solvent was evaporated to leave reddish brown residue. Recrystallization from hot water gave colorless needles, mp 87—90°. Yield before purification was almost quantitative. After drying on phosphorus pentoxide *in vacuo* at 60° for 48 hr, melting point raised to 116—120°. Elementary analyses of the substance of the lower melting point was consistent with Ib with a half mole of H_2O. *Anal.* Calcd. for $C_{10}H_{13}O_3N \cdot \frac{1}{2}H_2O$: C, 58.82; H, 6.86. Found: C, 58.98; H, 7.07. Elementary analyses of the substance of the higher melting point corresponded to anhydrous Ib. *Anal.* Calcd. for $C_{10}H_{13}O_3N$: C, 61.54; H, 6.67; N, 7.18. Found: C, 61.16; H, 6.58; N, 7.27.

β-Hydroxybutyranilide (Ic)——Ic was obtained by the reduction of IIc with the same procedure described above. The crude product was obtained quantitatively. Recrystallization from benzene gave colorless needles, mp 109° (lit.[11] mp 119°; with 1 mole H_2O, mp 112—113°). According to the results of analyses, Ic thus obtained was identical with anhydride. *Anal.* Calcd. for $C_{10}H_{13}O_2N$: C, 67.04; H, 7.26; N, 7.82. Found: C, 66.76; H, 7.04; N, 7.82.

Ia was supplied by Takeda Chemical Industries, Ltd., mp 157—158° (lit.[10] mp 160°).

Thin-Layer Chromatography

Silica gel B-5 of Wako Pure Chemical Industries, Ltd. was spread about 250 μ thick and dried at 110° for 1.5 hr. Solvent systems and spraying reagent used are shown in Table I.

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Drug Absorption, Metabolism, and Excretion. IV.[1] Pharmacokinetic Studies on Renal Transport. (1). Simultaneous Chemical Reaction and Diffusion (SCRD) Model for Uphill Transport

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In 1964 an excretion study of sulfonamides from the human kidney was carried out by one of the authors of the present report, and it was observed that five among seventeen slufonamides were secreted to renal tubules besides the glomerular filtration.[3] Since the drug concentration in the renal tubular fluid is much greater than that in plasma, the renal tubular secretion of the drug must have been performed against the concentration gradient. This is uphill transport. Despopoulos[4] suggested that the tubular secretion of sulfonamides was carried out by the same process as *p*-aminohippuric acid (PAH) secretion.

As for PAH, the functional characteristics of the tubular transport process involved have been studied extensively, particularly with respect to the tubular excretion.[5]

According to Beyer,[6] the process of PAH secretion is as follows, Fig. 13 of his review being reproduced in Fig. 1 of this report.

1) Part III: J. Shibasaki, T. Koizumi, T. Tanaka, and M. Nakatomi, *Chem. Pharm. Bull.* (Tokyo), **16**, 2277 (1968).
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3) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 428 (1964).
4) A. Despopoulos, and P.X. Callahan, *Am. J. Physiol.*, **203**, 19 (1962).
5) I. Sperber, *Pharmacol. Rev.*, **11**, 109 (1959); J.V. Taggart, *Am. J. Med.*, **24**, 774 (1958).
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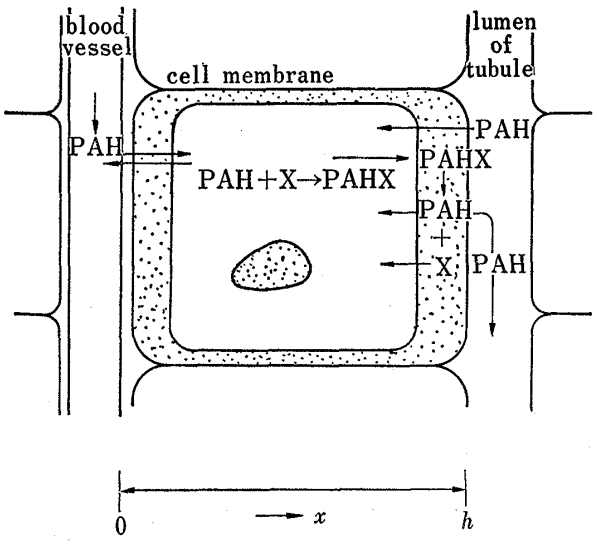

Fig. 1. A Diagrammatic Representation of the PAH Transport
Reproduced from Fig. 13 of (K.H. Beyer, *Pharmacol. Rev.*, 2, 227 (1950)).

PAH diffuses into the cell and the labile conjugate is formed within the cell (PAH→PAHX) through the expenditure of high phosphate bond energy, which permits the building up of a concentration of the intermediary metabolite within the cell that will enable it to diffuse toward the cell boundaries under its own gradient. Direction or orientation is given to the cellular secretory process by the concentration of an enzyme at the luminal border which is capable of splitting the conjugate thus releasing the original compound (PAH) within the interstices of the membrane or in close proximity thereto in sufficient concentration to permit the diffusion of the agent into the surrounding medium.

The present report describes the kinetic and the quantitative interpretation of the above scheme. Requirement for the uphill transport is also discussed.

Theory

The simultaneous chemical reaction and diffusion (SCRD) method[7] was employed for the mathematical interpretation of Beyer's model for PAH transport. Fig. 2 describes qualitatively the steady state situation of the model.

Assumptions employed for mathematical development are; (1) The rate of conjugate formation and that of breaking are expressed by Eq. 1 and 2.

$$\mathrm{PHA} + \mathrm{X} \xrightarrow{v_f} \mathrm{PAHX}$$

$$\mathrm{PAHX} \xrightarrow{v_b} \mathrm{PAH} + \mathrm{X}$$

$$v_f = k_f \cdot (\mathrm{PAH}) \cdot (\mathrm{X}) \qquad (1)$$

$$v_b = k_b \cdot (\mathrm{PAHX}) \qquad (2)$$

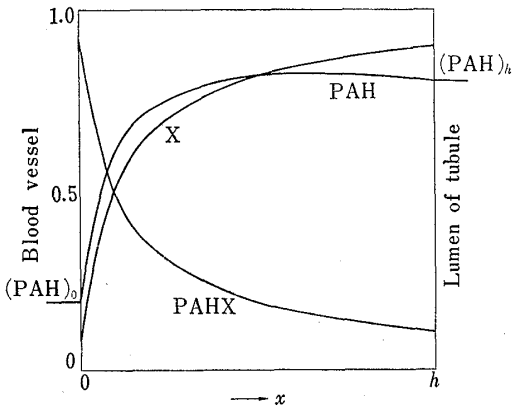

Fig. 2. Concentration Profile during the Steady State Uphill Transport of PAH (See text)

The apparent rate constant for conjugate breaking, k_b, depends on the concentration of the enzyme which is capable of splitting the conjugate. Therefore, if distribution of the enzyme is not uniform, its concentration is a function of position coordinate (x). Consequently the dynamic equilibrium constant, K, is also a function of x.

$$K = \frac{k_f}{k_b} = \frac{(\mathrm{PAHX})}{(\mathrm{PAH})(\mathrm{X})} = f(x) \qquad (3)$$

(2) Carrier, X, and conjugate, PAHX, are unable to cross the bounderies. These species exist only within the cell, or in the membrane. PAH, however, is freely diffusable into and out of the cell.

7) W.I. Higuchi, E.L. Parrott, D.E. Wurster, and T. Higuchi, *J. Am. Pharm. Assoc.*, **47**, 376 (1958); W.I. Higuchi, E. Nelson, and J.G. Wagner, *J. Pharm. Sci.*, **53**, 333 (1964).

(3) Each species PAH, PAHX and X diffuse individually according to their own concentration gradient.

(4) At $x=0$, $(\mathrm{PAH})=(\mathrm{PAH})_0$, $(\mathrm{PAHX})=(\mathrm{PAHX})_0$, and $(\mathrm{X})=(\mathrm{X})_0$. At $x=h$, $(\mathrm{PAH})=(\mathrm{PAH})_h$, $(\mathrm{PAHX})=(\mathrm{PAHX})_h$ and $(\mathrm{X})=(\mathrm{X})_h$.

For the steady state case, the application of Fick's law[8] leads to the following set of equations:

$$\left.\begin{aligned}\frac{d(\mathrm{PAH})}{dt}&=D_{\mathrm{PAH}}\frac{d^2(\mathrm{PAH})}{dx^2}+\phi_1=0\\ \frac{d(\mathrm{PAHX})}{dt}&=D_{\mathrm{PAHX}}\frac{d^2(\mathrm{PAHX})}{dx^2}+\phi_2=0\\ \frac{d(\mathrm{X})}{dt}&=D_{\mathrm{X}}\frac{d^2(\mathrm{X})}{dx^2}+\phi_3=0\end{aligned}\right\} \quad (4)$$

D_s is the diffusion coefficient of species s in the system. The ϕ's are the source or sink functions.

For proper material balance:

$$\phi_1=-\phi_2=\phi_3 \quad (5)$$

Therefore,

$$\left.\begin{aligned}D_{\mathrm{PAH}}\frac{d^2(\mathrm{PAH})}{dx^2}+D_{\mathrm{PAHX}}\frac{d^2(\mathrm{PAHX})}{dx^2}&=0\\ D_{\mathrm{PAHX}}\frac{d^2(\mathrm{PAHX})}{dx^2}+D_{\mathrm{X}}\frac{d^2(\mathrm{X})}{dx^2}&=0\end{aligned}\right\} \quad (6)$$

Eq. 5 means that for a given amount of reaction of PAH and X in any volume element, equivalent amount of PAHX is produced.

The solution to Eq. 6 obtained by integration are

$$D_{\mathrm{PAH}}\frac{d(\mathrm{PAH})}{dx}+D_{\mathrm{PAHX}}\frac{d(\mathrm{PAHX})}{dx}=C_1 \quad (7)$$

$$D_{\mathrm{PAHX}}\frac{d(\mathrm{PAHX})}{dx}+D_{\mathrm{X}}\frac{d(\mathrm{X})}{dx}=C_2 \quad (8)$$

where the C's are the integration constants.

The boundary conditions at $x=0$ and $x=h$ are, by the assumption (2) described above;

$$D_{\mathrm{PAHX}}\frac{d(\mathrm{PAHX})}{dx}+D_{\mathrm{X}}\frac{d(\mathrm{X})}{dx}=0 \quad (9)$$

Therefore $C_2=0$.

The steady state net transport rate of PAH from plasma to tubular fluid, or that for the direction of the coordinate, R, is,

$$R=-\left[\frac{d}{dx}\{D_{\mathrm{PAH}}\cdot(\mathrm{PAH})+D_{\mathrm{PAHX}}\cdot(\mathrm{PAHX})\}\right]_{x=0 \text{ or } x=h} \quad (10)$$

Therefore $C_1=-R$.

Now the integration of Eq. 7 and 8 with $C_1=-R$ and $C_2=0$ yields,

$$D_{\mathrm{PAH}}\cdot(\mathrm{PAH})+D_{\mathrm{PAHX}}\cdot(\mathrm{PAHX})=-R\cdot x+L_1 \quad (11)$$

$$D_{\mathrm{PAHX}}\cdot(\mathrm{PAHX})+D_{\mathrm{X}}(\mathrm{X})=L_2 \quad (12)$$

where L_1 and L_2 are integration constants.

8) W. Jost, "Diffusion," Academic Press Inc., New York, 1960, p. 3.

By applying the assumption (4), L_2 is evaluated:

$$L_2=D_{\mathrm{PAHX}}\cdot(\mathrm{PAHX})_0+D_{\mathrm{X}}(\mathrm{X})_0=D_{\mathrm{PAHX}}(\mathrm{PAHX})_h+D_{\mathrm{X}}(\mathrm{X})_h \tag{13}$$

Combining Eq. 12 with Eq. 3, and solving for (PAHX), one obtains

$$(\mathrm{PAHX})=\frac{L_2\cdot K\cdot(\mathrm{PAH})}{D_{\mathrm{X}}+D_{\mathrm{PAHX}}\cdot K\cdot(\mathrm{PAH})} \tag{14}$$

which when combined with Eq. 11 at $x=0$ yields the expression for L_1. Now taking this expression for L_1 and solving for R employing Eq. 11 at $x=h$ results in the final expression.

$$R=\frac{1}{h}\left[D_{\mathrm{PAH}}\{(\mathrm{PAH})_0-(\mathrm{PAH})_h\}+D_{\mathrm{X}}D_{\mathrm{PAHX}}\cdot L_2\cdot\frac{K_0\cdot(\mathrm{PAH})_0-K_h(\mathrm{PAH})_h}{\{D_{\mathrm{X}}+D_{\mathrm{PAHX}}\cdot K_h\cdot(\mathrm{PAH})_h\}\{D_{\mathrm{X}}+D_{\mathrm{PAHX}}\cdot K_0(\mathrm{PAH})_0\}}\right] \tag{15}$$

Discussion

Eq. 15 is the mathematical expression of PAH secretion rate, according to the model proposed by Beyer.

Mass transfer occurs for the direction of coordinate, or PAH is secreted from plasma to tubular fluid, when R has positive value, and vice versa.

When equilibrium constant, K, is independent of position, or at least $K_0=K_h$, Eq. 15 is,

$$R=D_{\mathrm{eff}}\frac{(\mathrm{PAH})_0-(\mathrm{PAH})_h}{h} \tag{16}$$

Eq. 16 is a well known expression for simple diffusion, of which the effective diffusion coefficient is

$$D_{\mathrm{eff}}=D_{\mathrm{PAH}}+\frac{D_{\mathrm{PAHX}}\cdot D_{\mathrm{X}}\cdot L_2\cdot K}{\{D_{\mathrm{X}}+D_{\mathrm{PAHX}}\cdot(\mathrm{PAH})_h\cdot K\}\{D_{\mathrm{X}}+D_{\mathrm{PAHX}}\cdot(\mathrm{PAH})_0\cdot K\}}$$

Uphill transport never occurs by simple diffusion, because R is positive only when $(\mathrm{PAH})_0$ is greater than $(\mathrm{PAH})_h$. When K_0 is greater than K_h, however, R could be positive even if $(\mathrm{PAH})_0$ is less than $(\mathrm{PAH})_h$ by Eq. 15, and uphill diffusion could occur depending on the relative values of $(\mathrm{PAH})_0$ and $(\mathrm{PAH})_h$.

For example, when $(\mathrm{PAH})_0=0.193622$, $(\mathrm{PAH})_h=0.800224$, $K_0=50.00$, $K_h=0.13850$, $D_{\mathrm{PAH}}=D_{\mathrm{PAHX}}=D_{\mathrm{X}}=1$, $h=1$ and $L_2=1$, then L_1 and R are calculated to be 1.1 and 0.2 respectively. Since R has positive value, uphill tarnsport does occur. Steady state concentration distribution of each species were calculated and are shown in Fig. 2.

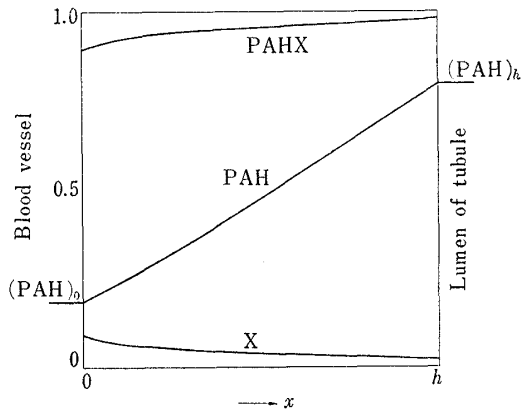

Fig. 3. Concentration Profile during the Steady State Transport of PAH (See text)

On the other hand, when $(\mathrm{PAH})_0=0.193622$, $(\mathrm{PAH})_h=0.800224$, $K_0=K_h=50.00$, $D_{\mathrm{PAH}}=D_{\mathrm{PAHX}}=D_{\mathrm{X}}=1$, $h=1$ and $L_2=1$, then L_1 and R are 1.1 and -0.67584 respectively. In this situation transport is from tubular fluid to plasma and down hill, because R has negative value. Concentration distributions of each species are shown in Fig. 3.

Various models have been proposed for the explanation of active transport mechanism from

biological and physiological point of view.[9] Present report is another proposal for uphill transport from the physicochemical stand point.

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Nuclear Magnetic Resonance Studies on Schiff Base and Related Compounds derived from Pyridoxal

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Biologically important reactions of amino acids such as transamination, racemization and so forth are catalyzed by enzymes containing pyridoxal. These reactions were found to be carried out nonenzymatically with metal ions as catalyst by Metzler, Ikawa and Snell[2] and were confirmed by many authors.[3] The first step of both enzymatic and nonenzymatic process is supposed to be reaction of amino group of amino acid and aldehyde of pyridoxal to form Schiff base.

In the former work,[4] reactions of pyridoxal with various amino acids and amines in methanol were studied by means of electronic absorption spectra. Methanol was chosen as a solvent, because Schiff base and other products were fairly stable in this solvent. Most amino acids and amines formed Schiff base as ultimate products. However, some amino acids or amines yielded products other than Schiff base, such as carbinolamine and substances having a tetrahydropyridoimidazole or a thiazolidine ring. Though biological significance of these compounds is open to future studies, some metal ion catalyzed reactions involving these compounds have been reported.[5]

In the present study, four compounds were prepared by conventional methods as examples of the products from pyridoxal and amino acids or amines; *i.e.* Schiff base of glycine (potassium pyridoxylideneglycinate), carbinolamine from sarcosine (potassium N-(3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridylhydroxymethyl) sarcosinate), cyclic product from histidine (potassium 4-(3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridyl)-4,5,6,7-tetrahydropyrido[3,4-*c*] imidazole-6-carboxylate) and thiazolidine derivative from cysteamine (2-(3-hydroxy-5-hydroxymethyl-2-methyl-4-pyridyl)thiazolidine). These four compounds as well as their component substances were studied by nuclear magnetic resonance (NMR) spectroscopy, to obtain further supports for the structures established by electronic absorption spectroscopy. Most NMR studies were carried out in tetradeuteromethanol (CD_3OD) solutions, but trifluoroacetic acid (TFA) and hexadeuterodimethylsulfoxide (DMSO-d_6) were also used as solvents.

1) Location: *Anagawa, Chiba,* Present address: *a) Faculty of Pharmaceutical Sciences, Kyushu Univer-sity, Katakasu, Fukuoka; b) Faculty of Pharmaceutical Sciences, University of Chiba, Yayoi, Chiba.*
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