The conditions were selected that gave the best yield for the shortest duration of reaction. Although these compounds could be obtained from glycine and cupric sulfate and the aldehyde as well as from cupric glycinate and aldehyde, the latter gave superior yields for the conditions studied. Lengthening the duration of reaction did not appear to improve the yield. Increasing the aldehyde concentration seems to result in larger formation of sticky material with no appreciable improvement of yield.

Removal of the cupric ion at the termination of the reaction by rapid heating gave inferior yields (8%). Use of the resin column for desalting can be eliminated when a smaller amount of sodium carbonate is used and the aqueous solution from which the sulfides have been largely removed is passed through a single cellulose column, but a smaller yield (8%) results.

The slow moving compounds appearing in trace amounts when β-hydroxyhomomethionine is chromatographed in the methyl ethyl ketone system are very likely the corresponding sulfoxides of the diastereomers. This is supported by the observation that the slower moving ninhydrin-positive material of each diastereomer is associated with the respective parent diastereomer during the separation procedure, being present when its parent compound is present and disappearing when its parent compounds disappear. When a small amount of diastereomer A and B was treated with hydrogen peroxide for a few minutes at room temperature and the resulting solution was chromatographed on paper in the methyl ethyl ketone system, a pronounced increase in the slower moving compound and a corresponding decrease in the parent compounds were noted. The ninhydrin-positive material that emerged ahead of β-hydroxyhomomethionine has not been identified.

Attempts have been made to prepare β-hydroxymethionine from methylthioacetaldehyde and cupric glycinate, starting with the diethylacetal of the aldehyde, but with no success thus far.

Enzymatic resolution of the racemates of each diastereomer of β-hydroxyhomomethionine and β-hydroxymethoxinine is now being studied in preparation for testing the pure enantiomers for activities in microbial antitumor screening systems and mammalian tumor systems.

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Influence of Repetitive Dosing and Altered Urinary pH on Doxycycline Excretion in Humans

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Abstract □ The effect of altered urinary pH on the renal excretion of doxycycline, with both single- and multiple-dose regimens, was studied in humans. Alkalinization of the urine resulted in higher cumulative amounts of doxycycline excreted during both single- and multiple-dose regimens. The increased excretion was reflected in larger renal clearances and shorter half-lives for both regimens when the alkaline condition was compared to the control condition. Half-life values increased from the single- to the multiple-dose regimen under both alkaline and control conditions. However, the increase in half-life values from single- to multiple-dose administration under the alkaline condition was shorter than the half-life change under control conditions.

Keyphrases □ Doxycycline—comparison of single-dose and multiple-dose regimens, effects of urine alkalinization on excretion, half-lives, humans □ Urinary pH—effects on excretion of doxycycline, single-dose and multiple-dose regimens, half-lives, humans □ Dosing regimens—comparison of single-dose and multiple-dose administration of doxycycline, effects of urinary pH on excretion, half-lives, humans □ Alkalinization, urine—effects on doxycycline excretion, comparison of single-dose and multiple-dose regimens, half-lives, humans □ Excretion, urinary—comparison of single-dose and multiple-dose doxycycline regimens, effects of alkalinization of urine, half-lives, humans

It was recently shown that altered urinary pH can significantly influence the urinary excretion of tetracycline and doxycycline (1). A treatment producing an alkaline urinary pH was shown to result in a 24% increase in cumulative tetracycline excretion when compared to a treatment producing an acidic urinary pH for the same time interval (48 hr). An even greater increase in cumulative excretion was shown for doxycycline under similar conditions. For doxycycline the alkaline urine condition resulted in a 54% increase in cumulative excretion as compared to an acidic urine condition.

This effect has been attributed to differences in the lipid solubilities of these antibiotics over the physiological pH range of the urine resulting in their increased or decreased reabsorption in the tubules of the kidney. The greater differences between alkalinization and acidification of the urine for the excretion of doxycycline than for tetracycline appear to confirm the theory that this antibiotic shows greater

lipid solubility at its isoelectric pH (5.6) than does tetracycline (2).

Although there is no evidence in the literature indicating the effect of altered urinary pH on the excretion of tetracyclines administered in multiple-dose regimens, it has been shown that the apparent biological half-lives of tetracyclines increase when a multiple-dose regimen is compared to a single dose (3). For example, tetracycline and doxycycline biological half-lives increased from 6.3 to 10.0 hr and from 8.3 to 14.5 hr, respectively, when a single-dose was compared to a multiple-dose regimen.

Since the tetracycline antibiotics are generally administered on a multiple-dose regimen, a more clinically relevant finding would be to determine the effect of altered urinary pH on the excretion of tetracycline antibiotics administered on a multiple-dose regimen. Also of importance would be the determination of changes in biological half-lives under controlled urinary pH conditions to find if these effects were similar to those observed with uncontrolled urinary pH conditions. Since altered urinary pH has been shown to influence doxycycline to a greater extent than tetracycline (1), changes in excretion during multiple dosing, if any, should be more apparent with this antibiotic.

Thus, the objectives of this study were to: *(a)* investigate the effects of urinary pH changes on serum levels, urinary excretion, and related pharmacokinetic parameters of doxycycline in humans during a multiple-dose regimen; and *(b)* determine whether the lengthening of half-life that has been observed for tetracyclines administered on a multiple-dose regimen occurs for doxycycline when the urinary pH is significantly altered.

EXPERIMENTAL

Subjects—Six adult, male volunteers with no known diseases, between 65.9 and 79.5 kg in weight, between 172.7 and 185.4 cm in height, and between 19 and 29 years of age, served as subjects. Clinical laboratory tests for creatinine clearance, hemoglobin, hematocrit, and serum albumin determined that all subjects had normal blood and renal function.

Procedure for Subjects on Single-Dose Regimen—To provide a control to determine if subjects had relatively normal urinary pH, determinations of urine pH were made every 6 hr beginning 14 hr prior to drug administration. Subjects on the alkaline protocol took 6.0 g of sodium lactate[1] every 6 hr for the entire test period beginning 14 hr before drug administration. The sodium lactate was given in a vehicle of syrup USP (15 ml) and licorice flavor compound[2] (0.1 ml) to reduce the saline taste of the alkalinizer.

Following the 14-hr control period, 200 mg of doxycycline (as two 100-mg capsules[3]) was administered orally with 240 ml of water. The subjects were requested not to eat food of any kind or drink any beverage, except water, for 3 hr before and 3 hr after doxycycline administration. Drug administration always occurred at 8:00 am.

Total urine voids were collected immediately prior to drug administration and 2, 4, 9, 12, and 16 hr following drug administration and then every 6 hr until drug could no longer be detected in the urine.

[1] Fisher Scientific Co., Fair Lawn, N.J.
[2] Fritzsche Dodge and Olcott, Inc., New York, N.Y.
[3] Vibramycin, Pfizer Laboratories Division, Chas. Pfizer and Co., New York, N.Y.

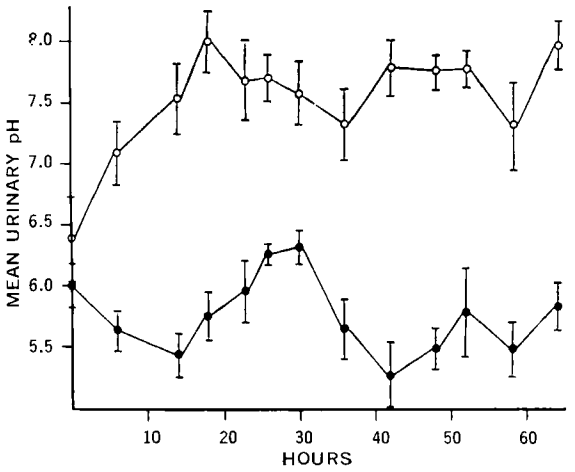

Figure 1—*Mean urinary pH* (±SE) *for six subjects on single-dose regimen. Key:* ●, *control condition; and* ○, *alkaline condition.*

Venous blood samples were taken immediately prior to the administration of the drug and 1, 2, 3, 4, 6, 9, 12, 22, 28, 34, 40, 46, and 52 hr following drug administration.

Procedure for Subjects on Multiple-Dose Regimen—Subjects followed the identical protocol as previously described for the single-dose regimen up to the time of administration of the test drug. At this time, an initial 200-mg loading dose of doxycycline was administered followed by 100 mg every 12 hr for a total of 12 doses. Drug administration always occurred at 8:00 am and 8:00 pm.

Urine voids were collected immediately prior to doxycycline administration and then every 6 hr for 72 hr. Urine voids were then collected at 73.5, 76, 77, 80, 82, and 84 hr after drug administration and then every 12 hr until 132 hr after administration of the initial drug dose (48 hr after the last dose).

Blood samples were taken immediately prior to the administration of the initial doxycycline dose and at 1.5, 3, 6, 9, 12, 24, 25.5, 27, 30, 33, 36, 48, 49.5, 51, 54, 57, 60, 72, 73.5, 75, 76, 77, 80, 82, 84, 96, 100, 104, 108, 120, 124, and 132 hr following the first dose of drug. Blood samples were always taken immediately prior to the administration of all doses (10, 12, 24, 36, 48, 60, 72, and 84 hr after the first dose).

Analytical—The analytical technique utilized in the determination of doxycycline in biological fluids was based on the method of Kohn (4), which involves the formation of an extractable complex of doxycycline with calcium ion and water-soluble barbiturate. The concentration of the resulting complex was then determined fluorometrically at an excitation wavelength of 400 nm and an emission wavelength of 520 nm[4]. A 0.5-ml serum sample and a 0.3-ml urine sample were employed for each determination. The assay is specific for unchanged doxycycline in blood or urine (5, 6).

Statistical Analysis of Data—Since each subject was tested under two different conditions (control and alkaline treatments), the statistical tests employed were by the method of paired observations or by a two-way analysis of variance (7). The t-test was employed for determining statistical significance of urinary pH values, cumulative excretion differences, and differences in various pharmacokinetic parameters. A two-way analysis of variance test was utilized for determining statistical significance for the blood level *versus* time curves in the excretory phases of the diagrams. The sources of variation in this analysis included time, the individual subjects, treatments to modify urinary pH, and residual.

RESULTS

The mean urinary pH for all subjects on the single-dose regimen under the two conditions is shown in Fig. 1. Throughout the entire excretion period, the sodium lactate treatment resulted in significantly higher mean pH values ($t = 11.68$, $df = 12$, $p < 0.01$) at all

[4] Model 110 Turner fluorometer, Turner Associates, Palo Alto, Calif.

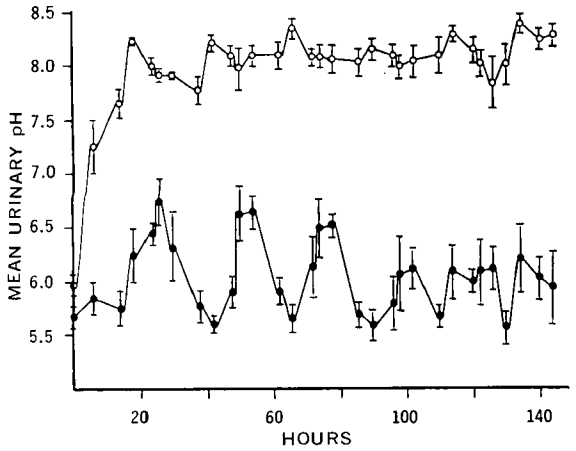

Figure 2—*Mean urinary pH* (±SE) *for six subjects on multiple-dose regimen. Key:* ●, *control condition; and* ○, *alkaline condition.*

time intervals when compared to the control treatment. A comparison of individual urine pH values under both conditions immediately prior to the alkalinizer treatment resulted in no significant difference.

Figure 2 shows mean urinary pH values for all subjects under the two conditions on the multiple-dose regimen. Over the entire excretion period, the sodium lactate treatment resulted in significantly higher mean pH values ($t = 22.26$, $df = 30$, $p < 0.01$) beginning with the first 12-hr period when compared with the control treatment.

The mean cumulative urinary excretion for all subjects under both conditions during the single-dose regimen is shown in Fig. 3. The alkaline treatment resulted in significantly higher mean cumulative excretion ($t = 15.61$, $df = 5$, $p < 0.01$) at the end of the 52 hr, the latest time at which all subjects excreted measurable amounts of drug. The control condition resulted in a mean of 37.1% of the dose excreted in the urine as compared to a mean of 60.8% excreted during the same time interval for the alkaline treatment. The values are in close agreement with similar work previously reported for doxycycline excretion (1).

Figure 4 shows the mean cumulative urinary excretion of doxycycline under both conditions during the multiple-dose regimen. The alkaline treatment resulted in significantly higher cumulative excretion ($t = 3.65$, $df = 5$, $p < 0.05$) at the end of the 132-hr test period. The control condition resulted in a mean excretion of

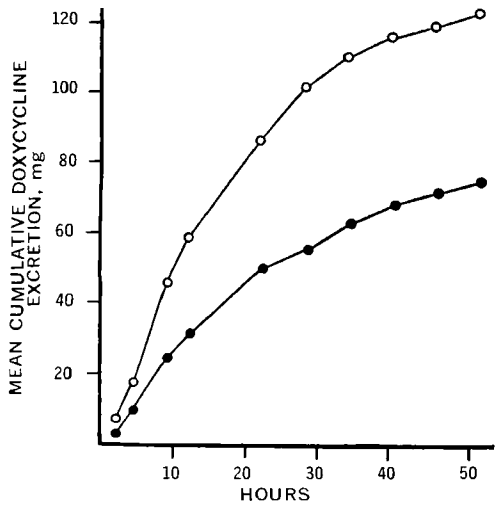

Figure 3—*Mean cumulative amounts of doxycycline excreted in the urine as a function of time for six subjects on single-dose regimen. Key:* ●, *control condition; and* ○, *alkaline condition.*

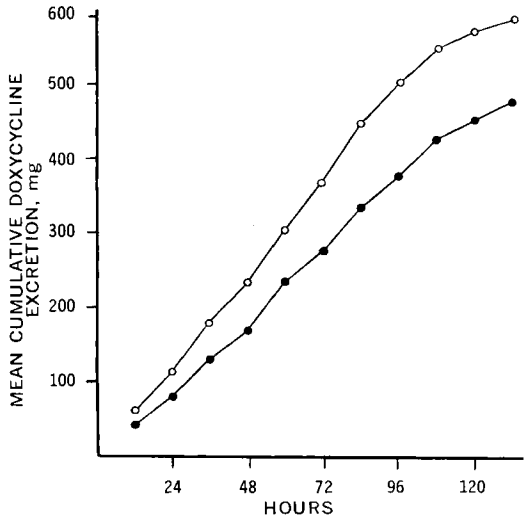

Figure 4—*Mean cumulative amounts of doxycycline excreted in the urine as a function of time for six subjects on multiple-dose regimen. Key:* ●, *control condition; and* ○, *alkaline condition.*

52.8% of the total administered doses over the entire collection period as compared to 65.0% for the alkaline condition.

Mean serum levels for the single-dose regimen under both conditions are shown in Fig. 5. Urinary alkalinzation resulted in significantly lower mean serum levels ($F = 13.20$; $df = 1,50$; $p < 0.01$) from 12 to 40 hr, the excretory phase for doxycycline, as compared to the control condition. A similar trend in doxycycline serum levels under controlled urinary pH conditions was shown in a previous study (1). Peak serum levels occurred at 3 hr during each treatment.

Figure 6 shows the mean serum levels for all subjects under both conditions during the multiple-dose regimen. As with the single-dose regimen, urinary alkalinization resulted in significantly lower mean serum levels ($F = 53.42$; $df = 1,140$; $p < 0.01$) as compared to the control condition. The values subjected to statistical analysis were those during the excretory phase for the drug after each dose of the multiple-dose regimen, which was always at least 10 hr after drug administration.

Both the single-dose (Fig. 5) and the multiple-dose (Fig. 6) semilogarithmic serum level *versus* time plots show appreciably different slopes during the postabsorptive phase (22–46 hr on the single-dose regimen and 100–132 hr on the multiple-dose regimen) when the two conditions are compared. These slopes (as determined by least-squares regression analysis) represent the elimination rate constants (K_E) of both regimens under both conditions (8). The single-dose regimen resulted in a mean elimination rate constant

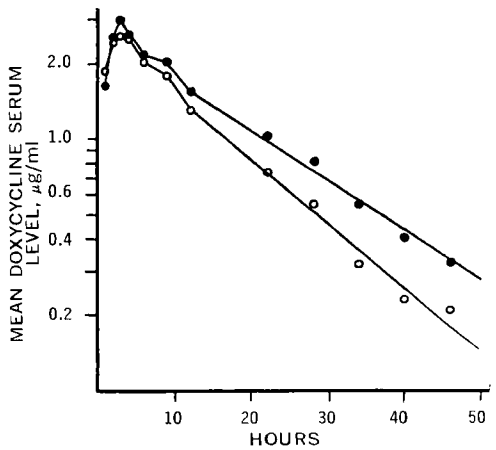

Figure 5—*Semilogarithmic plot of mean serum levels as a function of time for six subjects on single-dose regimen. Key:* ●, *control condition; and* ○, *alkaline condition.*

Table I—Pharmacokinetic Parameters for Doxycycline for Six Subjects during Single-Dose Regimen

Subject	Volume of Distribution Ratio, ($V_d^c/V_d^{OH^-}$)	Control Condition: Renal Clearance (R_c), ml/min	Control Condition: K_E, hr^{-1}	Control Condition: $t_{1/2}$, hr	Alkaline Condition: Renal Clearance (R_c), ml/min	Alkaline Condition: K_E, hr^{-1}	Alkaline Condition: $t_{1/2}$, hr
A	1.25	25.3	0.0568	12.2	61.8	0.0103	6.7
B	1.19	22.5	0.0337	12.4	51.5	0.1123	6.2
C	1.38	41.5	0.0583	11.9	60.0	0.1147	6.0
D	0.70	30.0	0.0701	9.9	42.5	0.0467	14.8
E	1.79	20.4	0.0400	17.3	24.8	0.0628	11.0
F	1.06	18.1	0.0446	15.5	43.6	0.0736	9.4
Mean	1.23	26.0	0.0542	13.2	47.4	0.0885	9.0
± *SE*	±0.15	±3.6	±0.0043	±1.1	±5.6	±0.0116	±1.4

Table II—Pharmacokinetic Parameters for Doxycycline for Six Subjects during Multiple-Dose Regimen

Subject	Volume of Distribution Ratio, ($V_d^c/V_d^{OH^-}$)	Control Condition: Renal Clearance (R_c), ml/min	Control Condition: K_E, hr^{-1}	Control Condition: $t_{1/2}$, hr	Alkaline Condition: Renal Clearance (R_c), ml/min	Alkaline Condition: K_E, hr^{-1}	Alkaline Condition: $t_{1/2}$, hr
A	1.61	26.2	0.0491	14.1	54.4	0.0988	7.0
B	0.88	10.7	0.0411	16.9	39.0	0.0689	11.3
C	0.55	20.0	0.0558	12.4	46.5	0.0502	13.8
D	0.91	18.2	0.0382	18.1	43.8	0.0555	12.5
E	1.21	21.8	0.0279	24.8	52.2	0.0535	13.0
F	0.98	29.4	0.0439	15.8	47.2	0.0543	12.8
Mean	1.03	21.1	0.0426	17.0	47.2	0.0618	11.8
± *SE*	±0.15	±2.7	±0.0038	±1.8	±2.3	±0.0074	±1.0

of 0.0542 hr^{-1} for the control condition as compared to 0.0885 hr^{-1} for the alkaline treatment, while the multiple-dose regimen resulted in mean elimination rate constants of 0.0426 and 0.0618 hr^{-1} for the control and alkaline conditions, respectively. Although a comparison of individual K_E values under the two different conditions on the single-dose regimen was just short of statistical significance, the multiple-dose regimen showed significantly different K_E values ($t = 2.57$, $df = 5$, $p < 0.05$) when the two treatments were compared.

Based on the K_E values, the alkaline treatment on the single-dose regimen resulted in a mean apparent biological half-life of 9.0 hr as compared to 13.2 hr for the control condition. The half-life for the control condition is shorter than most of those reported in the literature [15.0 hr (9, 10) and 15.1 hr (11)] but is much longer than that reported by Doluisio and Dittert [8.1 hr (3)]. The mean half-life for the alkaline and control conditions for the multiple-dose regimen were 11.8 and 17.0 hr, respectively. A comparison of individual half-lives for this regimen resulted in a significantly shorter half-life ($t = 2.91$, $df = 5$, $p < 0.05$) for the alkaline treatment as compared to the control condition. A comparison of the individual half-life values for the control conditions (single *versus* multiple dose) yielded a significantly longer half-life ($t = 2.69$, $df = 5$, $p < 0.05$) for the multiple-dose regimen. A comparison of the alkaline treatments (single *versus* multiple dose) showed a trend of longer half-life values for the multiple-dose regimen but this trend was short of statistical significance.

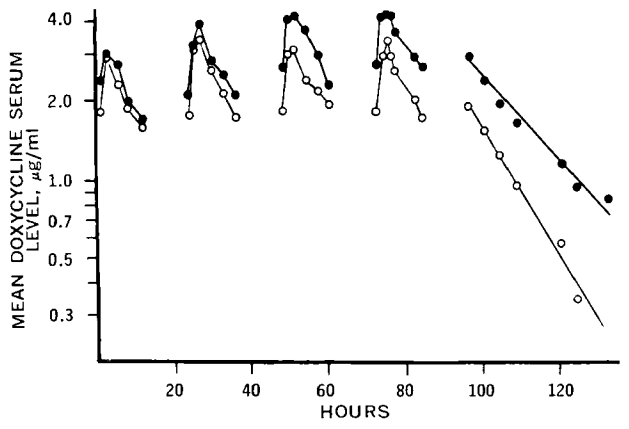

Figure 6—*Semilogarithmic plot of mean serum levels for six subjects as a function of time on multiple-dose regimen. Key: ●, control condition; and ○, alkaline condition.*

Renal clearance (R_c) values for the single-dose regimen were calculated based on Eq. 1 (8):

$$R_c = \frac{(Au)^{\infty}}{(AUC)_0^{\infty}} \qquad \text{(Eq. 1)}$$

where $(Au)^{\infty}$ is the total amount of drug excreted in the urine, and $(AUC)_0^{\infty}$ is the total area under the serum level–time curve.

Renal clearance values for the multiple-dose regimen were calculated based on Eq. 2 (8):

$$R_c = \frac{Au_{t(2)-t(1)}}{(AUC)_{t(1)}^{t(2)}} \qquad \text{(Eq. 2)}$$

where $(Au)_{t(2)-t(1)}$ is the amount of drug excreted in the urine during the steady-state interval between 72 and 84 hr, and $(AUC)_{t(1)}^{t(2)}$ is the area under the serum level–time curve during the same interval. Renal clearance, K_E, and biological half-life ($t_{1/2}$) for all subjects under both conditions for the single- and multiple-dose regimens are shown in Tables I and II, respectively.

The renal clearance values under both conditions on the single-dose regimen are significantly higher ($t = 4.43$, $df = 5$, $p < 0.01$) for the alkaline as compared to the control condition. Mean renal clearances were 26.0 and 47.4 ml/min for the control and alkaline treatments, respectively. A significant increase in renal clearance ($t = 14.6$, $df = 5$, $p < 0.01$) was also found for the multiple-dose regimen where the control and alkaline conditions showed mean renal clearances of 21.1 and 47.2 ml/min, respectively. No significant difference was observed when the renal clearance values from

the single- and multiple-dose studies under control conditions were compared. Likewise, no significant difference occurred when the alkaline conditions of the single- and multiple-dose regimens were compared.

In a previous paper (1), an equation for the volume of distribution ratios ($V_d^c/V_d^{OH^-}$) was derived to determine if the effects shown in this study were due to distribution changes. Ratio values of approximately unity indicate that the volume of distribution differences under each condition are negligible. As can be seen from Tables I and II, these changes were minimal for both the single- and multiple-dose regimens.

DISCUSSION

The findings of this study indicate that altered urinary pH can significantly change the urinary excretion of doxycycline administered in multiple doses to humans. It also confirms results of previous single-dose (1) and multiple-dose (3) studies.

As was expected on the basis of previous work (1), alkalinization of the urine (mean pH range 7.4–8.0) in the single-dose study resulted in 60.8% of the dose excreted in the urine as compared to the more acidic control condition (mean pH range 5.3–6.3) where only 37.1% of the dose was excreted. This effect is thought to be due to doxycycline having its maximum lipid solubility at its isoelectric pH of 5.6 (2) where enhanced tubular reabsorption would be favored. The shift to the alkaline pH condition resulted in a smaller fraction of doxycycline being present in the lipid-soluble form and hence less reabsorption and greater excretion.

This trend was also shown for the multiple-dose regimen where the alkaline condition (mean pH range 7.7–8.4) resulted in 65.0% excretion of the administered dose as compared to 52.8% of the dose excreted on the control condition (mean pH range 5.6–6.7). The trend of greater excretion of doxycycline due to alkalinization of the urine was also reflected in significantly lower serum levels with both regimens.

Although renal clearances of doxycycline are significantly greater for the alkaline condition as compared to the control condition under either regimen, renal clearance values are essentially identical when comparing conditions on the single dose with the same conditions on the multiple dose. This would indicate that the renal clearance of doxycycline is not influenced by repetitive dosing, at least not with the regimens employed in this study.

Mean elimination rate constants for the single-dose as compared to the multiple-dose regimen are lower with both conditions. This decrease is reflected in increased biological half-lives during the multiple-dose regimen as compared to the single-dose study. A mean increase of 3.8 hr occurred when control conditions for single- and multiple-dose regimens were compared in this study. A greater increase in biological half-lives of doxycycline (6.2 hr) for the uncontrolled urinary pH condition was shown in a previous study (3), although these apparently greater differences could possibly be attributed to the relatively short sampling times employed in the single-dose portion of the study. This could lead to an underestimation of the biological half-life and cause the change from single to multiple dosing to appear greater than actually was the case.

Alkaline urinary pH conditions also resulted in an increase in mean biological half-lives (2.8 hr) when the single-dose regimen was compared to the multiple-dose regimen in this study. Thus, altered urinary pH will also show a trend of increased biological half-lives between single and multiple dosing, but this difference is not as great as that observed under normal (uncontrolled) urinary pH conditions.

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