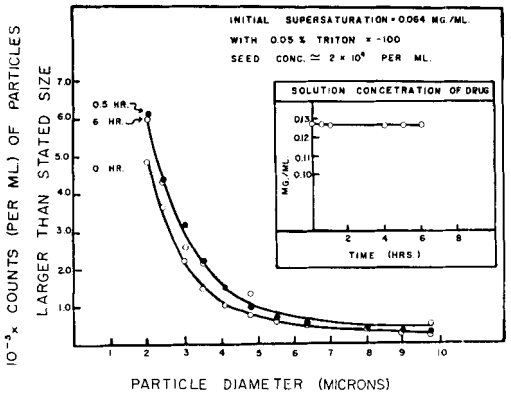

Fig. 4.—Growth of methylprednisolone suspensions at 25° in seeded supersaturated solutions. Main plots are number integral size distribution as function of time. Insets give data on relief of supersaturation over same time ranges.

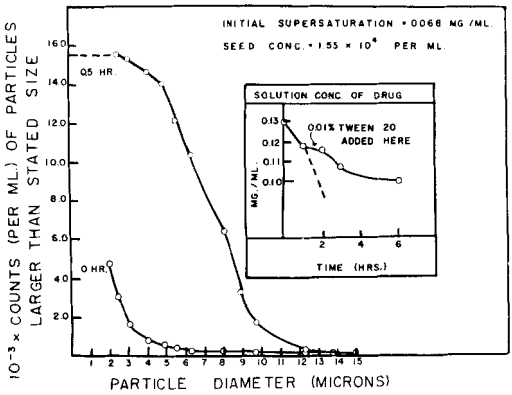

Fig. 5.—Growth of methylprednisolone suspensions at 25° in seeded supersaturated solutions. Main plots are number integral size distribution as function of time. Insets give data on relief of supersaturation over same time ranges.

fate elicited effects similar to Tergitol 4. Solubility studies carried out showed that none of these surfactants increased the solubility of methylprednisolone by more than 0.01 mg./ml. at the concentrations used in these studies. Thus, these effects are not due to reduction in the driving force for growth but probably due to the creation of some kind of an interfacial barrier.

In most instances, particularly for those cases in which the growth rates were relatively small, the rate law given by Eq. 6 appeared to be approximately obeyed. In terms of the plots of the cumulative counts *vs.* particle size, conformation of the data to Eq. 6 would mean that the curves would be displaced to the right at a constant rate with respect to time. The results shown in Figs. 2 and 3 appear to do just that. This suggests strongly that in these cases the growth rates are proportional to the surface area of the particles.

In the experiments with Tergitol 4, it was observed that, while a given ordinate value moved constantly with time to the right, the upper portions of the curves moved more slowly than the lower. For reasons mentioned earlier, this was probably not due to aggregation of the particles. This type of behavior appears to be analogous to the theoretical case leading to Eq. 8. At any given time t the smaller particles appear to be growing more slowly than the larger ones. Further studies are necessary to establish the situation unambiguously for this type of growth behavior.

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Control of Urine pH and Its Effect on Sulfaethidole Excretion in Humans

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A technique is described whereby urine can be excreted by human subjects, at a pH near 5.0 or 8.0, with a variation of not more than 0.1 or 0.2 pH unit over periods of at least 15 hours. The significance of controlled urinary pH in humans with respect to the excretion kinetics of a weak acid, sulfaethidole, has been determined. The harmonic mean $t_{1/2}$ for SETD at a controlled urinary pH of 5.0 is 11.4 hours; the $t_{1/2}$ for this drug at a controlled urinary pH of 8.0 is 4.2 hours.

THIS STUDY was undertaken to establish a technique whereby urine would be voided at a constant pH over a protracted time. Using this technique, the elimination of sulfaethidole (SETD) from human subjects was studied to determine the influence of controlled urinary pH on its excretion kinetics. That the elimination processes of some drugs are influenced by administration of agents which affect the acidity or alkalinity of the urine is well known. For example, Smith, Gleason, Stoll, and Ogorzalek (1) and others (2–6) have demonstrated that renal

Received April 17, 1962, from the School of Pharmacy, Temple University, Philadelphia, Pa.

Accepted for publication May 10, 1962.

Adapted from prize-winning manuscript submitted by Joel B. Portnoff in the Eastern Region, 1961 Lunsford Richardson Pharmacy Awards Competition.

Presented at Symposium on Drug Absorption, Metabolism, and Excretion, A.PH.A., Las Vegas meeting, March 1962.

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clearance of salicylates is increased when sodium bicarbonate or other alkalinizing agents are employed to increase pH of the urine. Similar results have been shown by Waddell and Butler (7) and by Mollaret, Rapin, Pocidalo, and Monsallier (8, 9) for phenobarbital, by Waddell and Butler (10) for 5,5-dimethyl-2,4-oxazolidinedione, by Gutman, *et al.* (11), for phenylbutazone analogs, and by Peterson, Finland, and Ballou (12) for sulfadiazine. It is generally accepted in clinical practice that the excretion process of chemotherapeutic sulfonamides can be hastened by administration of sodium bicarbonate. Also, it is well known that the elimination of many basic drugs is hastened by agents which acidify the urine (13–18).

The influence of urinary pH on the elimination of a drug such as SETD can be attributed to the pH dependence of renal tubular transfer of the drug. A large portion of SETD in the human body is contained in extracellular fluids, of which the blood is a large component. Blood passing through the nephron of the kidney is filtered of many of its components. The capillary networks of the glomeruli act as filter beds for the plasma, whereby most of the nonprotein substances filter passively through the glomeruli into the renal tubules. Many of the normal components of the glomerular filtrate are, in part, reabsorbed into the circulation from the tubules; absorption is effected by both active and passive processes, depending upon the substance in question. In addition, tubules may actively and passively secrete substances from the blood into the tubular fluid.

Drugs may be filtered through the glomeruli, secreted into the tubules, and reabsorbed from the tubules in ways similar to those which apply to normal blood components; however, knowledge of the active or passive nature of drug secretion and reabsorption at the renal tubule level is limited. For many drugs it is thought that passive diffusion may be more important than active transport in their excretion and reabsorption at the renal tubule level.

A difference in pH between plasma and tubular fluid can result in a considerable difference in the fraction of undissociated weakly acidic or weakly basic drug in these two fluids. In the absence of a specific transport mechanism it is usually the undissociated form of the drug which most readily diffuses between the two fluid compartments. The concentration gradient of the nonionized drug across the cellular barrier separating plasma from tubular fluid is, therefore, considered to be of primary importance in passive transport. Though it is difficult to determine the pH of fluid at sites in the tubule intermediate of the glomeruli and the bladder, the pH of the beginning and terminal processes can be readily determined by measuring plasma and urine pH. Plasma pH in humans is generally very constant, near 7.4, whereas urine pH may vary through a range of about 4.5 to 8.5 under different natural or experimental conditions.

Milne, Scribner, and Crawford (19) have shown that the pH of the tubular fluid and the pKa and partition coefficient of the drug would be expected to influence the renal tubular transfer of many weak acids and bases, just as such factors influence the absorption and secretion of drugs in the gastrointestinal tract (20–24). It was thought that by devising a technique for maintaining urine pH constant at several pH values, a convenient method would become available for assessing quantitatively, in kinetic terms, the effect of pH on the excretion of drugs. Since SETD is a weak acid which is eliminated virtually completely *via* the urinary route, and methodology for the kinetic elimination studies has been established (25–28), this appeared to be a suitable drug for such study.

EXPERIMENTAL

Human Studies.—Twelve healthy young adult white male human volunteers were employed in the excretion trials of this study. For most trials, food and fluid intake were not controlled.

Ammonium chloride and sodium bicarbonate were used, in the manner previously reported (29), to maintain the desired acidic or alkaline condition of the urine throughout the studies. Normally, a 3- to 4-Gm. dose of one of these agents was given in tablet form for 2 or 3 hours, until measurement of urine pH indicated that pH values of 5 or 8 had been attained. Smaller doses of these agents were administered, usually each hour, to maintain the pH at the desired value. The pH of the urine was monitored, usually every hour, to determine whether succeeding doses should be increased or decreased to maintain a constant pH.

After the desired urine pH was attained, a single 2-Gm. dose of powdered SETD suspended in water was administered orally. Samples of urine were collected, usually at hourly intervals, for at least 10 hours. Volumes of urine voided, pH, and free SETD excreted were measured in a total of 16 trials at a urinary pH near 8, and in 17 trials at a urinary pH near 5. All subjects were used in at least one trial at each of the two urinary pH values. One subject was studied to determine the effect of induced diuresis on the excretion profile. Another variation involved an excretion profile study with one subject where, in a single trial, the urine was maintained first at an acid pH and then at an alkaline pH.

Analytical Methods.—Urine pH measurements were made with a Beckman model G pH meter.

TABLE I.—ILLUSTRATION OF TYPICAL DOSAGE REGIMENS OF SODIUM BICARBONATE AND AMMONIUM CHLORIDE REQUIRED TO ATTAIN AND MAINTAIN CONSTANT URINARY pH IN HUMAN SUBJECTS

	Dosage of Ammonium Chloride, Gm.	Time, hr.	Urinary pH	Dosage of Sodium Bicarbonate, Gm.	Time, hr.	Urinary pH
Subject A	4.0	−1.25	...	4.2	−2.00	...
	...	−0.25	5.25	4.2	−1.00	...
	3.0	0	5.15	3.0	0	7.80
	2.0	1.17	5.07	1.2	1.00	8.10
	1.0	2.17	5.07	1.2	2.00	8.10
	1.0	3.33	5.03	1.2	3.00	7.95
	1.0	4.17	4.97	3.0	4.00	7.60
	1.0	5.00	5.00	3.0	5.00	7.90
	1.0	6.00	5.02	1.8	6.00	8.10
	1.0	7.00	5.03	1.8	7.00	8.10
	...	8.00	5.09	1.8	8.00	8.00
	...	9.33	4.87	1.8	9.00	8.10
	...	10.00	4.82	1.8	10.00	8.10
	...	12.00	4.82	...	11.00	8.00
Subject B	4.0	−1.00	...	4.2	−3.00	...
	3.0	0	...	4.2	−2.00	...
	1.0	1.00	5.30	4.2	−1.00	...
	1.0	2.00	5.30	4.2	0	7.50
	1.0	3.50	5.30	3.0	1.00	8.05
	1.0	4.00	5.30	3.0	2.00	8.00
	1.0	5.00	5.20	3.0	3.00	8.00
	1.0	6.00	5.10	1.2	4.00	8.10
	1.0	7.00	5.20	1.2	5.00	8.10
	1.0	8.00	5.30	1.2	6.00	8.00
	1.0	9.00	5.30	1.2	7.00	8.00
	...	10.00	5.30	1.8	8.00	8.00
	...	...	...	1.8	9.00	7.95
	...	...	...	...	9.50	7.85

Each urine sample, when collected, was rapidly brought to room temperature and the pH was determined immediately.

Urine samples were assayed for free SETD using a modification of the Frisk macro method (30). Spectrophotometric measurements of the final colored solutions, involving use of the Bratton-Marshall reagent (31), were made with a Beckman model B spectrophotometer, at a wavelength of 550 mμ.

RESULTS

Control of Urine pH.—Typical dosage regimens and the resulting urine pH are presented in Table I. Subjects were somewhat variable with regard to the quantities of ammonium chloride or sodium bicarbonate required to attain and maintain a constant pH; however, after gaining experience with this technique, and by monitoring the urine pH at about hourly intervals, it was a relatively easy matter to maintain urine pH within ±0.2 units over periods of at least 15 hours. The occasional incidence of nausea from the large doses of sodium bicarbonate and ammonium chloride was the principal difficulty encountered in employing this technique.

While good control of urine pH can be attained near pH 5 or pH 8, it was more difficult to maintain constant pH at intermediate values. It was concluded that this could not be accomplished conveniently to the degree of constancy desired for the excretion studies; therefore, studies on the SETD excretion profile were limited to values near pH 5 and pH 8.

Excretion Studies.—Typical data for the SETD excretion profile studies under conditions of constant pH are presented in Tables II and III. Since it has been shown previously (26, 27, 32) that oral doses of SETD are virtually completely absorbed, almost totally excreted in the urine, and that virtually all of the drug in the urine is measurable by use of the Bratton-Marshall reagent, interpretation of the excretion data was not difficult. As shown previously for this drug, rate of elimination can be expressed as

$$dW/dt = -k_b W \qquad \text{(Eq. 1)}$$

where W is the amount of drug remaining in the body at any time, t, or W = (dose − amount excreted), and k_b is the specific velocity constant for disappearance of drug from the body. Since

$$\log W = -(k_b/2.303)t + \text{constant} \qquad \text{(Eq. 2)}$$

k_b can be determined from the slope of the straight line resulting from a plot of log W *vs.* time (27). The biologic half-life, $t_{1/2}$, is determined for each trial from $t_{1/2} = 0.693/k_b$.

Plots of logarithm mg. of unexcreted drug *vs.* time, for single subjects, gave straight line plots after 1 or 2 hours following administration. Table IV illustrates the biologic half-life values obtained from inspection of the exponential plots of the urinary excretion data and the average urine pH as observed for each trial. Average acid urine pH values for subjects in 17 trials ranged from pH 4.5 to pH 5.3. When two trials are eliminated from the summary data, 15 trials fall within the pH range 4.8 to 5.2. The harmonic mean for $t^1/_2$ values observed in the 15 trials at pH 5 ± 0.2 is 11.4 hours with fiducial limits (26) of 9.9 to 13.5 hours. This corresponds to an arithmetic average k_b of 0.061 ± 0.004 hr.$^{-1}$, where ± designates standard error.

Average alkaline urine pH values for subjects in 13

TABLE II.—TYPICAL DATA FOR URINARY EXCRETION OF SETD,[a] URINE MAINTAINED AT pH 5

Dosage of Ammonium Chloride, Gm.	Hours	Urinary pH	Urine Volume, ml.	SETD per Sample, Mg.	Total Unexcreted SETD, mg.
4.0	−3.33	...	...	...	...
...	−1.83	5.3	...	...	...
1.0	0	...	...	...	...
1.0	1.33	5.1	248	22.3	1978
1.0	2.75	5.1	235	87.0	1891
1.0	3.75	5.1	114	124.3	1766
1.0	4.92	5.0	108	140.8	1626
...	6.08	5.0	119	132.1	1494
1.0	7.08	5.0	80	88.0	1406
1.0	8.08	5.1	100	90.0	1316
0.5	10.08	4.9	192	151.7	1164
...	11.08	4.8	149	82.0	1082
...	12.08	4.9	78	60.8	1021
...	13.23	5.0	57	51.3	970
0.5	14.08	5.0	50	37.0	933
...	15.08	4.9	75	45.6	887
...	17.75	...	391	104.0	783
...	23.00	...	946	200.6	582
...	25.59	...	489	86.1	497

[a] SETD, 2 Gm., administered orally at zero hour.

TABLE III.—TYPICAL DATA FOR URINARY EXCRETION OF SETD,[a] URINE MAINTAINED AT pH 8

Dosage of Sodium Bicarbonate, Gm.	Hours	Urinary pH	Urinary Volume, ml.	SETD per Sample, mg.	Total Unexcreted SETD, mg.
4.2	−2.75	...	...	...	...
4.2	−1.50	6.4	...	...	...
3.6	−0.25	8.1	...	...	...
0.6	0	8.6	...	...	...
3.6	1.00	8.1	70	294.0	1706
3.0	2.00	8.1	62	235.6	1470
3.6	3.08	8.1	95	247.0	1223
3.0	4.08	8.1	113	180.8	1043
3.0	5.08	8.1	99	142.6	900
2.4	6.08	8.1	86	123.9	776
2.4	7.66	8.1	130	179.4	597
...	8.75	8.0	100	108.0	489
3.0	9.25	8.0	44	44.0	445
...	10.59	8.1	130	75.4	369
...	11.59	8.0	105	39.9	329
...	13.59	8.0	130	85.8	244
...	14.83	7.6	158	63.2	180
...	19.75	...	580	118.9	62

[a] SETD, 2 Gm., administered orally at zero hour.

trials ranged from pH 7.4 to pH 8.1. When the three trials at the lowest pH are excluded, 10 trials are observed to fall within the average pH range of 7.9 to 8.1. The harmonic mean for $t_{1/2}$ values in the 10 trials at pH 8 ± 0.1 is 4.2 hours, with fiducial limits of 3.7 to 4.8. Average k_b is 0.165 ± 0.009 $hr.^{-1}$

Figure 1 is a presentation of the composite urinary excretion data from which Table IV values for $t_{1/2}$ were determined. It is a rather dramatic illustration of the adherence of the urinary excretion process, of many trials, to first-order kinetics in the time range studied. In addition, it gives a clear visual illustration of the dependence of the process upon the magnitude of the equilibrium urine pH. Figure 2 also illustrates this very well where an abrupt change in the slope of the excretion curve occurs when urine, being collected during a single trial, was suddenly changed from a pH of 5 to a pH of 8.

Though previous data had supported the opinion that, in the presence of adequate urine flow, elimination kinetics of SETD is relatively independent of urine volume (27), in the early phase of this study experimental verification of this point seemed desirable. During the course of an excretion study, a single subject who had consumed approximately 100 ml. of water each hour was suddenly changed to a regimen where he consumed almost 1000 ml. per hour for several hours. Data presented in Fig. 3 indicate that SETD excretion was essentially unchanged even by tenfold or greater increases in urine volume, so long as precautions were taken to maintain constant urine pH.

DISCUSSION

The fact that urine pH influences net elimination of SETD to the degree indicated in Table IV indicates that renal tubular transport of the drug plays an important role in the elimination process. Approximately 95% of the SETD in plasma is

TABLE IV.—BIOLOGIC HALF-LIFE FOR SETD IN HUMAN SUBJECTS AS A FUNCTION OF URINARY pH

Acid Range		Basic Range	
Av. Urine pH	$t_{1/2}$, hr.	Av. Urine pH	$t_{1/2}$, hr.
4.5	14.2	7.4	6.6
4.8	12.5	7.5	4.7
4.8	12.6	7.8	5.4
4.8	8.9	7.9	5.4
4.8	19.8	7.9	3.7
4.8	12.5	8.0	3.3
4.8	10.0	8.0	5.5
4.9	21.3	8.0	4.4
4.9	8.9	8.0	4.1
4.9	10.7	8.0	3.7
5.0	20.1	8.1	5.3
5.0	11.4	8.1	4.1
5.0	10.3	8.1	3.9
5.1	8.0	...	...
5.1	10.4	...	...
5.2	9.2	...	...
5.3	10.6	...	...
For 15 trials within pH range 4.8 to 5.2, Av. $t_{1/2}$, 11.4 hr.[a]		For 10 trials within pH range 7.9 to 8.1, Av. $t_{1/2}$, 4.2 hr.[a]	

[a] Harmonic mean $t_{1/2}$.

bound (33) and, therefore, only a small fraction of the total SETD is cleared in any single pass of fluids through the glomeruli. While the concentration of diffusible drug in the glomerular filtrate must be low, a condition favoring diffusion of SETD back into general circulation might be created through water reabsorption in the proximal tubule, thus increasing the concentration of drug in tubular fluid over that of unbound drug in the plasma. Furthermore, a decrease in pH of tubular fluid would favor reabsorption of drug by increasing the concentration of the more readily diffusible undissociated form of the drug.

The pKa of SETD, determined by potentiometric titration, is about 5.5, and the fraction of SETD which is undissociated is given by

$$(H^+)/[(H^+) + Ka]$$

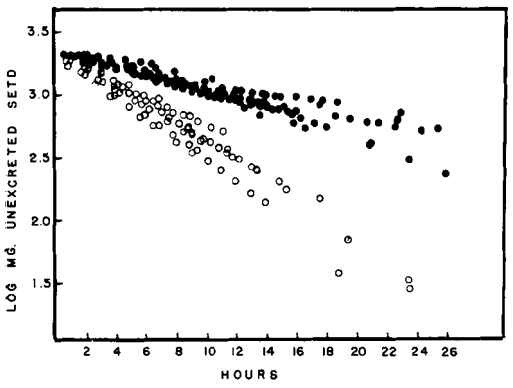

Fig. 1.—Exponential plot of the composite urinary excretion data for sulfaethidole following single 2-Gm. oral doses to 12 subjects in 30 trials. Darkened circles indicate data at constant urine pH near 5 and open circles indicate data where urine pH was maintained near 8 as described in the text.

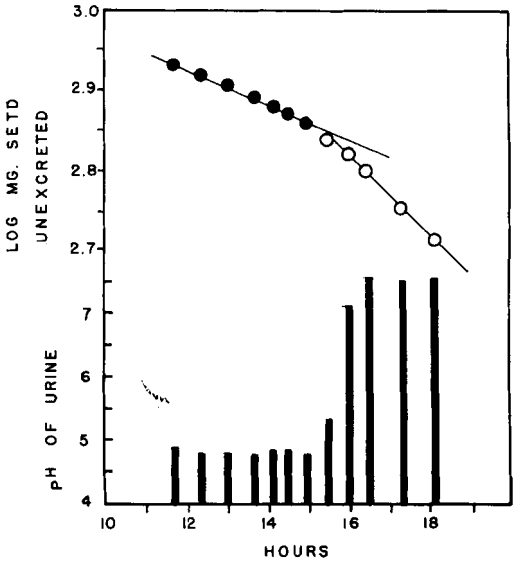

Fig. 2.—Exponential plot of SETD urinary excretion data for a single human subject after a 2-Gm. oral dose. The figure illustrates the abrupt change in slope of the exponential process when urine pH is changed from about pH 5 to pH 8.

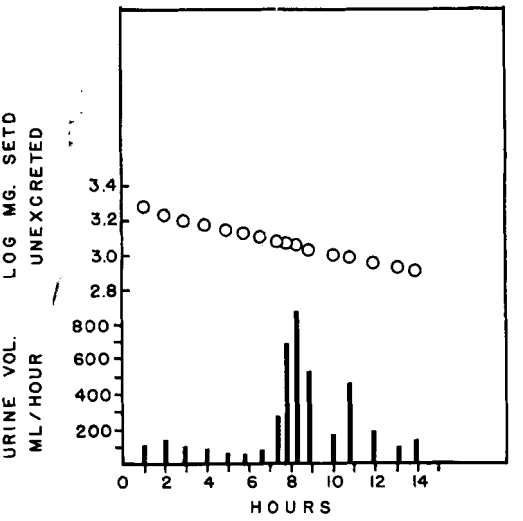

Fig. 3.—Exponential plot of SETD urinary excretion data for a single human subject after a 2-Gm. oral dose when urine pH was maintained near 5, but where the rate at which urine is voided was changed appreciably. The figure illustrates that the elimination rate is relatively independent of urine volume voided.

The fraction of SETD undissociated in plasma and urine under conditions of this study is as follows: plasma, pH 7.4, 1.2% free SETD is nonionized; urine, pH 8.0, 0.3% SETD is nonionized; urine, pH 5.0, 76% SETD is nonionized.

Although the pH of tubular fluid at sites for reabsorption of SETD is unknown, it seems fairly certain that under conditions of this study the fluid

would be alkaline when urine pH was maintained at pH 8 and acid when urine was maintained at pH 5. The longer biologic half-life for SETD under conditions of acid urine suggests that reabsorption of the drug from tubular fluids is favored by higher concentrations of undissociated drug. This concept is similar to those concerning gastrointestinal absorption which have seen considerable discussion in recent literature by Schanker, Brodie, Hogben, *et al.* (20–24). Milne, Scribner, Crawford (19), Gutman, *et al.* (11), and others have developed such thinking regarding factors influencing net drug elimination *via* the urinary route.

Though the pKa of SETD and other acidic and basic drugs apparently plays an important role in the mechanism of drug elimination, and though controlled urinary pH can exert a profound effect on the rate of net drug elimination, the intrinsic lipid solubility of the undissociated drug, concomitant administration of other drugs, and other factors must also be important in influencing the net elimination rate. It would seem important to obtain a clearer understanding of the mechanism of drug excretion by the kidney, and of the interrelationships of the physical-chemical properties of drugs and the mechanisms which come into play in body elimination of drugs. Such knowledge, coupled with additional knowledge of other body mechanisms for eliminating drugs and drug activity, has a bearing on the intelligent design of drugs and their dosage forms for controlling their duration of action.

The results of this study supplement and support, in a number of respects, previously reported findings concerning SETD excretion kinetics (25–28, 30, 32). In previous studies without benefit of controlled urinary pH, and using both blood concentrations and urinary excretion data, the $t_{1/2}$ for SETD in human subjects was shown to be about 8.4 hours (27). This value is about midway between the values of 4.2 and 11.4 hours, at controlled urine pH of 8 and 5, respectively, determined in this study. These results also give further reinforcement to the opinion that the $t_{1/2}$ values of drugs determined experimentally in human subjects are not to be construed as fixed values. Rather, they are subject to variation by a number of factors about which much additional data are necessary for a better understanding of elimination mechanisms and kinetics.

SUMMARY

1. A technique has been developed to permit control of the pH of urine being excreted by human subjects to ±0.1 pH unit, in the vicinity of pH 5 or pH 8, over periods of at least 15 hours.

2. Constant urinary pH in the vicinity of pH 5.0 can be attained by administering several grams of ammonium chloride each hour until the desired pH has been attained, and then increasing or decreasing the maintenance dose as indicated by hourly monitoring of urine pH. Constant urinary pH in the vicinity of pH 8.0 can be attained by employing sodium bicarbonate in a similar fashion.

3. Modification of urine pH in human subjects has a profound effect on the rate of elimination of sulfaethidole from the body.

4. The harmonic mean $t_{1/2}$ for SETD is 11.4 hours when urine pH is maintained at pH 5.0; the harmonic mean $t^{1}/_{2}$ is only 4.2 hours when urine pH is maintained at pH 8.0.

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