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* Present address: University of Southern California, School of Pharmacy, Department of Pharmacology, University Park, Los Angeles, CA 90007

Effect of Sex on Penicillin Blood Levels in Dogs

JOHN W. POOLE*

Abstract ☐ Studies concerned with the oral absorption characteristics of dicloxacillin from various pharmaceutical formulations in beagle dogs suggest that the female of this species shows consistently higher and more prolonged blood serum levels of the antibiotic than the corresponding male. An investigation was initiated to determine the extent of this phenomenon with respect to sodium dicloxacillin monohydrate and other penicillins. The results of these studies suggest that the monobasic penicillin molecules show blood serum level variations after oral administration that are related to the sex of the animal, while a similar correlation does not appear to exist for the amphoteric penicillins. Determination of the biological half-life of the various penicillins in the male and female after intravenous administration indicates that no sexual differences occur with respect to the disappearance of active drug from the blood.

Keyphrases ☐ Penicillin blood levels—dogs ☐ Sex effect, dogs—penicillin blood levels ☐ Blood levels, half-life, corticosteroids—sex effect, dogs ☐ Microbiological test method—analysis

Minor sexual differences in drug response among the animals of a species are frequently encountered in toxicity experiments. For example, L-thyroxin produces a more pronounced depressant effect on weight gain in male than in female rats (1), and the toxicity of hypoglycemic agents is enhanced in female and male rats pretreated with diethylstilbestrol (2). These differences are sometimes quite significant. For example, the antibiotic acetoxycyclohexamide was shown by Pallotta *et al.* (3), in acute and subacute tests, to be about four times as toxic for young female rats as it is for males. In many cases, these differences in response between the sexes can be traced to differences in enzyme activities and rate of metabolism. Male rats metabolize hexobarbital much faster than do females, and the average sleeping time of the male after receiving the drug is only about one-fourth that of the female (4, 5). Recently, in a report by Kernohan and Todd (6), it was suggested that women bleed more readily than men during heparin therapy. A similar conclusion, that women have a higher risk of bleeding with heparin than do men, was made from the studies of Jick *et al.* (7).

The results obtained in a series of investigations on the effect of dosage-form variables on biological avail-

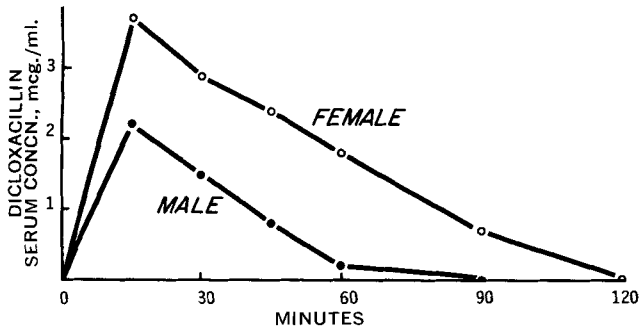

Figure 1—*Mean blood serum concentration of dicloxacillin in male and female beagle dogs after oral administration of 250-mg. doses.*

ability of dicloxacillin in beagle dogs indicated that the female of this species demonstrated consistently better utilization of this drug than the corresponding male. This was shown by higher peak blood serum levels and greater areas under the blood serum level–time curves after oral administration of the antibiotic to female dogs than after similar dosing to the males. A study was initiated to determine the extent of this phenomenon with respect to penicillin compounds as a class.

Specifically, the blood serum levels attained after oral administration of the monobasic penicillins, dicloxacillin and nafcillin, and the amphoteric penicillin, ampicillin, to male and female beagle dogs were determined. The biological half-lives of these drugs in this species were also determined in both sexes after intravenous administration of these compounds. In addition, the results obtained with several other penicillins used in various studies designed to evaluate dosage-form variables have been considered with respect to a difference in biological utilization of these agents between male and female beagle dogs.

EXPERIMENTAL

Compounds—The following were used: ampicillin, anhydrous (Wyeth Laboratories, C-10575); sodium nafcillin monohydrate (Wyeth Laboratories, W-663989); and sodium dicloxacillin monohydrate (Wyeth Laboratories, C-10651).[1]

Procedure—The general procedure used in the blood serum level studies follows. Six dogs, three of each sex, were utilized in each experiment. The test animals were dosed with the appropriate drug in a strict crossover manner. The dogs were fasted overnight; the antibiotic was administered by means of a stomach tube, followed by a standard amount of distilled water. Blood samples were drawn at the appropriate times postadministration and analyzed by a microbiological method utilizing *Sarcina lutea* as the test organism.

In studies in which the drug was administered intravenously, the penicillin was dissolved in an appropriate amount of physiological saline solution just prior to administration.

RESULTS AND DISCUSSION

In a series of investigations concerned with the effect of dosage-form variables on the biological utilization of dicloxacillin, oral administration of the various dosage forms containing this agent to female beagle dogs resulted in significantly higher and greater area under the blood serum level–time curves than after similar administration to male animals of the same species. A typical example of such a study is shown in Fig. 1. In this instance the female animals showed a mean peak serum level of the antibiotic

[1] Dosage forms of the several penicillins employed in these studies were prepared by the Development Section of the Pharmacy Research and Development Division, Wyeth Laboratories, Inc.

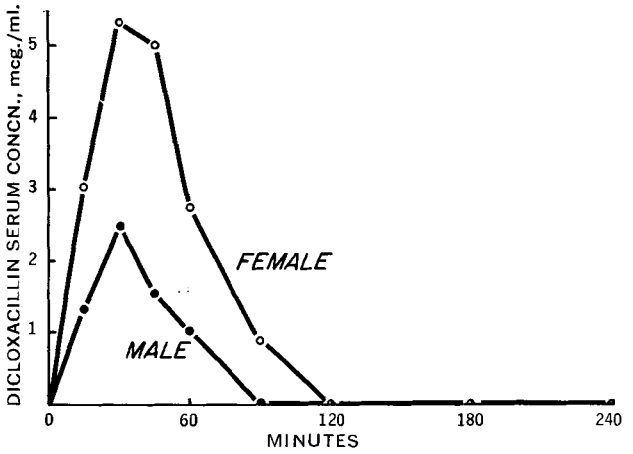

Figure 2—*Mean blood serum concentrations of dicloxacillin in male and female beagle dogs after oral administration of 25-mg./kg. doses.*

of 3.7 mcg./ml., while the male animal showed a mean peak serum level of 2.2 mcg./ml. The areas under the blood serum level–time curves calculated for each sex were 1.20 and 3.34 mcg./ml. × hr. for the male and female dogs, respectively. These results were influenced to some extent by the fact that discrete dosage units containing 250 mg. of the active moiety were administered to each animal in a crossover experiment. Because the females were, generally, slightly smaller than the male animals, this factor may have had a significant effect on the results observed. In addition, the possibility that the females used were "good" absorbers of this agent and the males "poor" absorbers was also considered.

To rule out the significance of these factors, a study was designed in which a 25-mg./kg. dose of penicillin was administered to the two sexes of this species in the form of a powder slurried in water. All the animals used in the initial experiments were omitted from this study. In addition to dicloxacillin, two other penicillins were included in the study, nafcillin and anhydrous ampicillin.

Figure 2 illustrates the difference in blood serum levels attained in this experiment with male and female animals after administration of dicloxacillin at the 25-mg./kg. dose. These results are in agreement with the earlier data obtained utilizing this drug in pharmaceutical dosage forms. The females in this instance showed a mean peak serum level of 5.4 mcg./ml., while the mean level with the males was 2.5 mcg./ml. Similarly, the areas under the blood serum level–time curves were determined to be 4.96 and 1.72 mcg./ml. × hr. for the female and male animals, respectively.

Figure 3 shows the results obtained when nafcillin was administered at the 25-mg./kg. level to beagle dogs of both sexes. In this instance, the females attained a mean peak serum level of 0.68

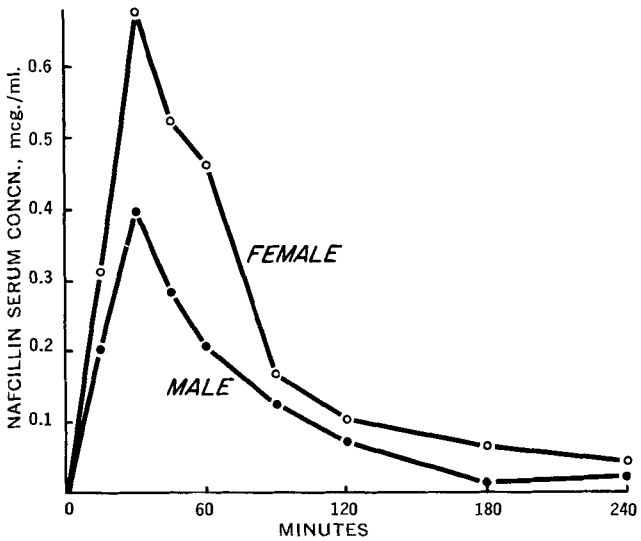

Figure 3—*Mean blood serum concentrations of nafcillin in male and female beagle dogs after oral administration of 25-mg./kg. doses.*

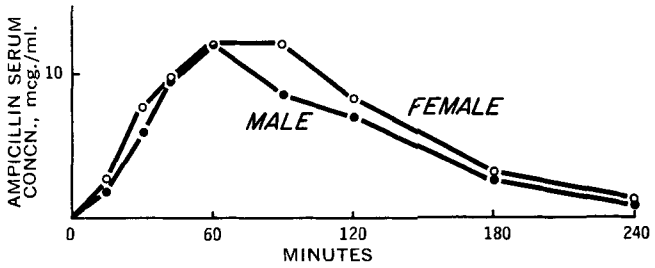

Figure 4—*Mean blood serum concentrations of ampicillin in male and female beagle dogs after oral administration of 25-mg./kg. doses.*

mcg./ml., while the males showed a mean peak serum level of 0.40 mcg./ml. The areas under the blood serum level–time curves for the females and males were calculated to be 0.81 and 0.46 mcg./ml. × hr., respectively.

However, as shown in Fig. 4, after administration of anhydrous ampicillin to animals of both sexes, no significant difference in mean peak serum levels attained or in the area under the blood serum level–time curves between the sexes was observed. The mean peak serum levels attained in this case were 12.1 and 12.2 mcg./ml., and the areas under the blood serum level–time curves were 21.8 and 26.0 mcg./ml. × hr. for the males and females, respectively. Table I summarizes the mean peak serum levels and curve areas obtained in this investigation. The blood serum level data obtained in this study indicate that the monobasic penicillins, such as dicloxacillin and nafcillin, show a significant difference in biological utilization of these agents between male and female beagle dogs. The results obtained with the amphoteric penicillin, ampicillin, did not demonstrate any significant differences in blood serum levels between the sexes.

The results obtained in subsequent experiments utilizing pharmaceutical dosage forms of penicillin G, a monobasic penicillin, and Wy-4508 [6-(1-aminocyclohexanecarboxamido)penicillanic acid], an amphoteric penicillin, are in agreement with the results summarized in Table I.

Figure 5 shows the mean blood serum levels obtained for penicillin G in male and female beagle dogs after oral administration of 500 mg. of the drug as buffered tablets. As was the case with the monobasic penicillins, the female showed a significantly better overall biological utilization of this agent than did the male animal. A mean peak serum level of 58 units/ml. was attained in the females, while a mean peak serum level of 25 units/ml. was noted in the males. Similarly, the areas under the blood serum level–time curves for the female and male animals were determined to be 80 and 48 units/ml. × hr., respectively.

Figure 6 illustrates the results obtained in a study of male and female beagle dogs after oral administration (250 mg.) of a pharmaceutical formulation of the amphoteric penicillin, Wy-4508. The results show that there is no significant difference in the blood serum levels attained between the male and female animals or in the overall biological utilization of this agent after such administration. The peak serum levels obtained were 31.5 mcg./ml. for the male and 27.5 mcg./ml. for the female. The areas under the blood serum level–time curves were 36.0 and 36.6 mcg./ml. × hr. for the males and females, respectively.

One possible mechanism by which the observed sex difference in blood serum levels could be explained is a difference in elimination rate (biological life) for the monobasic penicillins in the males and females. To test this possibility, an intravenous dose of the two

Table I—Peak Blood Serum Level and Areas under Blood Serum Level–Time Curve after Oral Administration of Various Penicillins (25 mg./kg.) to Male and Female Beagle Dogs

Compound	Peak Level, mcg. ml. Male	Peak Level, mcg. ml. Female	Area under Curve, mcg./ml. × hr. Male	Area under Curve, mcg./ml. × hr. Female
Nafcillin[a]	0.40	0.68	0.46	0.81
Dicloxacillin[a]	2.5	5.4	1.72	4.96
Ampicillin	12.1	12.2	21.8	26.0

[a] Administered as the sodium salt of the monohydrate.

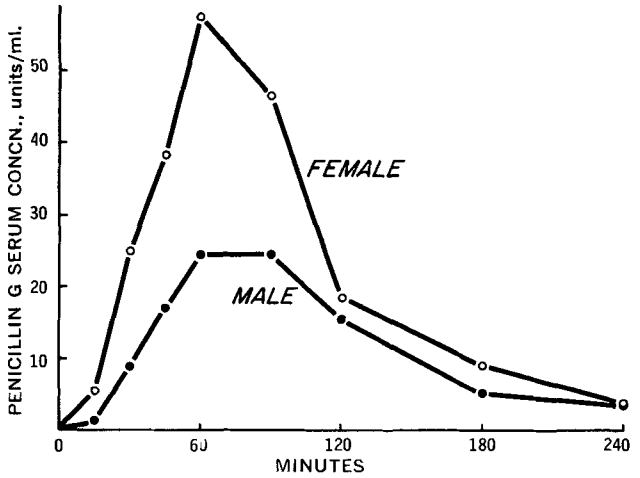

Figure 5—*Mean blood serum concentrations of penicillin G in male and female beagle dogs after oral administration of 500-mg. doses.*

monobasic penicillins, nafcillin and dicloxacillin, along with the amphoteric penicillin, ampicillin, was administered to beagle dogs of each sex and the blood serum level decay followed. The dogs used in this study were the same animals employed in the oral absorption studies with these penicillins. The biological half-life for ampicillin in both sexes was similar, as was expected, on the basis of the oral blood level data. The average half-life values were 35 and 34 min. for the male and female, respectively. However, neither of the monobasic penicillins, dicloxacillin or nafcillin, employed in this study showed a difference in the biological half-life for the male and female animals. The average biological half-life for nafcillin was determined to be 10 min. in each sex, while administration of dicloxacillin resulted in an average biological half-life of 16 min. for the male and 18 min. for the female. The results of these studies are summarized in Table II, where the average biological half-life for the various penicillin products in the male and female animals is listed along with the range of half-lives obtained. These results indicate that the differences in peak serum levels and areas under the blood serum level–time curves noted for the male and female dogs with monobasic penicillins are apparently not related to a difference in elimination rate of these drugs in the two sexes. Other possible mechanisms which may be involved in the differences in serum levels noted are: (*a*) a difference in the metabolism of the monobasic penicillins in the gut wall of the male and female animals or (*b*) a difference in acidity in the gastrointestinal tract of the male and female beagle dogs. This latter hypothesis could account for the lack of any sex difference noted with amphoteric penicillins which would not be as sensitive to the acid environment of the gastrointestinal tract as monobasic penicillins.

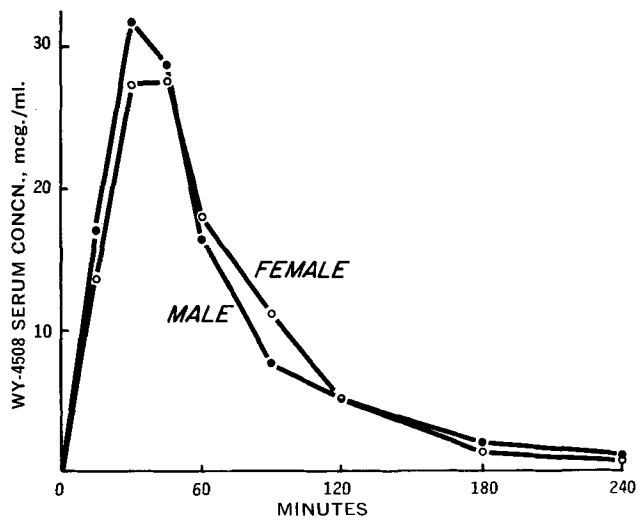

Figure 6—*Mean blood serum concentrations of Wy-4508 in male and female beagle dogs after oral administration of 250-mg. doses.*

Table II—Biological Half-Life of Various Penicillins in Male and Female Beagle Dogs after Intravenous Administration

Sex	Half-Life, min.		
	Nafcillin[a]	Dicloxacillin[a]	Ampicillin
Male	10 (9–11)[b]	16 (12–20)[b]	35 (29–39)[b]
Female	10 (9–11)[b]	18 (15–22)[b]	34 (28–38)[b]

[a] Administered as the sodium salt of the monohydrate. [b] Range of half-life observed.

In any event, the sex of the test animal should be considered in studies concerned with *in vivo* effects of monobasic penicillins in which dogs, particularly beagles, are used. The effect of the animal's sex on the biological utilization of such compounds in other species and in human subjects is not known at this time. Studies in humans are of interest since differences of the magnitude observed in the animal studies may be clinically significant.

SUMMARY

The biological utilization, as demonstrated by blood serum levels and area under the serum level–time curves, for a series of penicillin compounds in male and female beagle dogs has been determined. Results obtained indicate that the female of this species attained a higher mean peak serum level and a greater area under the blood serum level–time curve than did the male after oral administration of the monobasic penicillins studied: dicloxacillin, nafcillin, and penicillin G. The amphoteric penicillins employed, ampicillin and Wy-4508, showed no significant difference in blood serum levels or curve areas after oral administration to male or female beagle dogs. There was no significant difference between the sexes in the elimination rate (half-life) of the monobasic penicillins, sodium dicloxacillin monohydrate and sodium nafcillin monohydrate, or of the amphoteric penicillin, ampicillin, after intravenous administration, which rules out this factor as an explanation for the difference in serum levels noted. A possible reason for the sex difference in serum levels in beagle dogs may be due to a difference in acidity of the gastrointestinal tract and/or to a difference in the gut wall metabolism of the monobasic penicillins in the male and female of this species.

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* Fellow, American Foundation for Pharmaceutical Education.

Pharmacokinetic Analysis of Potentiating Effect of Phenylbutazone on Anticoagulant Action of Warfarin in Man

ROBERT A. O'REILLY* and GERHARD LEVY†

Abstract □ Warfarin is eliminated more rapidly but its anticoagulant effect is increased by concomitant administration of phenylbutazone. Pharmacokinetic analysis by recently developed techniques shows that the prewarfarin synthesis rate and the normal degradation of prothrombin complex activity are not affected by phenylbutazone, but that this drug has a pronounced effect on the relationship between synthesis rate of prothrombin complex activity and plasma–warfarin concentration. These observations are consistent with the assumption that phenylbutazone competitively displaces warfarin from nonspecific binding sites in the plasma and tissues (particularly the liver) and thereby increases the interaction of the anticoagulant with its pharmacologic receptor and metabolizing enzyme system.

Keyphrases □ Warfarin activity—phenylbutazone effect □ Phenylbutazone effect, warfarin activity—pharmacokinetics □ Pharmacokinetics—phenylbutazone potentiation, warfarin activity □ Biologic half-life, warfarin—phenylbutazone effect

Drug interactions are usually of three types. A drug may increase or decrease the elimination rate constant of another by stimulating or inhibiting drug metabolizing enzymes, by changing urine pH or the flow rate of urine or bile, and/or by affecting the distribution of the other drug in the body. Another type of drug interaction involves the potentiation or inhibition of the pharmacologic effect of one drug by another without measurably affecting its kinetics of elimination. The third type of drug interaction is one where the gastrointestinal absorption of one drug is increased or decreased by the other. The coumarin anticoagulants exemplify all three of these effects. Induction of microsomal enzymes by heptabarbital increases the rate of elimination of warfarin and bishydroxycoumarin without affecting the relationship between anticoagulant effect and plasma–coumarin concentration in man (1, 2). Heptabarbital apparently also decreases the gastrointestinal absorption of bishydroxycoumarin (3) by mechanisms which are still being studied.

A particularly interesting interaction is that between warfarin and phenylbutazone. The former is more rapidly eliminated in the presence of phenylbutazone, but its anticoagulant effect in man is increased (4).