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Kinetic Study on Ureolysis in Rats

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After the oral and intraperitoneal administration of urea-^{14}C and sodium bicarbonate-^{14}C to rats, the radioactivity of carbon dioxide expired in breathing air and that of urea transported into the blood were measured. Ureolysis was examined quantitatively on the basis of a compartment model analysis, where the hydrolysis and transport of substances were assumed to be first-order processes. Ureolysis *in vivo* was found not only in the lumen, but also in other tissues such as juxtamucosa, kidney and liver; the former rate constant (h^{-1}) was 0.759 (k) and the latter 0.0122 (k'). The constant k observed in normally fed rats was 9.5-fold higher than k in fasted rats, but k' was low in both cases. The administration of nicotino-hydroxamic acid as a urease inhibitor completely depressed k, but k' was not affected. On the bases of 71.0 $mg \cdot kg^{-1} \cdot h^{-1}$ of urea excreted in urine and the rate constants obtained for transport and hydrolysis of urea, the amounts of urea hydrolyzed in the lumen and other tissues were estimated to be 4.92 and 5.04 $mg \cdot kg^{-1} \cdot h^{-1}$, respectively. Consequently, it was calculated that urea hydrolyzed in the body corresponded to 12.3% of urea synthesized in the liver.

Keywords——ureolysis; hydroxamic acid; urea-^{14}C; kinetics; intestinal urease; rate constant; rat

Most of the urea synthesized in the liver is excreted into urine, but a part of it is excreted into the gastrointestinal tract and hydrolyzed to ammonia and carbon dioxide.[1] The ureolysis in the gut is attributable to urease (urea amidohydrolase EC 3.5.1.5) produced by intestinal microorganisms, because oral administration of antibiotics[2-4] or hydroxamic acid (HXA),[5,6] a specific inhibitor of urease, potently depressed this hydrolysis. Furthermore, no ureolysis was observed *in vivo* or *in vitro* in germ-free animals.[7,8]

It has been established that urea is the major source for ammonia production in the gut[9] and ammonia produced is one of the pathogens of hyperammonemia in patients with liver cirrhosis,[10] uremia,[3,11] or stagnant loop syndrome.[3] Therefore,it is important to elucidate quantitatively the absorption, excretion and hydrolysis of urea in the gut.

This paper presents a quantitative analysis of ureolysis in rats based on computer calculations with appropriate compartment models.

Materials and Methods

Animals——Female rats (Wistar strain, about 200 g body weight) were maintained on commercial feed (Nihon Clea, CE-2); before the experiments unless otherwise stated.

Compounds, Dose and Administration——Urea-^{14}C (2.5 μCi, 100 μmol per rat) or $NaHCO_3$-^{14}C (0.7 μCi, 53 μmol per rat) was orally or intraperitoneally administered to rats. Nicotino-HXA (30 mg/kg), which was synthesized in our laboratory, was orally administered to rats at the indicated time.

Assay of Radioactivity in Breathing Air and Blood——Carbon dioxide in breathing air was trapped in ethanolamine for a period of six h and its radioactivity was assayed as described in our previous report[4] with Bray's reagent. Whole blood was obtained from the arterial cardiac vein of the rat. Each aliquot of blood was mixed with an equal volume of 1N H_2SO_4 and the radioactivity of this preparation was assayed by the same methods.

Programing for Compartment Models——Compartment models for ureolysis are shown in Fig. 1, according to Charlwood[12] with a minor modification. In these analyses, both carbon dioxide and urea were assumed to be homogeneously distributed in the lumen and body fluid. C (carbon dioxide) and U (urea) were

defined as the concentrations of the substances in the lumen and body fluid, which were designated by subscripts G and BF, respectively. Transport of the substances between these compartments was assumed to be governed by a first-order process, but intraperitoneal urea was considered to be instantaneously diffused into the body fluid.[13]

Model I, which describes the behavior of carbon dioxide, is mathematically expressed by the following two equations:

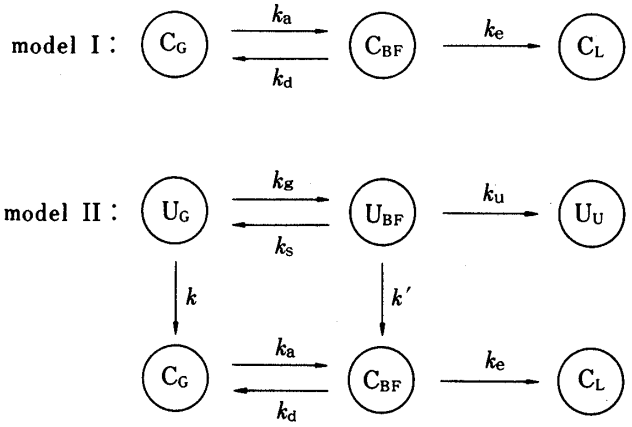

Fig. 1. Pharmacokinetic Compartment Models Used for Analysis of Ureolysis

$$\frac{dC_G}{dt} = -k_a C_G + k_d C_{BF} \quad \text{(Eq. 1)}$$

$$\frac{dC_{BF}}{dt} = -(k_d + k_e) C_{BF} + k_a C_G \quad \text{(Eq. 2)}$$

In these equations, k_a, k_d and k_e are the rate constants (h^{-1}) of absorption from the lumen into body fluid, excretion from body fluid into the lumen and excretion from body fluid into breathing air, respectively.

For model II, which expresses the behavior of urea and carbon dioxide produced by the ureolysis, the following five equations apply:

$$\frac{dU_G}{dt} = -(k_g + k) U_G + k_s U_{BF} \quad \text{(Eq. 3)}$$

$$\frac{dU_{BF}}{dt} = -(k_u + k_s + k') U_{BF} + k_g U_G \quad \text{(Eq. 4)}$$

$$\frac{dC_G}{dt} = k U_G - k_a C_G + k_d C_{BF} \quad \text{(Eq. 5)}$$

$$\frac{dC_{BF}}{dt} = -(k_e + k_d) C_{BF} + k' U_{BF} + k_a C_G \quad \text{(Eq. 6)}$$

$$\frac{dC_L}{dt} = k_e C_{BF} \quad \text{(Eq. 7)}$$

In these equations, k_g, k_s and k_u are the rate constants (h^{-1}) of absorption of urea from the lumen into body fluid, excretion of urea from body fluid into the lumen and into urine, respectively. k and k' are the rate constants(h^{-1}) of ureolysis in the lumen and in other tissues, respectively.

Using these equations, regression analyses were performed according to Atkins' program[14] with a digital computer (FACOM, 230-4S type).

Results

Determination of Rate Constants

Based on the data relating to oral or intraperitoneal administration of $NaHCO_3$-^{14}C, the rate

constants of absorption into body fluid (k_a) and of excretion into expired air (k_e) and into the lumen (k_d) were calculated according to Eq. 1 and Eq. 2 (Table I). The values of k_a and k_e were markedly higher than that of k_d, suggesting that HCO_3^--^{14}C in the lumen was rapidly absorbed into body fluid and was immediately expired as $^{14}CO_2$, but the excretion from body fluid into the lumen was negligible (k_d=0). Other rate constants in the cases of oral and intraperitoneal administration of urea-^{14}C were also determined according to Eq. 3 through Eq. 7 of model II, in which k_a, k_e and k_d were substituted by the values obtained from the equations in model I. The absorption rate constant (k_g) from the lumen into body fluid showed the same value in both normally fed rats and fasted rats which had been starved for 18 h before the experiment. The excretion rate constants from body fluid into the lumen (k_s) and into urine (k_u) in fasted rats were 6.3 and 2.6 times higher than those in fed rats, respectively. These values, however, were lower than k_g, suggesting that the absorption of urea was faster than the excretion. On the other hand,

TABLE I. Rate Parameters obtained by the Least-Squares Method

k (h^{-1})	Control		Nicotino-HXA
	Fed	Fasted	Fed
k_a	1.271±0.103	1.819±0.143	1.271
k_e	3.901±0.243	1.615±0.125	3.901
k_d	0.0	0.0	0.0
k_g	1.494±0.398	1.558±0.503	1.494
k_s	0.0355±0.0618	0.221±0.256	0.0355
k_u	0.171±0.0629	0.451±0.479	0.171
k	0.759±0.181	0.0791±0.0226	0.0
k'	0.0122±0.0171	0.0105±0.00819	0.0130±0.00374

After the administration of $NaHCO_3$-^{14}C or urea-^{14}C, the experimental values of $^{14}CO_2$ in the expired air or urea-^{14}C in the blood were used for calculation on a digital computer according to Atkins' program.

the rate of ureolysis in the lumen (k) was 9.5 times higher in fed rats than in fasted rats. Ureolysis in other tissues (k'), however, was remarkably low in both cases.

Excretion curves of $^{14}CO_2$ *versus* time calculated using these parameters (Table I) coincided well with the experimental data, as shown in Fig. 2.

Effect of Urease Inhibitor on the Rate Constants

Figure 3 shows the time course of $^{14}CO_2$ excretion after successive oral administration of nicotino-HXA to fed rats. HXA is a strictly specific inhibitor of urease[15] and its administration did not alter the amount of urea excreted into urine. Based on these findings, the decomposition rate constants, k and k' were evaluated from Eq. 3 through Eq. 7, in which other rate constants except k and k' were fixed to the values listed in Table I. The result that k decreased to zero indicates the complete depression of ureolysis by HXA in the lumen, but the absence of change in k' suggests inefficacy of HXA on ureolysis in other tissues.

Quantittive Aspects of Ureolysis in Fed Rats

Since dx/dt in the seven equations (Eqs. 1 to 7) is zero in the steady state, ureolysis was estimated quantitatively from the rate constants (Table I) and the experimental value of urea excreted into the urine in fed rats, which was measured to be 71.0 $mg \cdot kg^{-1} \cdot h^{-1}$, corresponding to $k_u U_{BF}$. The amount of urea lost from body fluid, $(k_u + k_s + k')U_{BF}$, was evaluated as 90.5 $mg \cdot kg^{-1} \cdot h^{-1}$, because 78.4% of the amount was excrete into urine. In addition, 16.2% of the amount was excreted into the lumen, representing ($k_s U_{BF}$ or $(k_g + k)U_G$), and of this, 33.4% was hydrolyzed in the lumen, based on the ratio of k to k_g. On the other hand, 5.57% ($k'U_{BF}$) of the amount of urea lost from body fluid was also hydrolyzed in other tissues. Consequently, the

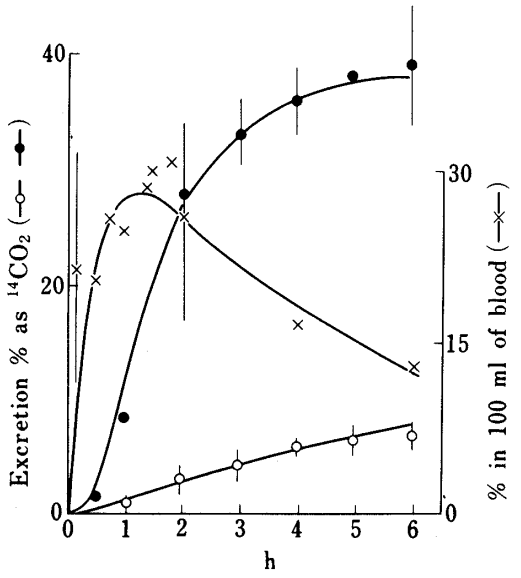

Fig. 2. Ureolysis in Fed Rats

The amounts of radioactivity in the expired air were measured after oral or intraperitoneal administration of urea-^{14}C (100 μmol, 2.5 μCi) to one group of six fed rats. The closed and open circles show the recovery % of the original dose after oral and intraperitoneal administration, respectively. The crosses show the amounts (percent) of urea-^{14}C transferred from the lumen in 100 ml of blood, which correspond to U_{BF}. All the data are averages with SD.

The curves are theoretical ones based on model II, in which k_a, k_e and k_d were taken to be 1.271, 3,901 and 0.0, respectively, and the values of the other parameters were obtained by regression analysis on a digital computer using Atkins' program.

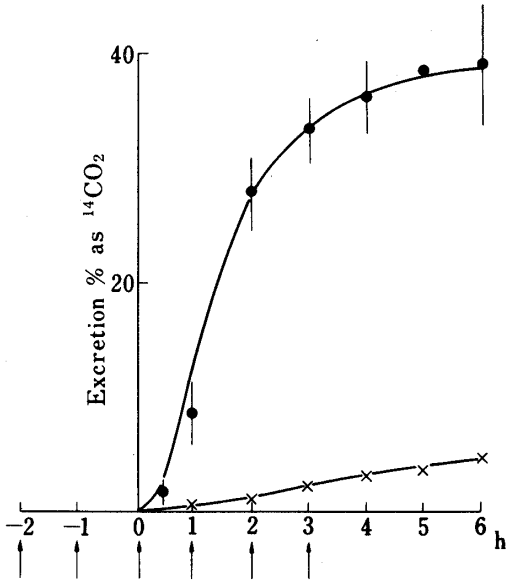

Fig. 3. Inhibition of Ureolysis in Fed Rats by the Administration of Nicotino-HXA

Nicotino-HXA (30 mg/kg) was administered orally every hour at the times indicated by the arrows, and urea-^{14}C was administered orally at time zero.

The closed circles and crosses represent the recovery % of $^{14}CO_2$ in the expired air of the control and treated groups, respectively. The curves are theoretical ones based on model II, in which the parameter values were set to k_a=1.271, k_e=3.901, k_d=0.000, k_g=1.494, k_s=0.0355 and k_u=0.171. The best-fit values for k and k' were examined on a digital computer using Atkins' program.

amounts of ureolysis in the lumen and in other tissues were estimated to be 4.92 and 5.04 mg·kg^{-1}·h^{-1}, respectively. From the sum of urea excreted in urine and hydrolyzed *in vivo*, urea synthesized was calculated to amount to 1943 mg·kg^{-1}·d^{-1}. Therefore, it is suggested that ureolysis *in vivo* corresponds to 12.3% of the total urea synthesized in liver

Discussion

The compartment models shown in Fig. 1 were used to analyze quantitatively urea transport and ureolysis, ignoring circadian rhythm of urea metabolism and assuming passive membrane transport of urea. Atkins[16] reported a successful theoretical analysis of the concentrations of glucose and insulin in plasma using such a simple compartment model. In these models, better-fitting theoretical values were obtained by locating ureolysis not only in the lumen (k) but also in other tissues (k'). The value of k' may represent the urease activity in juxtamucosa and in other tissues. Urease activity in juxtamucosa has been reported to be important in ureolysis *in vivo*.[9,17] We have also found urease activity in other tissues, for example, in the liver and kidney; this was due to bacterial infection, because no activity was detected in germ-free rats. The ratio of k to k' was 60 : 1 in normally fed rats and 8 : 1 in fasted rats. Although k' was markedly smaller than k, the urease activity in tissues other than the lumen is considered to play an important role in ureolysis *in vivo* because, even in fed rats, half of the total amount of urea hydrolyzed depended on the urease in other tissues. Moreover, this k' value was hardly affected by the administration of HXA. This suggests that urease exists in some tissues into which HXA can not be transported, or that HXA is rapidly metabolized[18] before reaching the tissues where urease is present. These suggestions are supported by our earlier observation that oral administration of HXA more strongly depressed ureolysis than did intraperitoneal administration.

Bodenlos,[1] Walser[11] and Jones[3] calculated the amount of ureolysis in the human gut by determining the specific radioactivity of urea in plasma after intravenous administration of urea-^{14}C, in which all reactions were assumed to progress according to a first-order process. In their reports, the amount of urea hydrolyzed in the gut corresponded to 22—29% of that of urea synthesized in liver. Gibson *et al.*[19] also calculated ureolysis in the gut to be 27% from the ratio of defference in specific radioactivity between urea administered and urea in urine to the specific activity of urea in plasma. Our result in the rat was 12.3%, which is much lower. However, the amount of urea hydrolyzed in humans calculated from our data in the rat was 14.2 g·60 kg body weight·d^{-1}, which is similar to the data in humans reproted by Gibson *et al.*[19]

We suggest, therefore, that in uremic patients, a high concentration of urea in body fluid results in an increase of the ureolysis corresponding to k', while in patients with stagnant loop syndrome, excessive growth of intestinal bacteria may cause an increase of the ureolysis corresponding to k.

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