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Drug Absorption, Metabolism, and Excretion. V.[1] Pharmacokinetic Studies on Renal Transport. (2). Analysis of *p*-Aminohippurate Run Out from the Kidney Slices by using Simultaneous Chemical Reaction and Diffusion Model

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Experimental data reported on PAH run out from the kidney slices were analyzed by applying the simultaneous chemical reaction and diffusion (SCRD) model. Various characteristic features of the experimental data were clearly explained by the proposed model.

In the previous communication,[1] simultaneous chemical reaction and diffusion (SCRD) expression was proposed for the mathematical interpretation of Beyer's model of *p*-aminohippurate (PAH) transport,[3] and the renal uphill transport of PAH was physicochemically explained. The model is as follows (Fig. 1). PAH is freely permeable to the cell membrane. Inside of the cell, PAH combines with substance X, which might be called a carrier and forms copmlex, PAHX. Formation of the complex is counteracted by the decomposition of the complex to the original substance PAH. At a given position coordinate, relative concentration of PAH, PAHX and X are determined by the values of the rate constant of complex formation and that of decomposition. Dynamic equlibrium ratio, K, is defined as the ratio of these rate constants. Each species diffuse through the intracellular fluid according to their own concentration gradients. At the proximity to the luminal border of the cell, however, the distribution of the enzyme that is capable of splitting the complex is much higher and breaking of the complex is faster than in the other side of the cell, consequently the dynamic equilibrium ratio, K, being larger at the luminal border of the cell. This results in the higher concentration of PAH at the luminal border and net transport of PAH from blood vessel border of the cell to the luminal border, even though the concentration in blood is lower than in tubular fluid. Substance X and complex PAHX can not cross the cell boundaries and remain inside of the cell conserving the total amount of X.

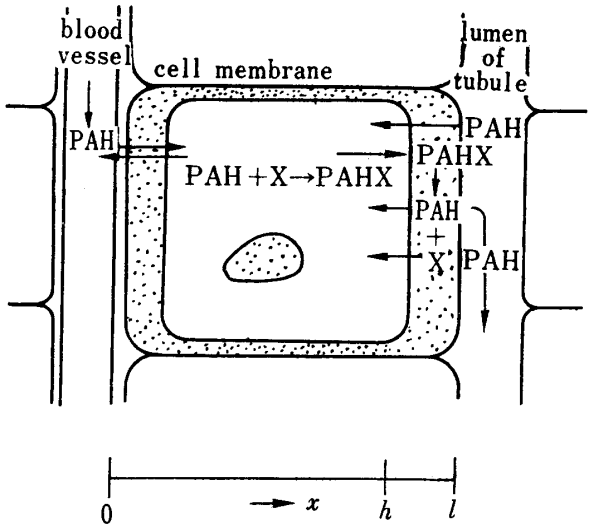

Fig. 1. Diagrammatic Representation of PAH Transport

Reproduced from (K.H. Beyer, *Pharmacol. Rev.*, 2, 227 (1950)).

1) Part IV: J.Shibasaki, T.Koizumi, and W.I.Higuchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 2273 (1968).
2) Location: *1-14 Bunkyo-cho, Nagasaki, 852, Japan.*
3) K.H.Beyer, *Pharmacol. Rev.*, 2, 227 (1950).

In order to examine the applicability of the SCRD model to the renal transport of PAH, experimental results of PAH run out from the kidney slices were analyzed by using this model.

Theory

Let $x=0$ be the position coordinate of blood vessel side boundary of the cell and $x=l$ be that of luminal border. The dynamic equilibrium ratio is K_0 at $0<x\leq h$, whereas it is K_l at $h<x<l$, due to uneven distribution of the enzyme that breaks the complex. Then the partial differential equations expressing the model proposed are;

$$\frac{\partial(\mathrm{PAH})}{\partial t}=D_{\mathrm{PAH}}\frac{\partial^2(\mathrm{PAH})}{\partial x^2}+\phi_{\mathrm{PAH}} \tag{1}$$

$$\frac{\partial(\mathrm{PAHX})}{\partial t}=D_{\mathrm{PAHX}}\frac{\partial^2(\mathrm{PAHX})}{\partial x^2}+\phi_{\mathrm{PAHX}} \tag{2}$$

$$\frac{\partial(\mathrm{X})}{\partial t}=D_{\mathrm{X}}\frac{\partial^2(\mathrm{X})}{\partial x^2}+\phi_{\mathrm{X}} \tag{3}$$

$$\phi_{\mathrm{PAH}}=-\phi_{\mathrm{PAHX}}=\phi_{\mathrm{X}} \tag{4}$$

$$\frac{(\mathrm{PAH})(\mathrm{X})}{(\mathrm{PAHX})}=\begin{cases}K_0 \text{ at } 0<x\leq h, \text{ for } 0<t\\ K_l \text{ at } h<x<l, \text{ for } 0<t\end{cases} \tag{5}$$

$$D_{\mathrm{X}}\frac{\partial(\mathrm{X})}{\partial x}+D_{\mathrm{PAHX}}\frac{\partial(\mathrm{PAHX})}{\partial x}=0 \qquad \text{at } x=0,\ x=l \text{ for } 0<t \tag{6}$$

where (PAH), (PAHX), and (X) are the concentration of *p*-aminohippurate, complex, and the carrier in the cell, respectively. ϕ's represent the rate of formation or disappearance of the respective species. Equation 4 is obtained for the appropriate material balance. Equation 6 is obtained because X and PAHX are insulated by the cell boundaries.

For the purpose of simple mathematical treatment, it is assumed that,

$$D_{\mathrm{PAH}}=D_{\mathrm{PAHX}}=D_{\mathrm{X}}=D \tag{7}$$[4]

And set;

$$(\mathrm{PAH})+(\mathrm{PAHX})=\mathrm{PX} \tag{8}$$

$$(\mathrm{PAHX})+(\mathrm{X})=\mathrm{XX} \tag{9}$$

Then equation 1 to 4 are rearranged as equation 10 and 11;

$$\frac{\partial \mathrm{PX}}{\partial t}=D\frac{\partial^2 \mathrm{PX}}{\partial x^2} \tag{10}$$

$$\frac{\partial \mathrm{XX}}{\partial t}=D\frac{\partial^2 \mathrm{XX}}{\partial x^2} \tag{11}$$

Though these partial differential equations were solved simultaneously under the steady state conditions in the previous report,[1] in order to express the phenomena of PAH run out from or uptake by the kidney slices, it is necessary to be solved under non-steady state conditions.

4) Diffusion coefficient is inversely proportional to the square root of the molecular weight,[5] necessarily D_{PAH} is not equal to D_{PAHX}. For the practical purposes, however, this assumption that the all diffusion coefficients are equal is permissible.[6]

5) E.Nelson, *J. Pharmacol. Exptl. Therap.*, **135**, 120 (1962).

6) W.I. Higuchi, E. Nelson, and J.G. Wagner, *J. Pharm. Sci.*, **53**, 333 (1964).

Run Out of PAH from the Kidney Slice

Although a kidney slice is composed of a number of cells each of which has the ability of PAH transport described above, it is approximated here as a single unit of which the thickness is l. As the slice is loaded with PAH prior to the run out experiment, the boundary conditions are;

$$(\mathrm{PAH})=0 \text{ at } x=0 \text{ and } x=l, \text{ for } 0<t \tag{12}$$

$$\mathrm{PX}=\mathrm{PX}_0 \text{ at } 0<x<l, \text{ for } t=0 \tag{13}$$

$$\mathrm{XX}=\mathrm{XX}_0 \text{ at } 0<x<l, \text{ for } t=0 \tag{14}$$

From equations 6, 11, and 14, it is evident that;

$$\mathrm{XX}=\mathrm{XX}_0 \text{ at } 0<x<l, \text{ for } t\leq 0 \tag{15}$$

Meaning of equation 15 is that the total concentration of the carrier, free and combined, is constant everywhere in the slice at anytime.

From equations 5 and 12, equations 16 and 17 are obtained,

$$(\mathrm{PAHX})=0 \text{ at } x=0 \text{ and } x=l, \text{ for } 0<t \tag{16}$$

$$\mathrm{PX}=0 \quad \text{at } x=0 \text{ and } x=l, \text{ for } 0<t \tag{17}$$

General solutions of the diffusion equation 18 under various boundary conditions have been given by Carslaw and Jaeger[7] and by Barrer.[8]

$$\frac{\partial C}{\partial t}=D\frac{\partial^2 C}{\partial x^2} \tag{18}$$

Under the boundary and initial conditions 19, the solution is given as equation 20,

$$\left.\begin{aligned} &C=C_1 \text{ at } x=0, \text{ for } 0<t \\ &C=C_2 \text{ at } x=l, \text{ for } 0<t \\ &C=f(x) \text{ at } 0<x<l, \text{ for } t=0 \end{aligned}\right\} \tag{19}$$

$$C=C_1+(C_2-C_1)\frac{x}{l}+\frac{2}{\pi}\sum_{n=1}^{\infty}\frac{C_2\cos n\pi-C_1}{n}\sin\frac{n\pi x}{l}\exp\left[-\frac{Dn^2\pi^2}{l^2}t\right]$$
$$+\frac{2}{l}\sum_{n=1}^{\infty}\sin\frac{n\pi x}{l}\exp\left[-\frac{Dn^2\pi^2}{l^2}t\right]\int_0^l f(x')\sin\frac{n\pi x'}{l}dx' \tag{20}$$

Therefore the solution of equation 10 under the conditions 13 and 17 is obtained from equation 20 by substitution: $C=\mathrm{PX}$, $C_1=C_2=0$, and $f(x)=\mathrm{PX}_0$. The result is;

$$\mathrm{PX}=\frac{2}{\pi}\mathrm{PX}_0\sum_{n=1}^{\infty}\frac{1-\cos n\pi}{n}\sin\frac{n\pi x}{l}\exp\left[-\frac{Dn^2\pi^2}{l^2}t\right] \tag{21}$$

The amount of PAH remaining in the slice per unit cross-sectional area, Q, at time t is obtained by integration of equation 21 with respect to x between 0 and l,

$$\left.\begin{aligned} &Q=\int_0^l \mathrm{PX}\,dx=\frac{4}{\pi^2}Q_0\sum_{n=1}^{\infty}\frac{1-\cos n\pi}{n^2}\exp\left[-\frac{Dn^2\pi^2}{l^2}t\right] \\ &Q_0=\mathrm{PX}_0\times l \end{aligned}\right\} \tag{22}$$

7) H.S. Carslaw and J.C. Jaeger, "Conduction of Heat in Solids," 2nd. ed., Oxford University Press, London, 1959, p. 99.

8) R.M, Barrer, "Diffusion in and through Solids," Cambridge Universtiy Press, London, 1951, p. 15.

where Q_0 is the initial amount of PAH, free and combined, in the slice.

For t sufficiently large, the first term in the series of equation 22 gives a good approximation.

$$Q=\frac{8}{\pi^2}Q_0\exp\left[-\frac{D\pi^2}{l^2}t\right] \tag{23}$$

Equation 22 is represented numerically in Table I and graphically on Fig. 2.

TABLE I. Q Values defined by the Following Equation

$$Q=\frac{4}{\pi^2}\sum_{n=1}^{\infty}\frac{1-\cos n\pi}{n^2}\exp(-n^2\tau)$$

τ	Q	τ	Q	τ	Q	τ	Q
0.00	1.000000	0.12	0.751157	0.28	0.619892	1.60	0.163652
0.01	0.928166	0.13	0.740996	0.30	0.606556	1.80	0.133986
0.02	0.898411	0.14	0.731219	0.40	0.545804	2.00	0.109698
0.03	0.875579	0.15	0.721785	0.50	0.492636	2.25	0.085433
0.04	0.856330	0.16	0.712661	0.60	0.445257	2.50	0.066536
0.05	0.839372	0.17	0.703818	0.70	0.402682	2.75	0.051818
0.06	0.824042	0.18	0.695231	0.80	0.364280	3.00	0.040356
0.07	0.809943	0.19	0.686879	0.90	0.329581	3.50	0.024477
0.08	0.796821	0.20	0.678745	1.00	0.298203	4.00	0.014846
0.09	0.784496	0.22	0.663066	1.20	0.244141	5.00	0.005462
0.10	0.772838	0.24	0.648084	1.40	0.199885	10.00	0.000037
0.11	0.761751	0.26	0.633716	1.50	0.180862		

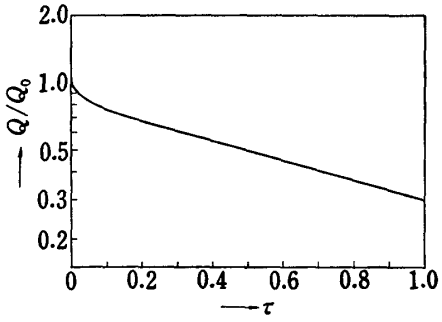

Fig. 2. Graphical Representation of Equation 22

$\tau=\frac{D\pi^2}{l^2}\cdot t$

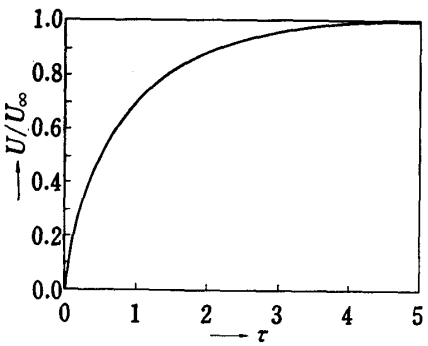

Fig. 3. Graphical Representation of Equation 30

$\tau=\frac{D\pi^2}{l^2}\cdot t$

Uptake of PAH by the Kidney Slice

Kidney slice free of PAH is immersed into the medium containing PAH. The capacity of the medium is such that the concentration of PAH is kept constant during the uptake experiment. Then the boundary conditions are;

$$\left.\begin{aligned}&(\mathrm{PAH})=(\mathrm{PAH})_0 \text{ at } x=0 \text{ and } x=l, \text{ for } 0<t\\&(\mathrm{PAHX})=(\mathrm{PAH})=0 \text{ at } 0<x<l, \text{ for } t=0\\&(\mathrm{X})=\mathrm{XX}_0 \text{ at } 0<x<l, \text{ for } t=0\end{aligned}\right\} \tag{24}$$

Therefore,

$$\mathrm{PX}=0 \text{ at } 0<x<l, \text{ for } t=0 \tag{25}$$

And it is assumed that;

$$PX = PX_0 \text{ at } x=0, \text{ for } 0<t \tag{26}$$

$$PX = PX_l \text{ at } x=l, \text{ for } 0<t \tag{27}$$

Evaluation of PX_0 and PX_l is performed as follows; as the sum of (X) and (PAHX) is constant and equal to XX_0, everywhere at anytime,

$$K_0 = \frac{(X)_{x=0} \times (PAH)_0}{(PAHX)_{x=0}} = \frac{\{XX_0 - (PAHX)_{x=0}\} \times (PAH)_0}{(PAHX)_{x=0}}$$

By rearrangement,

$$(PAHX)_{x=0} = \frac{XX_0 \times (PAH)_0}{K_0 + (PAH)_0}$$

Therefore,

$$PX_0 = (PAH)_0 + \frac{XX_0 \times (PAH)_0}{K_0 + (PAH)_0} \tag{28}$$

Similarly,

$$PX_l = (PAH)_0 + \frac{XX_0 \times (PAH)_0}{K_l + (PAH)_0} \tag{29}$$

The solution of equation 10 under the conditions 25 to 27 is obtained from equation 20 by substitution, and the cumulative amount of PAH uptaken by the kidney slice per unit cross-sectional area, U, is;

$$\left.\begin{aligned} U &= U_\infty \left\{1 - \frac{4}{\pi^2} \sum_{n=1}^{\infty} \frac{1-\cos n\pi}{n^2} \exp\left[-\frac{Dn^2\pi^2}{l^2} t\right]\right\} \\ U_\infty &= \frac{PX_0 + PX_l}{2} \times l \end{aligned}\right\} \tag{30}$$

where U_∞ is the total amount of PAH ultimately uptaken by the kidney slice at a given medium concentration. Equation 30 is represented graphically on Fig. 3.

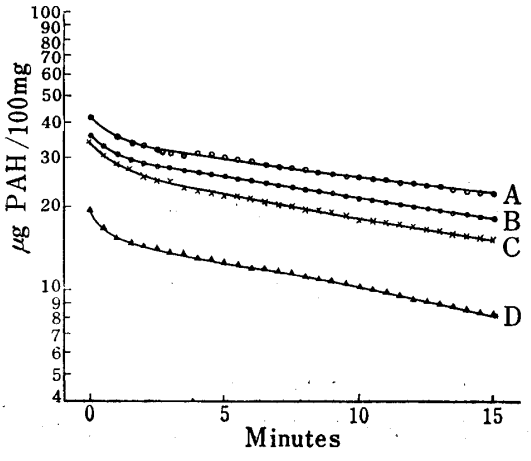

Fig. 4. PAH Run Out Curve from Renal Slices reproduced from Farah's Report

Loading with PAH was done at 25° in phosphate buffer containing 6×10^{-4} M (curves A,B, and C) or 2×10^{-4} M PAH (curve D)for 90 min. Run out rate betermined at 25° into phosphate buffer, gas phase 100% oxygen. Slices were transferred every 0.5 min from one beaker to the next.

Abscissa: time in minutes; ordinate: PAH content of slices (per 100 mg) plotted on a logarithmic scale

Discussion

Run Out Data by Farah

Run out of PAH from the dog kidney slice was investigated extensively by Farah, *et al.*[9] Slices preliminary loaded with PAH were transferred to the first of a series of small beakers containing 2 ml of PAH free buffer and after 30 seconds slices were transferred to the next beaker. All beakers had been equilibrated for 30 minutes at the desired temperature with the gas phase. By these experimental procedure the boundary conditions 12 and 17 were satisfied. Farah's results are reproduced on Fig. 4 of the present paper.

According to Farah, (a) the release curve consists of an early fast, followed by a slower component. (b) The slow release component when plotted on a semilogarithmic scale gave

9) A. Farah, M. Franzer, and M. Stoffel, *J. Pharmacol. Exptl. Therap.*, **139**, 120 (1963).

a straight line (c) the slope of which was not influenced by the initial cocentration of PAH in the slices.

All of these characteristic observations are fully satisfied by equation 22. (a) is obvious from Fig. 2, (b) and (c) are easily shown by equation 23, where the slope is $D\pi^2/l^2$ and independent of the initial concentration. Equation 22 of SCRD model also predict much more facts.

For all the release curves shown on Fig. 4, terminal straight line portion was extrapolated to $t=0$, in every case the readings at the intercept was about 80% of the initial concentration of PAH (Table II).

Table II. Q_0 Values, Intercept Readings and the Percentage Values of the Intercept respect to Q_0 Values

Curve	Q_0 value	Intercept reading	$\frac{\text{Intercept}\times 100}{Q_0}$
A	41.9	33.7	80.4
B	35.2	29.6	84.1
C	33.7	27.2	80.7
D	19.2	15.4	80.2

Data were obtained directly from the Fig. 2 of Farah's report. [9]

This fact is predicted by equation 23, the equation of the terminal straight line portion. At $t=0$, right hand side of equation 23 is equal to $8Q_0/\pi^2$ which is 81.057% of the initial value, Q_0.

The thickness of the slice was approximately 0.5 mm.[10] The diffusion coefficient is around 5×10^{-6}(sucrose) to 0.5×10^{-7} (Tobacco mosaic virus) cm^2/sec.[11] Therefore $D\pi^2/l^2$ lies in the range of 0.012 to 1.2 min^{-1}, corresponding value in Farah's report is k for which 0.04 to 0.117 min^{-1} at 25° were listed.[9] Agreement between predicted value and experimental data is satisfactory.

Effect of temperature on the diffusion coefficient is expressed by equation 31.[12]

$$D=A\times\exp\left[-\frac{E}{RT}\right] \tag{31}$$

Therefore Fig. 3 of Farah's report, which is reproduced on Fig. 5 of the present paper, is also explainable by the present model of SCRD. Logarithm of k value on Fig. 5 was plotted against reciprocal of temperature. E value of equation 31 calculated from the slope of the resulting straight line was around 5.3 kcal which is a reasonable value for the activation energy for diffusion.[12]

Farah also reported the effect of various substances on the rate of run out of PAH from the dog renal slices. This effect can be explained by the present model as follows. Farah's rate of run out, k, corresponds to $D\pi^2/l^2$ of our model, therefore effect of substances on the rate means effect on D for SCRD model. By Stokes–Einstein equation[12] D is expressed as a function of viscosity.

$$D=\frac{KT}{6\pi r\eta} \tag{32}$$

10) C.R. Ross and A. Farah, *J. Pharmacol. Exptl. Therap.*, **151**, 160 (1966).
11) W. Jost, "Diffusion in Solids, Liquids, Gases," Academic Press, New York, N.Y., 1960, p. 477.
12) S. Glasstone, K. Laidler, and H. Eyring, "The Theory of Rate Process," McGrow–Hill, New York, N.Y., 1941, p. 516

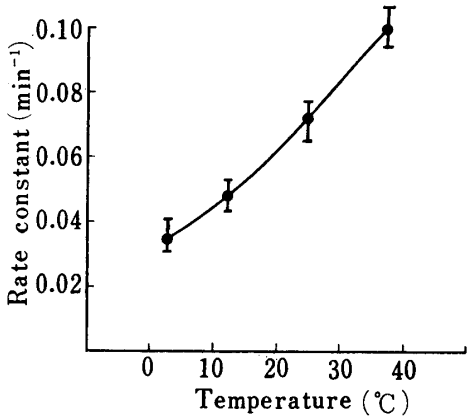

Fig. 5. The Effect of Temperature on the Rate Constant of PAH Run Out from Dog Renal Slices taken from Farah's Report

PAH loading done in phosphate buffer containing 0.6×10^{-3}M PAH for 90min. Run out rate was determined into phosphate buffer; gas phase 100% oxygen. Interval between slice transfer was 0.5 min. Each point represents the average of 5 to 7 determinations. Lines through each point represent twice the standard error.

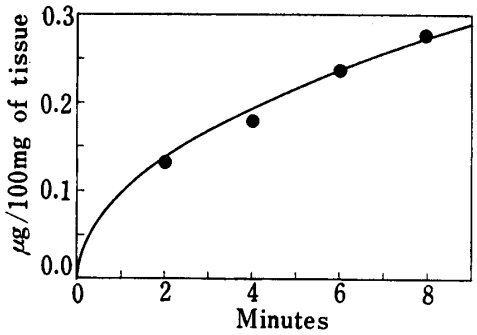

Fig. 6. Comparison of Theoretical Values and Experimental Data for PAH Uptake by Kidney Slices

Theoretical curve was drawn by equation 30, where $U\infty = 0.605$ μg/100 mg and $\frac{D\pi^2}{l^2} = 0.05$ min^{-1}. Experimental data were taken from Ross's report.[10]

abscissa: time in minutes; ordinate: PAH content

where K is Boltzman constant, T is absolute temperature, r is the radius of the diffusing particle and η is the viscosity of the medium. The change in viscosity of the intracellular fluid due to the added substances will cause the change in D and consequently in rate of run out. Furthermore, if D_X, D_{PAH}, and D_{PAHX} are not equal to each other,[4] addition of an accelerator or inhibitor of the enzyme which split the complex, causes the change of the relative amount of free and combined PAH and that will cause the change in run out rate. Because diffusion rate will be determined by D_{PAH} when the concentration of PAH is predominant, whereas diffusion will proceed by D_{PAHX} if PAHX is the main particle. Quantitative treatment of the experimental data from this point of view with the different D values is now in preparation.

Uptake Data

Uptake data of PAH by the kidney slice reported by Ross, *et al.*[10] are insufficient and estimation of D/l^2 is not easy as the case of run out. By choosing 0.05 min^{-1} for $D\pi^2/l^2$ which is consistent with the results of run out experiment and 0.605 μg/100 mg for U_∞, theoretical curve agrees with the experimental data. Comparison is made on Fig. 6, continuous curve is calculated by equation 30 and points are replotted from Ross's report.[10]

Conclusion

On the basis of results discussed above, SCRD model proposed for the renal transport of PAH agrees very well with the experimental data of PAH run out from or uptake by the kidney slices, and it was proved that the model is applicable to the kinetic investigation of PAH transport at renal tubule.