# NOTE

## Comparison between the Rate Constants for Acetylation of Sulfonamides *in Vivo* and O/W Partition Coefficient

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It has been shown in recently reported work that the kinetics of the disposition of at least three sulfonamides by humans in the post-absorptive and post-equilibrative phases of drug distribution can be described within experimental error by a model that consists of two competitive apparent first-order processes for elimination of unchanged drug, one of which is acetylation and the other elimination of free drug by the kidney.[1-3] The acetylated drug is removed by the kidney by another apparent first-order process. This communication reports kinetic studies on the disposition of two other sulfonamides, sulfaethylthiadiazole [$N^1$-(5-ethyl-1,3,4-thiadiazol-2-yl)-sulfanilamide] and sulfisomidine [$N^1$-(2,6-dimethyl-4-pyrimidyl)-sulfanilamide] in normal humans. Also reported are the results of determinations made to attempt to correlate the rate constants for acetylation to heptane-aqueous phase at pH 7.4 or chloroform-aqueous phase at pH 7.4 partition coefficients.

### Experimental

Finely powdered drug in 0.5-g. doses was orally ingested in water suspension by normal adult human test subjects under the same conditions as used in other work.[1-3] Other test conditions and urine analyses for drug were also essentially the same as previously reported.[1-3]

Partition coefficients were determined by two independent measurements at 24–26° by allowing the aqueous phases containing the sulfonamides in concentrations of 10 to 120 mg./100 ml. to equilibrate with equal volumes of the organic phase. Both phases were assayed by the Bratton and Marshall procedure.[4] In the case of the organic phase, the solvent was removed by gentle heating and the residue redissolved in water for assay. Prior to assay, the pH of the aqueous phase was measured using a Beckman Model G pH meter freshly standardized

(1) E. Nelson and I. O'Reilly, *J. Pharmacol. Exptl. Therap.*, **129**, 368 (1960).
(2) E. Nelson and I. O'Reilly, *J. Pharm. Sci.*, **50**, 417 (1961).
(3) E. Nelson, *ibid.*, in press.
(4) The assistance of Mr. Gerald Reichelderfer is gratefully acknowledged.

at pH 7.0. The partition coefficients determined were corrected to represent this quantity for a system with only the undissociated acid in the aqueous phase, that is, the intrinsic partition coefficient $K^*$, by equation (1).[5]

$$K^* = K(1 + 10^{pH-pK_a}) \quad (1)$$

where $K$ was the coefficient measured. The same equation was rearranged to calculate $K$ at pH 7.4.

## Results and Discussion

Cumulative free and acetylated sulfonamide excretion is listed in Table I. The partition coefficients are given in Table II. Excretion of unchanged drug was examined for ability to be described by equation (2).[1]

$$Se = Se^0 f(1 - \exp[-kt]) \quad (2)$$

where $Se$ is the amount of unchanged sulfonamide excreted at time $t$ after time zero, $Se^0$ is the amount of sulfonamide in the body at zero time, $f$ the fraction of a dose excreted as unchanged material, $t$ is time in hr. and $k$ is the apparent first order rate constant for removal of sulfonamide from the body and equal to $k_1 + k_3$, where $k_1$ and $k_3$ are, respectively, the apparent first order rate constants for acetylation and excretion of free drug.

Experimentally observed excretion and theoretically predicted excretion as described by equation (2) were in good agreement and this agreement was similar to that found in past work with other sulfonamides.[1-3] Since only very small amounts of acetylated drug were formed and excreted, it was not deemed practical to determine the rate constant for excretion of acetylated drug as has been done in the past.[1-3] The values of the various constants in equation (2) are listed in Table III for both sulfonamides studied.

TABLE I

CUMULATIVE MG. FREE (F) AND ACETYLATED (A) SULFONAMIDES AND ML. URINE EXCRETED BY TEST SUBJECTS IN VARIOUS TIMES IN HOURS FOLLOWING INGESTION OF 0.5-G. DOSES IN WATER SUSPENSIONS[a]

| | Sulfisomidine, mg. excreted at time in hours | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | | 4 | | 6 | | 8 | | 12 | | 24 | | 36 | | 48 | |
| Subject | F | A | F | A | F | A | F | A | F | A | F | A | F | A | F | A |
| C | 78 | 3 | 179 | 7 | 248 | 13 | 296 | 15 | 367 | 23 | 435 | 29 | 453 | 32 | 454 | 33 |
| P | 70 | 2 | 155 | 5 | 216 | 11 | 255 | 12 | 331 | 16 | 402 | 26 | 429 | 30 | 432 | 30 |
| B | 62 | 1 | 153 | 4 | 222 | 6 | 264 | 9 | 330 | 13 | 412 | 28 | 432 | 38 | 437 | 40 |
| Mean | 70 | 2 | 162 | 5 | 229 | 10 | 272 | 12 | 343 | 17 | 416 | 28 | 438 | 33 | 441 | 34 |

(5) L. C. Craig, *J. Biol. Chem.*, **150**, 33 (1943).

TABLE I (*Continued*)

Urine, ml., at time in hours

| Subject | 2 | 4 | 6 | 8 | 12 | 24 | 36 | 48 |
|---|---|---|---|---|---|---|---|---|
| C | 510 | 840 | 1030 | 1390 | 1820 | 2310 | 2790 | 3390 |
| P | 140 | 270 | 430 | 780 | 1600 | 1800 | 2260 | 3660 |
| B | 50 | 250 | 470 | 640 | 810 | 1650 | 2130 | 3100 |
| Mean | 230 | 450 | 640 | 940 | 1410 | 1920 | 2390 | 3380 |

Sulfaethylthiadiazole, mg. excreted at time in hours

| | 2 | | 4 | | 6 | | 8 | | 10 | | 12 | | 24 | | 60 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject | F | A | F | A | F | A | F | A | F | A | F | A | F | A | F | A |
| C | 27 | 0 | 97 | 0 | 168 | 0 | 227 | 0 | 279 | 1 | 321 | 2 | 422 | 3 | 514 | 6 |
| P | 42 | 1 | 153 | 3 | 211 | 6 | 262 | 7 | 297 | 9 | 321 | 11 | 392 | 14 | 515 | 22 |
| E | 38 | 1 | 66 | 1 | 118 | 2 | 154 | 3 | 206 | 6 | 235 | 9 | 355 | 11 | 474 | 15 |
| Mean | 36 | 1 | 105 | 1 | 166 | 3 | 214 | 3 | 261 | 5 | 292 | 7 | 390 | 9 | 501 | 14 |

Urine, ml., at time in hours

| Subject | 2 | 4 | 6 | 8 | 10 | 12 | 24 | 60 |
|---|---|---|---|---|---|---|---|---|
| C | 390 | 680 | 860 | 970 | 1080 | 1180 | 1650 | 4180 |
| P | 500 | 1040 | 1450 | 2030 | 2350 | 2500 | 2840 | 5220 |
| E | 250 | 440 | 570 | 650 | 1830 | 2070 | 3640 | 7950 |
| Mean | 380 | 720 | 960 | 1220 | 1750 | 1920 | 2710 | 5780 |

[a] Excretion of acetylated drug given in terms of unchanged drug.

TABLE II

O/W PARTITION COEFFICIENTS AND RATE CONSTANTS FOR ACETYLATION OF SEVERAL SULFONAMIDES[1]

| | Rate constant hr.$^{-1}$ | Heptane/aqueous | | Chloroform/aqueous | |
|---|---|---|---|---|---|
| | | Intrinsic ($K^*$) | At pH 7.4 ($K$) | Intrinsic ($K^*$) | At pH 7.4 ($K$) |
| Sulfathiazole | 0.0302 | $2.48 \times 10^{-5}$ | $2.07 \times 10^{-5}$ | $1.83 \times 10^{-1}$ | $1.52 \times 10^{-1}$ |
| Sulfisoxazole | 0.0233 | $2.71 \times 10^{-4}$ | $2.36 \times 10^{-5}$ | 1.18 | $1.03 \times 10^{-1}$ |
| Sulfamethylthiadiazole | 0.0458 | $1.49 \times 10^{-4}$ | $1.54 \times 10^{-5}$ | $3.68 \times 10^{-1}$ | $3.81 \times 10^{-2}$ |
| Sulfaethylthiadiazole | 0.0020 | $2.87 \times 10^{-3}$ | $2.59 \times 10^{-5}$ | 1.23 | $1.11 \times 10^{-1}$ |
| Sulfisomidine | 0.0097 | $1.42 \times 10^{-4}$ | $7.75 \times 10^{-5}$ | $3.02 \times 10^{-1}$ | $1.65 \times 10^{-1}$ |

TABLE III

RATE AND OTHER CONSTANTS INVOLVED IN ACETYLATION AND EXCRETION OF SULFONAMIDES BY TEST SUBJECTS

| | Sulfaethylthiadiazole | | | | Sulfisomidine | | | |
|---|---|---|---|---|---|---|---|---|
| | Subjects | | | | Subjects | | | |
| Constant | C | P | E | Mean | C | P | B | Mean |
| $k_1$ (hr.$^{-1}$) | 0.001 | 0.003 | 0.002 | 0.002 | 0.010 | 0.008 | 0.011 | 0.0097 |
| $k_2$ (hr.$^{-1}$) | 0.086 | 0.073 | 0.057 | 0.072 | 0.137 | 0.120 | 0.117 | 0.125 |
| $k$ (hr.$^{-1}$) | 0.087 | 0.076 | 0.059 | 0.074 | 0.147 | 0.128 | 0.128 | 0.134 |
| $Se^0$ (mg.) | 492 | 487 | 444 | 478 | 377 | 362 | 375 | |
| | 0.989 | 0.959 | 0.968 | 0.972 | 0.932 | 0.934 | 0.916 | 0.927 |

An attempt was made to relate rate constant for acetylation to O/W partition coefficient (aqueous phase at pH 7.4) for the substances reported on now and sulfonamides studied in the past.[1-3] A kinetic analysis of sulfonamide acetylation data as has been conducted in this laboratory does not by itself allow assignment of the rate-limiting step found to a particular step in the many steps which drugs must go through to be acetylated even though useful information is gained.[6] It seemed a possibility that the rate-limiting step in acetylation may have been penetration of drug to the site of acetylation. This explanation is consistent with the nature of the process found for acetylation, *i.e.*, the process was apparent first order and penetrations by indifferent substances proceed by first order, or as often referred to, by passive processes. If the acetylation process was indeed passive, then it would be expected that increases in rate constant should be associable with increases in organic phase-aqueous phase at pH 7.4 partition coefficient. A brief examination of the data of Table II, where rate constants for acetylation available from present and past work are listed along with partition coefficients, will show that this correlation does not exist in the series of compounds studied.

The determination of the rate constant for acetylation is independent of the presence of other metabolites of the sulfonamides such as *N*-glucuronides[7] even though the presence of these substances would cause the rate constant for excretion of free drug to represent the sum of the rate constants for excretion of free drug and glucuronide formation when the usual assay is conducted to determine sulfonamide titer in urine.

(6) E. Nelson, *Nature*, **189**, 928 (1961).
(7) E. W. Maynert, *Ann. Rev. Pharmacol.*, **1**, 45 (1961).