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62. Tamotsu Koizumi, Takaichi Arita, and Kiichiro Kakemi : Absorption and Excretion of Drugs. XXI.*1 Some Pharmacokinetic Aspects of Absorption and Excretion of Sulfonamides. (3). Excretion from the Kidney.

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Previous reports[1] of this series demonstrated that the blood level-time course of orally administered drug was related to the balance between gastrointestinal absorption and urinary excretion.

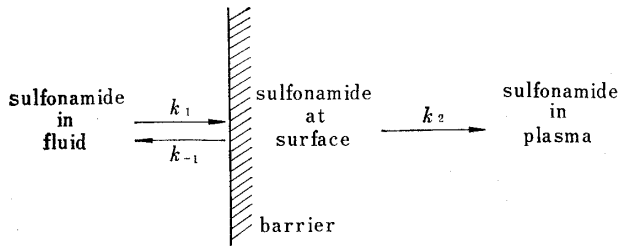

Fig. 1. Model proposed Concerning Absorption of Sulfonamide

On the absorption, transport process of unionized sulfonamides shown in Fig. 1 was proposed and which was supported by the data obtained from the experiments carried out with rat stomach[2] and small intestine.[3]

Now it should be considered the mechanism of excretion of sulfonamides, and this is also important from the view point of prolongation of the drug action. Nelson[4] reported on kinetic treatment of urinary excretion of sulfisoxazole and sulfamethizole, the model proposed consisted of two competitive first order process, respectively for acetylation and excretion of unchanged drug and a two step consecutive first order process, the first step of which was the formation of acetylated drug and the second the urinary excretion of this material.

Present report describes the kinetic mechanism of the excretion of passively transported sulfonamides from the kidney, and active transport is also discussed.

Beyer[5] described the functional characteristics of renal transport that essentially three processes are involved in the formation of urine by the kidney. They are 1) glomerular filtration, 2) tubular secretion, and 3) tubular reabsorption, both active and passive.

And according to Despopoulos,[6] most of the sulfonamides experimented in this series exclude the renal active transport.

The composition of the fluid as it passes from the blood stream at the glomerular capsule and is presented to the most proximal cells of the tubule is ordinarily the same as that of the plasma from which it was filtered, except for the absence of most of proteins and protein bound drugs.[5] And Koch[7] described that as the glomerular filtrate enters the proximal tubule, transtubular transport of solute and water begins. As a result of the active transport of Na^+, both Cl^- and HCO_3^- are reabsorbed. The proximal tubule is freely permeable to water, and, as solute is reabsorbed, water is reabsorbed

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*1 Part XX : This Bulletin, **12**, 421 (1964).

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1) K. Kakemi, T. Arita, T. Koizumi : Yakugaku Zasshi, **81**, 172 (1961).
2) *Idem* : This Bulletin, **12**, 413 (1964).
3) *Idem* : *Ibid.*, **12**, 421 (1964).
4) E. Nelson : J. Pharmacol. Exptl. Therap., **129**, 368 (1960).
5) K. Beyer : Pharmacol. Revs., **2**, 227 (1950).
6) A. Despopoulos, P. Callahan : Am. J. Physiol., **203**, 19 (1962).
7) A. Koch : "Medical Physiology and Biophysics" T.C. Ruch and J.F. Fulton. Philadelphia and London : W.B. Saunders Co., 1960, Chapter 39 The Kidney, page 844.

at an equivalent rate. Sulfonamide, however, whose molecule is not so small as Na^+ or H_2O, moves more slowly than water and hence concentration gradient occures between tubular fluid and plasma, and this concentration gradient works as the driving force for passive reabsorption of the drug.

Thus the excretion of sulfonamides can be systematized that unbound drug in blood stream is filtered at glomeruly, reabsorbed passively at tubule, and the rest is excreted in urine. These processes are shown in Fig. 2.

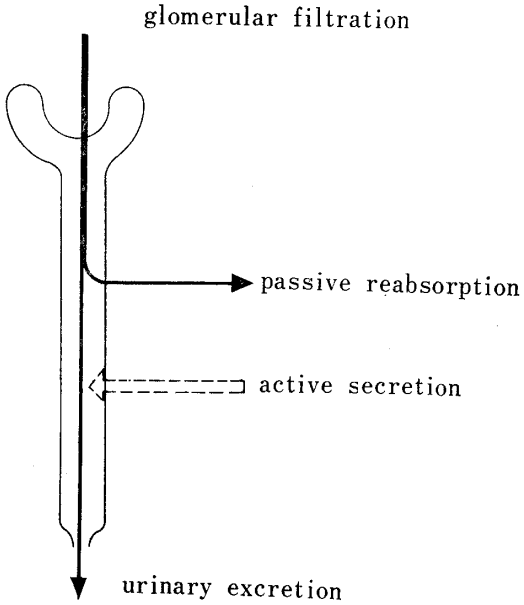

Fig. 2. Excretion System of Sulfonamides

For the convenience of consideration and kinetic treatment of the process, following are assumed : a) glomerular filtration rate is constant during the experiment, b) rate of water absorption at proximal tubule is constant and large enough compared with that of sulfonamide, c) volume of fluid in tubule where the drug is passively reabsorbed is constant, d) reabsorption of the drug is first order process, and e) fraction of protein bound drug is constant within the extent of blood level experimented.*3

In following equations there will be used the terms (units in parentheses) : t, time after zero time (hr.); V, volume of whole blood (ml.); v, volume of fluid in tubule (ml.); k_f, glomerular filtration rate (ml./hr.); k_w, rate of water reabsorption at the proximal tubule (ml./hr.); D, amount of unchanged drug free from protein in the body (mg.); S, amount of unchanged sulfonamide in the body (mg.); Se, amount of unchanged sulfonamide excreted in urine (mg.); f_p, fraction of protein bound drug; C_p, plasma concentration of the unchanged drug free from protein (mg./ml.); C_t, drug concentration in tubular fluid (mg. /ml.); Kr, reabsorption rate constant (hr^{-1}); K_3, excretion rate constant of unchanged drug described in Nelson's equation[4] (hr^{-1}).

From assumption a) and b), equation (1) can be described.

$$C_t=\frac{k_f}{k_f-k_w}C_p \qquad (1)$$

From the assumption d), equation (2) is described.

$$\frac{dC_t}{dt}=Kr(C_t-C_p) \qquad (2)$$

And from the assumption e), equation (3) is described.

$$D=(1-f_p)S \qquad (3)$$

Amount of unchanged drug filtered during time dt is given as equation (4).

$$d(\text{filtered drug})=D\frac{k_f}{V}dt \qquad (4)$$

Amount of drug reabsorbed at tubule during time dt is given as equation (5), considering equation (1) and (2).

*3 Protein binding of sulfonamides follows Klotz's equation,[8] and the fraction of protein bound drug depends on the drug concentration. But in the extent of blood level after oral administration of 1 g. of the drug the fraction of bound drug can be considered constant within the magnitude of experimental error.

8) I.M. Klotz, F.M. Walker : J. Am. Chem. Soc., **70**, 943 (1948).

$$vdC_t=\frac{k_w}{k_f-k_w}\frac{v}{V}KrDdt \tag{5}$$

Consequently amount of drug excreted into urine during time dt is given as equation (6).

$$dSe=D\frac{k_f}{V}dt-\frac{k_w}{k_f-k_w}\frac{v}{V}KrDdt$$
$$=\left(\frac{k_f}{V}-\frac{k_w}{k_f-k_w}\frac{v}{V}Kr\right)Ddt \tag{6}$$

k_f, k_w, v, and V are independent of the kind of the drugs, so that they are included in a constant a, and D is substituted with $(1-f_p)S$, then equation (6) gives equation (7).

$$dSe=\left(\frac{k_f}{V}-aKr\right)(1-f_p)Sdt \tag{7}$$

Nelson[4] presented the equation (8) on the excretion of sulfisoxazole.

$$dSe=K_3Sdt \tag{8}$$

From equation (7) and (8), equation (9) is obtained.

$$aKr=\frac{k_f}{V}-\frac{K_3}{(1-f_p)} \tag{9}$$

The right side of equation (9) is directly proportional to reabsorption rate.

Experimental and Results

Determination of K_3——Adult men in apparent good health ingested 1 g. of finely powdered drug with 100 ml. of H_2O. And K_3 was determined by Nelson's method.[4] Results are shown in Table I.

Table I. Nelson's Excretion Rates of Sulfonamides and Results from S/M Ratio Experiments

	Sulfonamide	K_3	f_p	$\frac{K_3}{1-f_p}$	$\left(\frac{k_f}{V}-\frac{K_3}{1-f_p}\right)=aKr$	$aKr\sqrt{M}$	Active transport	$P_{CHCl_3}^{pH6\ 37°}$
1	Sulfanilamide	0.0317	0.060	0.0337	0.751	9.84	—[a]	0.04
2	Sulfanilacetamide	0.0358	0.185	0.0439	0.741	10.8	±[a]	0.04
3	Sulfaguanidine	0.0763	0.124	0.0871	0.698	10.6	—	0.01
4	Sulfapyridine	0.0113	0.267	0.0154	0.770	12.1	—[a]	1.06
5	Sulfadiazine	0.0503	0.318	0.0738	0.711	11.2	—[a]	0.89
6	Sulfamethoxazole	0.0406	0.587	0.0983	0.687	10.9	—	1.43
7	Sulfathiazole	0.0900	0.643	0.252	0.533	8.53	—[a]	0.14
8	Sulfamerazine	0.0126	0.766	0.0538	0.731	11.8	—[a]	2.14
9	Sulfisoxazole	0.0776	0.888	0.693	0.092	1.50	+[a]	0.44
10	Sulfamethizole	0.399	0.856	2.77	—	—	+[a]	0.20
11	Sulfisomidine	0.0824	0.894	0.777	0.008	0.13	±	0.27
12	Sulfamethazine	0.0061	0.746	0.0240	0.761	12.7	—[a]	5.26
13	Sulfamethoxypyridazine	0.0068	0.884	0.0586	0.726	12.1	—[a]	3.76
14	Sulfamonomethoxine	0.0118	0.879	0.0975	0.689	11.5	—	1.90
15	Sulfaethidole	0.0802	0.981	4.22	—	—	+	0.84
16	Sulfadimethoxine	0.0145	0.989	1.32	—	—	—	26.0
17	Sulfaphenazole	0.0231	0.905	0.243	0.542	9.59	—	19.2
18	Sodium thiosulfate	0.785	0	—	—	—	—	—

a) Results by A. Despopoulos[6]

Determination of k_f/V——It is evident from equation (9) that filtration rate k_f/V is nothing but K_3 divided by $(1-f_p)$ when Kr is zero. According to Gilman, Philips and Koelle,[9] $Na_2S_2O_3 \cdot 5H_2O$ is not reabsorbed at all at tubule, and glomerular filtration rate was determined using thiosulfate.

After intravenous injection of 2 g. of $Na_2S_2O_3 \cdot 5H_2O$, urine was collected periodically, and amount of excreted thiosulfate was determined.

Table II. Data of Thiosulfate Excretion used for Calculation of k_f/V

Time (hr.)	Excrtd. (mg.)	Accmld. (mg.)	Residue (mg.)
0.5	310.47	310.47	115.46
1	77.58	388.05	37.88
1.5	21.92	409.97	15.96
2	10.14	420.11	5.82
3	5.34	425.45	0.48
4	0.48	425.93	0.0
5	0.0	—	—

administered $Na_2S_2O_3$ 1250 mg.

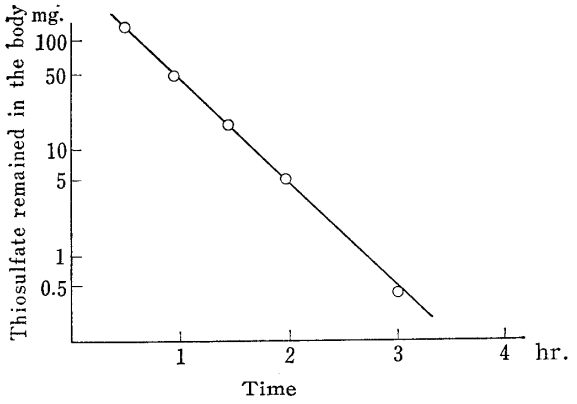

Fig. 3. Excretion Rate of Thiosulfate

Results are shown in Table II. Data were treated following Nelson's method of calculating K_3. Procedure of treatment is shown below. Logarithm of the fourth column of Table II were plotted against time and Fig. 3 was obtained. The slope of the obtained straight line was 1.0. Fraction of recovered thiosulfate, f, was 0.341 as shown in equation (10).

$$f = \frac{\text{Collected thiosulfate in urine}}{\text{Administered thiosulfate}} = \frac{425.93\ \text{mg.}}{1250.00\ \text{mg.}} = 0.341 \qquad (10)$$

Slope of the straight line in Fig. 3 was multiplied with 2.303 and with f, and the product gave k_f/V, as f_p of thiosulfate is zero.

$$\frac{k_f}{V} = 1.0 \times 2.303 \times 0.341 = 0.785\ (\text{hr}^{-1}) \qquad (11)$$

Determination of f_p——One ml. of sulfonamide solution was mixed with 4 ml. of blood plasma and ultrafiltrated for 1 hr. in Visking tube by 4000 r.p.m., concentration of sulfonamide in filtrate was called α (mg./ml.), another 1 ml. of sulfonamide solution was mixed with 4 ml. of phosphate buffer solution (pH 7.4), instead of plasma, and treated as above, concentration of sulfonamide in the filtrate was called β (mg./ml.). Then $\beta-\alpha$ is amount of the drug bound by proteins in 1 ml. of plasma, and α is amount of unbound drug in 1 ml. of plasma.

Since protein binding of sulfonamide follows Klotz's equation (12), reciprocal of $\beta-\alpha$ was plotted against reciprocal of α, and K and ν were calculated from straight line obtained. Then f_p is expressed as equation (13). Calculated f_p's are shown in Table I.

$$\frac{1}{\beta-\alpha} = \frac{1}{K\nu\alpha} + \frac{1}{\nu} \qquad (12)$$

$$f_p = \left(\frac{\beta-\alpha}{\beta}\right)_{\max} = \lim_{\beta \to 0} \frac{\beta-\alpha}{\beta} = \frac{K\nu}{1+K\nu} \qquad (13)$$

Analytical Method——Sulfonamides and acetyl sulfonamides : Urine was diluted properly and treated as reported previously.[2] Thiosulfate : Gilman's method was followed.[9]

9) A. Gilman, F. S. Philips, E. S. Koelle : Am. J. Physiol., **146**, 348 (1946).

Kidney Slice Experiments——Kidney cortical slices were prepared and treated following the method of previous reports.[6,10,11]

Accumulation of sulfonamides to guinea pig kidney slice was experimented, and S/M ratio was measured which indicates the presence and absence of active transport. Results are shown in Table I.

Discussion

$\{k_f/V-K_3/(1-f_p)\}$ was multiplied with $\sqrt{M}$ in order to compensate the diffusion process,*4 and plotted against partition coefficient between $CHCl_3$ and H_2O determined at 37° and pH 6 which is average pH of urine experimented. And Fig. 4 was obtained. In the figure solid circles represent presence of active transport, and open circles absence of active transport, measured by S/M ratio experiments.

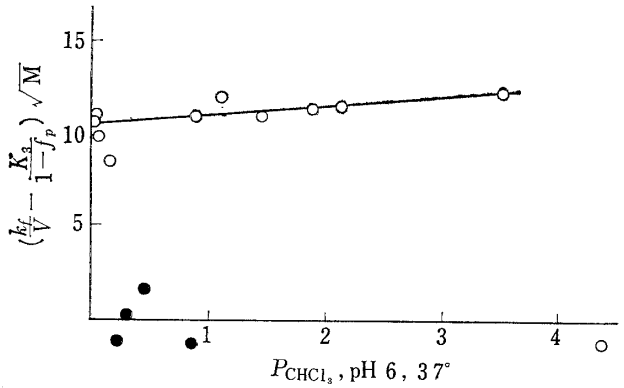

Fig. 4. Extent of Reabsorption Rate *vs.* Partition Coefficient Plot

Solid circle represents active transport measured by S/M ratio experiments, and open circle represents absence of active transport.

Examination of Fig. 4 shows that spots are on a flat line indicating that the model proposed in previous paper[2,3] concerning about absorption of sulfonamide from rat stomach and small intestine is also available for tubular reabsorption, as far as passive transport is concerned. Almost flat line shows that in passive tubular reabsorption, partition process is fast enough and diffusion process is rate limitting.

With sulfonamides actively secreted, solid circle dropped far below the line of passive reabsorption, indicating slow reabsorption rate or fast excretion rate.

Sulfadimethoxine with which active transport is negative from the results of S/M ratio measurement, shows too slow reabsorption rate. This is due to high glucuronidation rate of this drug. It is reported that 60 to 70% of administered sulfadimethoxine was excreted as glucuronide during 24 hours.[12] And glucuronides are reported to be accomplished by active tubular transport.[13]

Summary

Kinetic mechanism of excretion of passively transported sulfonamide from kidney is described.

Urinary excretion rate of the drug was determined after oral administration to man. Some of the data obtained fit the mechanism proposed. And drugs which did not fit the mechanism were revealed to participate in the active transport.

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*4 If the mechanism proposed for the absorption of sulfonamide from rat stomach is also available for the tubular reabsorption, equation (14) will be obtained;

$$\sqrt{M}\left(\frac{k_f}{V}-\frac{K_3}{1-f_p}\right)=\frac{abP}{1+aP} \qquad (14)$$

where M is molecular weight of the drug, P partition coefficient and a and b are constants.

10) R. J. Cross, J. V. Taggart: Am. J. Physiol., **161**, 181 (1950).
11) A. Despopoulos: *Ibid.*, **184**, 396 (1956).
12) S. Okamoto: Saishin Igaku, **15**, 1882 (1960).
13) L. Peters: Pharmacol. Revs., **12**, 1 (1960).