process will be as follows (Fig. 5): Two membranes are situated in series in mucosa, one for H^+ production and one for anion exchange. In the first membrane, H^+ is produced by the dissociation of the metabolic hydrogen ($H \rightarrow H^+ + e^-$) *via* the electron transport system of the respiratory chain, as postulated by several workers[17–19]. The electron separated is transferred to O_2, consequently causing accumulation of OH^-. This concentrated OH^-, first exchanged with Cl^- bound to anion carrier E and then facilitated by CO_2 ($OH^- + CO_2 \rightarrow HCO_3^-$) promotes the circulation of the anion-exchange carrier within the second membrane. In other words, in the absence of CO_2 the concentrated OH^- will leak back and recombine with the separated H^+ on the secretory side but the presence of CO_2 will prevent this recombination. Thus the action of CO_2 lies probably in the step distal to the OH^- pump, where carbonic anhydrase, a strong candidate for the carrier, presumably will aid the reaction.

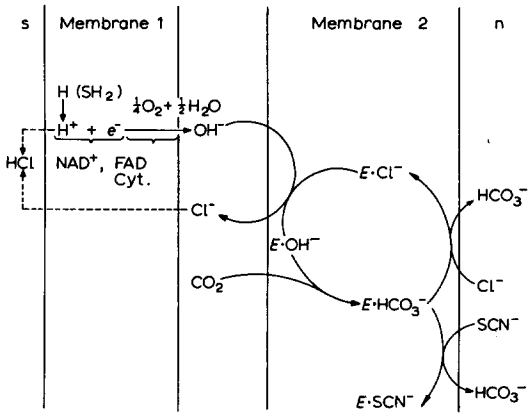

Fig. 5. A supposed process of acid secretion. The active electron transport process in Membrane 1 and the passive facilitated Cl^- transfer in Membrane 2 are coupled in the OH^-–Cl^- exchange process. CO_2 combines with OH^- bound to carrier (E) and aids the latter's circulation.

The view that the mechanisms underlying the transport of H^+ and Cl^- in mucosa are separated but coupled in the OH^-–Cl^- exchange process, may agree with the observation by SACHS *et al.*[19] that Cl^- transport is relatively insensitive to amytal, suggesting a qualitative distinction between the two mechanisms.

Although the model is in a sense a variant of the forced anion exchange presented by HOGBEN[20], it shows qualitative agreement with the present and previous observations as well as with other phenomena in this field. However, a quantitative comparison is still lacking and the model takes no consideration of either potential difference or the short-circuit current. Nevertheless, neglect of these factors would not change the qualitative predictions of the model significantly. Since the actual effect of nutrient HCO_3^- upon SCN^- inhibition contradicted the prediction of the model, and since the slight modification tried of the model with respect to the blocked

carrier form has not improved the situation yet, there remains the possibility that SCN^- inhibition may be of quite a different nature. Further examination of this possibility is desirable.

In conclusion, the primary motive force of Cl^- transport will be provided by the OH^- concentration, which is created by the respiratory electron transfer chain. Therefore, it is probably better to regard the Cl^- transport as a sort of passive facilitated transfer, the counter transport of which is HCO_3^- transport, both being mediated by the anion-exchange carrier. Then the active process, which promotes the circulation of the carrier in the later stages, will essentially be the electron transport through the redox system, though the action of CO_2 is certainly indispensable.

APPENDIX

Starting from Eqn. 4, Eqn. 5 is obtained as follows: If the reduced concentrations S', R' and β (Eqn. 6) are used and the total concentrations of the carrier on the secretory and nutrient sides are designated as C_{t1} and C_{t2}, respectively, they can be written

$$C_{t1} = [C_1] + [CS_1] + [CR_1] + [C'] = [C_1]\,(1 + S_1' + (1 + \beta)R_1') \tag{7}$$

$$C_{t2} = [C_2] + [CS_2] + [CR_2] = [C_2]\,(1 + S_2' + R_2') \tag{8}$$

Using Eqns. 1, 2, 6, 7 and 8, Eqn. 4 becomes

$$J_1 = [CS_1]D = \frac{[C_1]\,[S_1]}{K_{CS}}D = D[C_1]S_1' = D\frac{C_{t1}S_1'}{1 + S_1' + (1 + \beta)R_1'}$$

and similarly

$$J_2 = D\frac{C_{t2}S_2'}{1 + S_2' + R_2'},\quad J_3 = D\frac{C_{t1}R_1'}{1 + S_1' + (1 + \beta)R_1'},\quad J_4 = D\frac{C_{t2}R_2'}{1 + S_2' + R_2'} \tag{9}$$

Since the total concentration of carrier, C_T, is constant

$$C_{t1} + C_{t2} = C_T \tag{10}$$

and the carrier cannot leak out of the membrane,

$$J_2 - J_1 = J_3 - J_4 \text{ or } J_1 + J_3 = J_2 + J_4 \tag{11}$$

Introduction of Eqn. 9 into Eqn. 11 yields

$$\frac{D(S_1' + R_1')}{1 + S_1' + (1 + \beta)R_1'}C_{t1} = \frac{D(S_2' + R_2')}{1 + S_2' + R_2'}C_{t2} \tag{12}$$

Solving the simultaneous Eqns. 10 and 12, we get

$$C_{t1} = C_T \cdot \frac{(1 + S_1' + (1 + \beta)R_1')\,(S_2' + R_2')}{(S_1' + R_1')\,(1 + S_2' + R_2') + (S_2' + R_2')\,(1 + S_1' + (1 + \beta)R_1')} \tag{13}$$

$$C_{t2} = C_T \cdot \frac{(1 + S_2' + R_2')\,(S_1' + R_1')}{(S_1' + R_1')\,(1 + S_2' + R_2') + (S_2' + R_2')\,(1 + S_1' + (1 + \beta)R_1')} \tag{14}$$

Putting Eqns. 13 and 14 in Eqn. 9, we finally obtain Eqn. 5.

Using Eqn. 5, the correlations between the substrates and the fluxes can be obtained as follows:

(a) Control ($\beta = 0$). From the relation that when $y = (c+dx)/(a+bx)$ and $ad-bc > 0$, then

$$\frac{\partial y}{\partial x} > 0 \tag{15}$$

we get

$$\frac{\partial J_1}{\partial S_1'} > 0, \quad \frac{\partial J_2}{\partial S_1'} < 0, \quad \frac{\partial J_3}{\partial S_1'} > 0, \quad \frac{\partial J_4}{\partial S_1'} > 0 \tag{16}$$

and since $(\partial/\partial S_1')\,(J_1-J_2) > 0$ and $(\partial/\partial S_1')\,(J_1-J_4) > 0$, clearly

$$\frac{\partial J_1}{\partial S_1'} > \frac{\partial J_2}{\partial S_1'} \text{ and } \frac{\partial J_1}{\partial S_1'} > \frac{\partial J_4}{\partial S_1'} \tag{17}$$

Thus, correlations between S_1' or S_1 and the four fluxes are obtained. Similarly, the correlations for the other three substrates can be obtained.

(b) SCN^- ($\beta \gg 1$). Using the above relation (Eqn. 15), it is easily shown that the signs of the correlations, different from those for the control period, are obtained only at two places, namely

$$\frac{\partial J_2}{\partial R_1'} < 0 \text{ and } \frac{\partial J_4}{\partial R_1'} < 0 \tag{18}$$

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