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Effect of Sleep on Bioavailability of Tetracycline

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Abstract □ The effect of sleep on the bioavailability of tetracycline was studied using 250-mg tetracycline hydrochloride capsules from two commercial sources. One capsule of each brand was given to each of 12 healthy volunteers on two separate occasions. On one occasion, the subject ingested the drug upon arising in the morning and remained ambulatory (ambulatory trial). On the other, the subject awakened during the night, ingested the capsule, and resumed sleep (sleep trial). Bioavailability was assessed from the cumulative amount of drug excreted in the urine during the 96 hr following drug intake. While there was no significant difference in the bioavailability of one brand between the ambulatory and sleep trials, the bioavailability of the other decreased an average 27% ($p < 0.01$) in the sleep trial. Also, the difference between the bioavailability of the two preparations was not discernible in ambulatory subjects, but it was statistically significant ($p < 0.05$) in the sleep trial. The impaired bioavailability appears to result from decreased dissolution of one brand in the high gastric pH occurring at night. These findings raise questions as to the predictability of bioavailability studies in ambulatory subjects relative to the actual use of drug products, which are frequently administered during conditions of bed rest or sleep.

Keyphrases □ Tetracycline—bioavailability, different commercial capsules, effect of sleep, humans □ Bioavailability—tetracycline, different commercial capsules, effect of sleep, humans □ Sleep—effect on bioavailability of tetracycline, different commercial capsules, humans □ Antibacterials—tetracycline, bioavailability, different commercial capsules, effect of sleep, humans

The pharmacokinetics and pharmacodynamics of several drugs including sulfonamides, penicillin, streptomycin, griseofulvin, minocycline, indomethacin, and levodopa (1–8) may be subject to diurnal variations and may display considerable dependency on changes in the posture and state of wakefulness. Observed changes in blood drug levels, urinary excretion, and pharmacological effects generally were attributed to changes in distribution or elimination. However, decreased systemic availability would also provide a reasonable explanation for some observed results.

Gastric pH increases at night (9), and body position may greatly affect gastric emptying (10). These changes might significantly affect the absorption of some drugs, such as tetracycline, whose dissolution is pH dependent. Absorption of tetracycline from solid dosage forms is dependent upon rapid dissolution in acidic gastric fluids and subsequent gastric emptying of dissolved drug into the upper small intestine where absorption occurs (11–15).

The solubility and dissolution rate of tetracycline are much smaller at duodenal pH (4–5) than gastric pH (1–3), which would result in the decreased absorption of products that are incompletely dissolved in gastric fluids when emptying occurs (14, 15). Furthermore, decreased absorption of tetracycline from solid dosage forms occurs when gastric pH is increased. Concomitant administration of sufficient sodium bicarbonate to increase the gastric pH to 4–5 resulted in a 50% decrease in the bioavailability of tetracycline (14). In this study, tetracycline absorption in one individual who returned to sleep after drug ingestion was dramatically less than in all other subjects (14).

These considerations prompted this study on the bioavailability of two tetracycline products in ambulatory and sleeping individuals.

EXPERIMENTAL

Twelve apparently healthy volunteers (11 males and one female) participated. Each subject was administered one capsule containing 250 mg (labeled amount) of tetracycline hydrochloride with 200 ml of water either upon arising in the morning (ambulatory trial) or during the night (sleep trial). Two preparations, Brands A[1] and B[2], containing the same labeled amount of the drug[3], were used in each ambulatory and sleep trial, so that each subject participated in four separate trials. Brand A was tested first, and the trial condition was assigned randomly to the subjects by drawing lots. Then Brand B was ingested by all subjects in the sleep trial, followed by its administration to the subjects while ambulatory. At least 2 weeks separated each trial.

In the ambulatory trial, the drug was ingested in the morning between 8:00 and 9:00 am following an overnight fast. No food was allowed for at least the next 4 hr, and the subjects remained ambulatory for the rest of the day. In the sleep trial, the subjects were instructed to awaken at about the midpoint of their sleeping time, ingest the capsule, and directly resume sleep. Breakfast was taken upon arising in the morning, after which the subjects remained ambulatory. The average sleeping time was 6–8 hr, and the drug was ingested at about 4:00 am. Therefore, in both trials, food was withheld for at least 4 hr before and after drug ingestion.

No attempt was made to control the composition of the meal of the evening before or the day after drug administration, except that milk and dairy products were strictly forbidden on the 1st day of the study. No other medication was allowed for at least 7 days before and throughout the study. Immediately before taking the drug, the subjects voided completely, and this sample was saved and used as the urine blank sample. Urine was collected every hour for the first 8 hr after drug ingestion in the ambulatory trial or upon arising in the sleep trial and then

[1] Lot 202-101, Lederle Labs, Pearl River, N.Y.
[2] Lot 23/G, Gyma Lab., Forest Hills, N.Y.
[3] Chemical assay indicated that the amounts of drug present in Brands A and B were 262 and 258 mg, respectively.

Table I—Cumulative Amounts (Milligrams) of Tetracycline Excreted in Urine in 4 Days

Subject	Sex	Age	Weight, kg	Height, m	Brand A		Brand B	
					Ambulatory	Sleep	Ambulatory	Sleep
A	M	24	109	1.72	194.6	50.2	121.3	97.1
B	M	34	84	1.73	197.9	164.8	165.1	169.0
C	M	24	66	1.75	92.3	112.4	109.9	107.0
D	M	23	61.3	1.62	154.6	91.0	120.0	108.8
E	M	22	60	1.7	122.8	128.0	65.7	61.5
F	M	22	70.3	1.72	160.0	100.7	156.3	53.0
G	M	24	63.5	1.7	145.2	133.8	78.4	73.8
H	F	25	64.4	1.62	147.2	131.3	177.1	120.0
I	M	24	63.5	1.73	125.2	152.4	154.9	124.3
J	M	25	77.2	2.6	171.4	119.8	171.2	104.3
K	M	23	68.1	2.67	97.5	133.0	98.3	22.7
L	M	31	72.1	1.72	126.8	84.0	52.2	26.4
Mean					144.6	116.8	122.5	89.0
SD					33.7	31.5	42.9	42.8

ad libitum for a total of 96 hr. The volume of each sample was measured and an aliquot was frozen until analyzed.

The amount of tetracycline in the two preparations and the unchanged drug excreted in urine were assayed spectrophotofluorometrically[4] by the method of Kohn (16). This assay is specific for tetracycline and has a high degree of precision, as indicated by the 3–6% variations in replicate determinations of biological samples (16).

The results were analyzed according to the analysis of variance of split-plot in time design (17). Differences between the means of the four trials were tested for significance by Duncan's new multiple-range analysis (18).

RESULTS

The cumulative amounts of intact tetracycline excreted in urine in 96 hr (Qu^{∞}) in the four trials are reported in Table I. Large inter- and intrasubject variations were seen in the urinary recoveries. Six subjects (A, B, D, F, H, and J) of the 12 ingesting Brand A in the sleep trial had between an 11 and 74% decrease in Qu^{∞} compared with the ambulatory trial of the same brand. However, in four other subjects (C, I, K, and L), a 22–36% increase in Qu^{∞} in the sleep trial was observed. Subjects E and G excreted essentially the same amount of tetracycline in the sleep and ambulatory trials.

When Brand B was taken in the sleep trial, eight subjects (A, D, F, H, I, J, K, and L) showed a 10–77% decrease in Qu^{∞} compared with drug ingestion while ambulatory. The other four subjects excreted virtually the same amount of tetracycline in the sleep and ambulatory trials. Unlike with Brand A, no increase in Qu^{∞} was observed when Brand B was taken in the sleep trial.

The overall mean and standard deviation of the Qu^{∞} obtained with Brand A in the ambulatory and sleep trials were 144.6 ± 33.7 and 116.8 ± 31.5 mg, respectively. The mean Qu^{∞} and standard deviation of Brand B were 122.5 ± 42.9 mg in ambulatory subjects compared to 89.0 ± 42.8 mg in the same subjects taking the drug in the sleep trial, representing a decrease of about 27%.

Analysis of variance of these data and the multiple-range comparison between means are shown in Table II. Sleep had a differential effect on the two brands of tetracycline. Whereas no statistically significant difference in the mean Qu^{∞} was observed between the sleep and ambulatory trials of Brand A, the average urinary recovery of Brand B in the sleep trial was significantly reduced ($p < 0.01$) compared with its mean Qu^{∞} in the ambulatory subjects. Most importantly, in spite of the lack of significant difference between the two preparations in the ambulatory subjects, the difference between their mean Qu^{∞} in the sleep trials was statistically significant ($p < 0.05$).

If urinary recovery from Brand A during the ambulatory trial is taken as a standard, then Brand B administered at night during sleep showed a highly significant ($p < 0.01$), almost 39% decrease in the cumulative amount of tetracycline excreted in urine (Fig. 1).

Figure 2 shows a representative plot (Subject J) of urinary excretion rates of tetracycline as a function of time in the sleep and ambulatory trials. The inset figure indicates that the elimination kinetics of the drugs in the postabsorptive-distributive phase were essentially identical for both brands in these trials. Similar results were observed in all other subjects.

DISCUSSION

Generally, a decrease in urinary recovery of a drug under different experimental conditions may result from changes in several events involved in its absorption and/or disposition[5] including: (*a*) decreased renal excretion, (*b*) increased extrarenal elimination from the systemic circulation such as hepatic metabolism and biliary or other clearances, and (*c*) a decrease in the amount of drug reaching the systemic circulation (bioavailability).

A decrease in bioavailability may be characterized further as resulting from a decrease in the fraction of drug available for absorption from the luminal contents or an increase in the fraction of drug metabolized, stored in intestinal tissue, or cleared by biliary secretion during the initial transit through the hepatoportal system (first-pass effect).

Decrease in Elimination—The parallel slopes of the urinary excretion rate–time plots (Fig. 2) rule out the occurrence of changes in elimination kinetics during the postabsorptive-distributive phase (~8–10 hr after drug administration). However, this finding does not rule out the possibility of changes occurring in the elimination of tetracycline from the systemic circulation during the first few hours following its administration when the subjects were either sleeping or ambulatory. Since urinary excretion parameters were employed in the present study, it is logical to consider the possibility of decreased renal elimination at night during sleep as a result of alteration in urine pH or renal function.

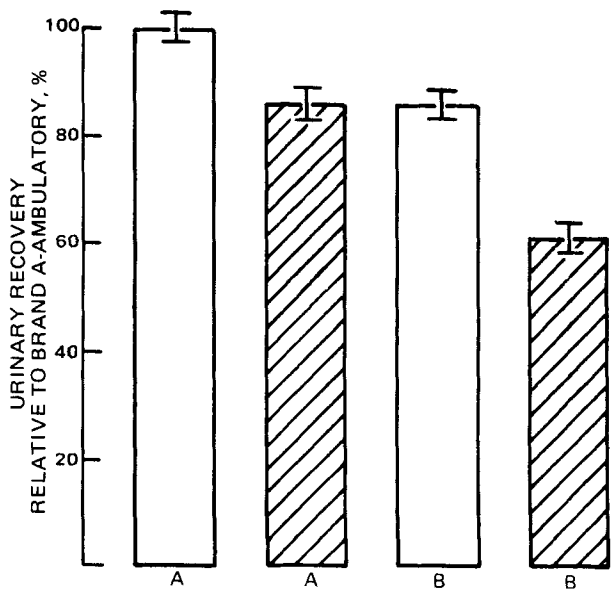

Figure 1—*Percent mean urinary recovery relative to Brand A administered to ambulatory subject when Brands A and B were administered to ambulatory subjects (open bars) and during sleep (hatched bars). The standard errors of the mean are indicated by the horizontal lines.*

[4] Aminco-Bowman model 4-8202, American Instrument Co., Silver Spring, Md.

[5] Changes in distribution are excluded from this discussion because of the obvious lack of reference to its parameters in the urinary excretion data.

Table II—Analysis of Variance[a] of the Cumulative Amounts of Tetracycline Excreted in Urine in 4 Days

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Sums of Squares	F	p
Subjects	11	34,601.9	3,145.6	3.27	<0.10
Conditions[b]	1	11,303.7	11,303.7	11.74	<0.05
Residual error	11	10,586.8	962.4		
Total	23	56,492.4			
Brands	1	7,465.0	7,465.0	8.49	<0.05
Brands × subjects	11	9,036.3	821.5	0.93	>0.10
Conditions[b] × brands	1	97.5	97.5	0.11	>0.10
Residual error	11	9,669.3	879.0		
Total	47	82,760.5			

Comparison of Trial Means[c]

$A_{ambulatory}$ *versus* A_{sleep}	Not significant at 0.05 level
$A_{ambulatory}$ *versus* $B_{ambulatory}$	Not significant at 0.05 level
$A_{ambulatory}$ *versus* B_{sleep}	Significant at 0.01 level
A_{sleep} *versus* $B_{ambulatory}$	Not significant at 0.05 level
A_{sleep} *versus* B_{sleep}	Significant at 0.05 level
$B_{ambulatory}$ *versus* B_{sleep}	Significant at 0.01 level

[a] Split-plot in time design (17). [b] Refers to ambulatory and sleep conditions. [c] Duncan's new multiple-range test (18).

Elliot *et al.* (19) observed a 0.77–2.48 unit rise in urinary pH in 12 of 18 normal subjects after awakening in the morning compared with urinary pH at night. This rise in pH, termed the morning tide, lasted 2–3 hr after arising (19), occurred irrespective of food intake (20), and was part of the diurnal variation in urinary pH, which appeared to follow a pattern characteristic for a given individual (21). A similar increase in urinary pH was observed in humans upon assuming a recumbent posture (22).

Alkalinization of urine pH (~7.5) over 48 hr significantly increased the cumulative amount of tetracycline excreted in urine (23). Based on this study and the presumed occurrence of high urinary pH in the morning, subjects who ingest the tetracycline capsules at 4:00 am should show an *increase* in urinary recovery of the drug compared with the ambulatory trials in which the drug is taken at 8:00–9:00 am. This assumption is made because the sleep trial drug absorption, which lasts between 3 and 4 hr (24), is essentially complete upon awakening and the presumably high urinary pH enhances tetracycline excretion in urine; the ambulatory subjects, who take the drug upon arising, cannot absorb significant amounts during the time their urinary pH remains alkaline.

The results shown in Table I do not support this logical corollary. Only four subjects (C, I, K, and L) displayed an increase in Qu^{∞} in the sleep trial of Brand A that was not manifested in the sleep trial of Brand B as would be expected from the consistency of the urinary pH profile of individuals (21). In fact, three of these subjects (I, K, and L) had a 20–77% decrease in the urinary recovery of Brand B and the fourth showed a meager 3% reduction. In addition, comparison between the means of the four trials indicates that sleep caused a significant differential reduction in Qu^{∞} of Brand B (Table II). These facts are difficult to reconcile with the notion that the observed decreases in urinary recovery of the drug in the sleep trials are due to its reduced renal excretion as a result of changes occurring in urinary pH.

The other factor that would account for the decreased urinary excretion of tetracycline in the sleep trial is the physiological alterations of kidney function. Little information is available on the effect of sleep, bed rest, or recumbent posture on the function of this organ. In a recent study employing seven catheterized sleeping patients, within 2 min of the onset of rapid eye movement (REM) epochs, there was a marked decrease in the urine flow rate; however, the glomerular filtration rate in these patients remained unaffected (25). At the termination of the REM state, the urine flow rate increased even beyond the baseline value.

In a study of renal function in 18 normal male subjects at 4-hr intervals over 24 hr (26), there was no change in renal plasma flow during sleep at night as compared to bed rest during the day. There was a slight, but statistically significant, drop in the glomerular filtration rates during the REM sleep between 12:00 midnight and 4:00 am, after which the glomerular filtration rate achieved a normal value. The urine flow rate, however, was diminished significantly during sleep, with the greatest drop occurring during the REM periods. This drop in the urine flow rate was attributed to increased tubular reabsorption of water (26). In another study, prolonged bed rest had no effect on the urine flow rate or kidney function (27).

These findings indicate that while the glomerular filtration rate of the subjects ingesting tetracycline at about 4:00 am could be considered reasonably similar to that of the ambulatory subjects, a comparison between their urine flow rates is difficult to make. It is particularly difficult to conjecture the effect of arising to ingest the drug and then resuming sleep on the urine flow rate. Nevertheless, other investigators found that changes in the urine flow rate had no effect on the renal clearance of tetracycline and three other analogs (28, 29). Although no information is available on the effect of this parameter on the fraction of tetracycline ultimately excreted in urine, it is inconceivable that such an effect would be demonstrated in the sleep trial of only one brand of the drug and not the other.

Increase in Extrarenal Elimination—The tetracyclines are not appreciably metabolized (30). In addition, biliary excretion of tetracycline in the rat does not constitute a major route of elimination, since 95% of the amount excreted *via* the bile is absorbed from the small intestine (31). Rather, excretion in the lower part of the small intestine and colon is believed to account for the extrarenal elimination, as stipulated for another analog of this antibiotic (32). If this is also the other extrarenal route of elimination of tetracycline in humans, then, to account for the observed decrease in Qu^{∞}, a compensatory increase in its elimination *via* this route must be expected. Although this possibility cannot be ruled out, some physiological modalities of sleep provide no support for its occurrence. For instance, blood pressure, which may affect organ perfusion, drops and intestinal function proceeds normally during sleep (33).

On the other hand, if extrarenal elimination of tetracycline in humans occurs *via* biliary excretion, this route of elimination may actually be diminished at night. Bile collected through a fistula in humans was lower at night, with a minimum between 1:00 and 5:00 am (34); the same was true for rabbits which are more active at night (35).

An increase in any other extrarenal route of elimination of tetracycline during sleep is unlikely because of the difficulty to explain its differential occurrence with two preparations of the same drug.

Decrease in Bioavailability—Previously (15) in ambulatory subjects, urinary excretion rates of tetracycline were proportional to serum concentrations, and the cumulative amount excreted in urine, Qu^{∞}, was proportional to the total amount absorbed as assessed by the area under the serum concentration–time curve (AUC). In both of these relationships, the proportionality constant, as predicted by theory, was equal to the renal clearance of the drug, namely 80–90 ml/min. Since changes in the glomerular filtration rate, urine pH, and renal and extrarenal elimination have been ruled out implicitly, the observed decrease in Qu^{∞} when tetracycline capsules are taken during sleep more likely represents a decrease in the bioavailability of the drug relative to its administration to ambulatory subjects.

Sleep reportedly causes a decrease in gastric, salivary, and lacrimal secretion, but it has no effect on intestinal function (33). The decrease in gastric acidity is likely to affect the dissolution of a drug such as tetracycline (14), which is favored by low pH, thereby causing a decrease in the extent of its bioavailability during sleep[6]. No information is available on the effect of sleep on gastric emptying, and its contribution to the decreased bioavailability at night cannot be ruled out. However, an increase in the fraction eliminated during the first pass through the hepatoportal system may be rejected on the basis of a lack of evidence

[6] Because of the inherent limitations of urinary excretion studies, alteration in the absorption rate of tetracycline at sleep cannot be established.

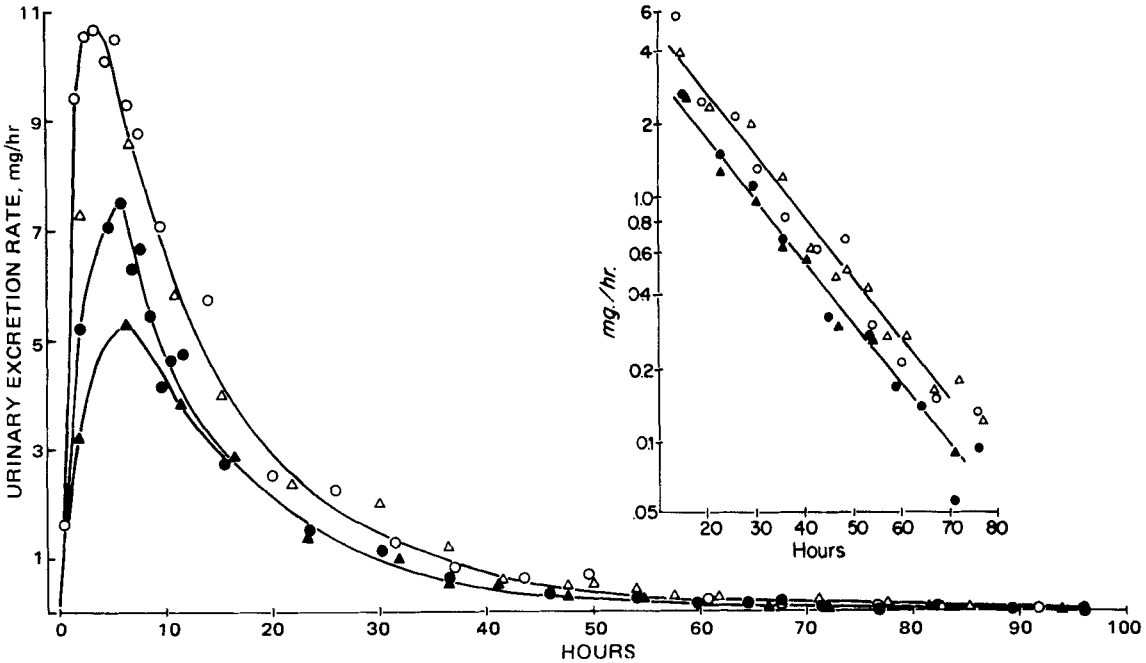

Figure 2—*Representative urinary excretion rates* versus *time plots for Subject J ingesting a tetracycline hydrochloride capsule while ambulatory (open symbols) and during sleep (closed symbols). Data for Brand A are designated by circles; Brand B by triangles. The excretion rates of the two brands in the ambulatory phase (open symbols) are superimposable.*

for the hepatic or biliary clearances of tetracycline (30–32).

In addition to dissolution, other important considerations suggest and possibly contribute to the decreased bioavailability of tetracycline during sleep. While the difference in the mean Qu^{∞} between the two brands in the 12 ambulatory subjects was not large enough to be statistically significant, the difference between them in the sleep trials was significant (Table II). In other words, differences between the bioavailability of the two brands conceivably could have been demonstrated in a larger number of *ambulatory* subjects, but only 12 *sleeping* subjects were needed to detect these bioavailability differences.

In addition, the bioavailability of Brand A in the ambulatory subjects was statistically similar to its bioavailability in the sleep trial, but it was almost 39% greater ($p < 0.01$) than the bioavailability of Brand B in the sleep trial (Fig. 1). Poor formulation, coupled with the sleep effect, could have produced the marked impairment in the bioavailability of Brand B. This situation is implied also by the lack of interaction between brands and conditions (Table II), which may characterize an additive effect between two factors (36).

The difference in formulation is illustrated also by the intersubject variability of the two brands. In general, this variability was smaller in Brand A than B. The coefficients of variation for A in the ambulatory and sleep trials were 23.3 and 27%, respectively; the coefficient of variation of B in ambulatory subjects was 35%, compared with 48% in the same subjects ingesting the drug during sleep. This increased variability is characteristic of products with low bioavailability (37).

CONCLUSIONS

The results of the present study involving two brands of tetracycline under the specific conditions of the trials indicate that the ingestion of one brand of tetracycline hydrochloride capsule during sleep resulted in a decrease in the bioavailability of the drug compared with its administration to ambulatory subjects. Obviously, these results cannot be extrapolated to other drugs or even other brands or lots of the same drug. In addition, although it was previously shown that differences between tetracycline products observed in single-dose studies are quantitatively predictive of multiple-dose plasma levels in ambulatory subjects (15), further studies are needed to determine whether the effect of sleep also is observed upon multiple-dose administration.

These findings, however, provide one example of an interaction between physiological and formulation variables which must be considered in the design and interpretation of bioavailability studies. Questions are raised as to the predictability of ambulatory studies relative to the actual clinical use of drug products frequently administered during conditions of bed rest or sleep. Most important is the fact that differences in products that were not statistically demonstrable in the usual ambulatory subjects were evident in the more clinically realistic situation involving sleep.

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Hydrolysis and Epimerization Kinetics of Hetacillin in Aqueous Solution

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Abstract □ Methods were developed for quantitating epimerization to epihetacillin and hydrolysis to ampicillin in the alkaline degradation of hetacillin, and both rates in deuterium oxide at 35° and in water at various temperatures were determined. In each case, plots of log k for the epimerization against pH or pD yielded straight lines with a positive slope, which verified the first-order dependence on the hydroxide ion or deuteroxide ion. The activation energy of the epimerization process was 21.2 kcal/mole. In aqueous solution at high pH, epimerization rather than conversion to ampicillin represents a major pathway of hetacillin degradation, although the β-lactam ring of the hetacillin molecule is highly resistant to attack by the hydroxide ion.

Keyphrases □ Hetacillin—epimerization and hydrolysis in aqueous solution, effect of pH and temperature □ Epimerization—hetacillin in aqueous solution, effect of pH and temperature □ Hydrolysis—hetacillin in aqueous solution, effect of pH and temperature □ Antibacterials—hetacillin, epimerization and hydrolysis in aqueous solution, effect of pH and temperature

Hetacillin, which was developed to improve the aqueous stability of concentrated ampicillin solutions and to increase oral absorption of ampicillin, is a condensation product of ampicillin and acetone (1) and is often used instead of ampicillin.

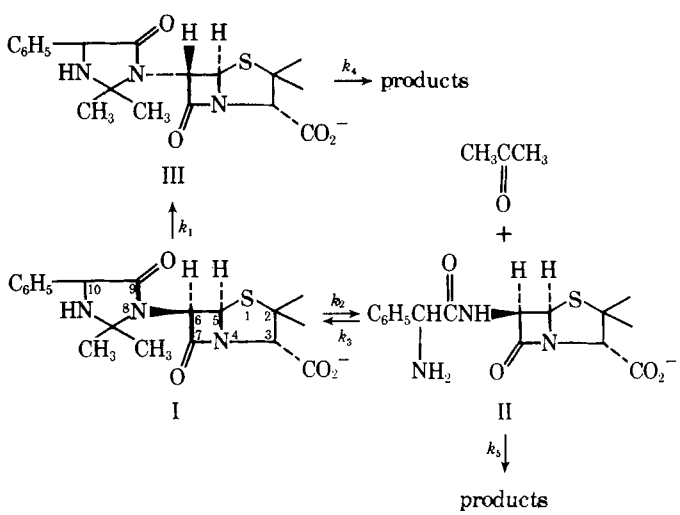
In the degradation of hetacillin (I) in aqueous solution, at least two reactions are possible: hydrolytic interconversion to ampicillin (II) (2, 3) and epimerization to 6-epihetacillin (III) (4), which produces inactive 6-epiampicillin (5). The rates of both hydrolysis (2, 3, 6) and epimerization (7) depend on pH and occur simultaneously (Scheme I). Although detailed kinetics of the hydrolysis of hetacillin to ampicillin were reported (2, 3, 6, 8–10), much less is known about the kinetic behavior of the C-6 epimerization reaction.

The present investigation was undertaken to develop methods for quantitating epimerization of hetacillin and to determine the rates of hydrolysis and epimerization of hetacillin in aqueous basic solution by utilizing NMR and optical rotatory dispersion spectroscopy. The preliminary NMR observations were reported previously (7).

EXPERIMENTAL

Materials—Hetacillin potassium[1] and ampicillin sodium[2] were used as supplied.

Epihetacillin was synthesized by a reported procedure (4). Two grams of hetacillin potassium was placed in 20 ml of distilled water, and the



Scheme I

[1] Banyu Pharmaceutical Co., Tokyo, Japan.
[2] Takeda Chemical Ind., Osaka, Japan.