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Plasma Protein Binding and Urinary Excretion of *R*- and *S*-Epimers of an Arylmalonylamino 1-Oxacephem II: In Rats

Keyphrases □ Stereoisomers, arylmalonylamino 1-oxacephem—new β-lactam antibacterial agent, renal clearance, plasma protein binding, rats □ Renal clearance—new antibacterial agent, arylmalonylamino 1-oxacephem stereoisomers, effect of plasma protein binding, comparison of rat and human data □ Protein binding, rat plasma—new antibacterial agent, arylmalonylamino 1-oxacephem stereoisomers, effect on renal clearance, comparison with human data

To the Editor:

In Part I (1), we reported that the *R*-epimer of an arylmalonylamino 1-oxacephem derivative {7β-[2-carboxy -2- (4-hydroxyphenyl)acetamido] -7α- methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl] -1- oxa-1-dethia-3-cephem-4-carboxylic acid disodium salt (I)}, a new antibacterial agent, was excreted into the urine faster than the *S*-epimer in humans. It was concluded that the phenomenon was due to the difference between the binding of human plasma protein with *R*- and *S*-epimers.

To confirm that conclusion, studies were carried out using rats in which I was not metabolized and was excreted mainly into the urine, as in humans. The extent of protein binding of the *R*- and *S*-epimers in rat plasma was measured by the same method described previously (1). Unlike in humans, there was no difference in plasma protein binding between the *R*- and *S*-epimers in rats (Fig. 1). Thus, taking into consideration our conclusion in the previous study (1), the urinary excretion rates of the *R*- and *S*-epimers in rats should be the same.

To confirm this prediction, the plasma concentration and urinary excretion rate of the *R*- and *S*-epimers after intravenous injection of I (70 mg/kg) to three rats were measured by the method described previously (1). The plasma concentration–time curves for the *R*- and *S*-epimers were the same (Fig. 2), and the renal clearance values were 0.85 ± 0.04 and 0.83 ± 0.04 ml/min/100 g for the *R*- and *S*-epimers, respectively. As expected, there was no difference in the renal clearance in rats between these epimers.

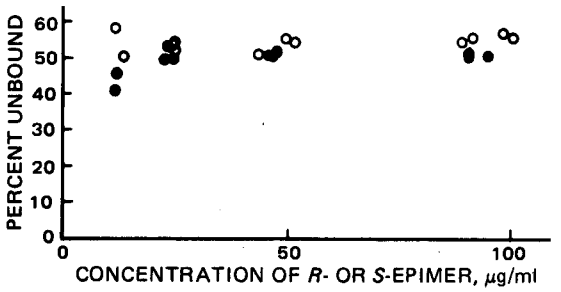

Figure 1—*Rat plasma protein binding of* R- (○) *and* S- (●) *epimers of I.*

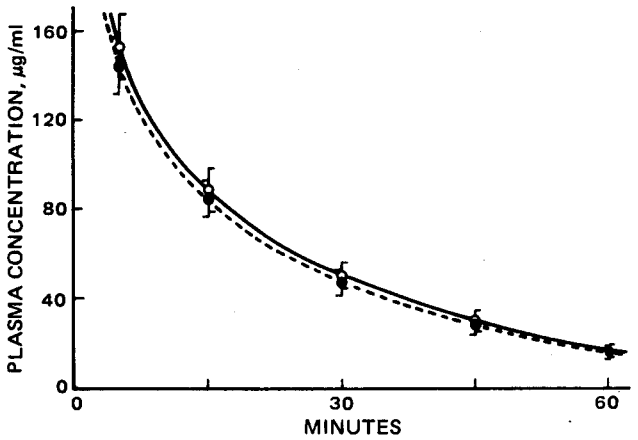

Figure 2—*Plasma concentration of* R- (—○) *and* S- (- -●) *epimers after intravenous administration of I. Each data point gives the mean and standard deviation of three rats.*

These stereospecific differences in plasma protein binding have been reported for other drugs (2–5) and vary between species.

In vitro studies[1] of the protein binding of I in dog plasma showed that the percentage of the unbound fraction of the *R*-epimer was larger than that of the *S*-epimer; these results were similar to those on human plasma protein binding. This finding suggested that urinary excretion of the *R*-epimer was faster than that of the *S*-epimer in dogs, as in humans, and this prediction was confirmed experimentally[1].

These findings support the previous conclusion that the difference in the urinary excretion rate between *R*- and *S*-epimers of I in humans is due to the difference in protein binding of human plasma between these epimers.

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Lowering Blood Urea Nitrogen with Amino Acid Supplementation

Keyphrases □ Amino acids—dietary supplementation, effects on blood urea nitrogen, rats □ Colorimetry—analysis of blood urea nitrogen in rat serum, effects of dietary supplementation of amino acids □ Dietary supplements—amino acids, effects on blood urea nitrogen in rats

To the Editor:

Currently we are studying the effect of dietary supplementation of amino acids on alcohol metabolism. The amino acid augmentation is based on the fasting plasma profile theory (1–4).

From past work on the metabolic effects of such diets, it was found that they produce a significant decrease in serum cholesterol in rats (5) and humans (6). This observed effect was theorized to be the result of an increase in net protein utilization.

While conducting the present study, we discovered that dietary supplementation of limiting amino acids to the third level (L-lysine, L-tryptophan, and L-threonine), based on the fasting plasma profile theory, produces a significant decrease in serum blood urea nitrogen.

Twenty male Sprague–Dawley rats, 250–275 g, were randomly divided into two groups of 10 each. Following a 2-week acclimation period during which both groups were fed a standard animal diet[1], the first group was fed a diet supplemented to the third level of limiting amino acid (Table I) for 2 additional weeks while the second group was maintained on the standard diet for the same period.

On the 15th day, blood collected by orbital sinus puncture was centrifuged, and the serum was retained. Blood urea nitrogen was measured colorimetrically by means of a standard kit[2].

The serum blood urea nitrogen for the treatment group was 8.45 ± 0.36 mg/100 ml (mean ± *SEM*) while that of the control group was 15.1 ± 0.49 mg/100 ml. A Student *t* test showed a significant difference to the $p < 0.01$ level.

It is theorized that the observed effects are caused by an increase in net protein utilization when the limiting amino acids are supplemented, based on the fasting plasma profile theory, and that this increase reduces the amount of underutilized amino acids that are otherwise available for energy metabolism or storage. The end result is a decrease in the amount of nitrogenous metabolic products (wastes) in serum.

[1] Purina Laboratory Chow.
[2] Sigma Chemical Co., St. Louis, MO 63178.

Table I—Amino Acid-Supplemented Animal Feed to the Third Level of Limiting Amino Acid

Ingredient	Amount, %
L-Tryptophan	0.309
L-Threonine	0.341
L-Lysine	1.667
Standard feed	97.683

This type of dietary supplementation can be of invaluable assistance to those who must reduce their blood urea nitrogen levels (dialysis patients, nephrotic patients, *etc.*).

The major advantage of the augmentation of the body's amino acids based on the fasting plasma profile theory is that it removes a lot of guesswork and establishes a hard mathematical formula for preparing the diet.

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Effect of Variation of Plasma Oleic Acid Concentration on Relative Concentration of Free and Protein Bound Warfarin

Keyphrases □ Warfarin—effect of plasma oleic acid concentration on free and protein bound warfarin concentration, protein binding, humans □ Protein binding—binding of warfarin to human serum albumin, effect of plasma oleic acid concentration on free and bound warfarin concentration □ Oleic acid—effect of concentration on free and protein bound warfarin concentration, humans

To the Editor:

Although the normal serum concentrations of free fatty acids are between 0.3 and 0.9 mmole/liter (1), pathological conditions such as diabetes, reduced renal function, cardiac infarction, and bacterial disease (2–12) can cause substantial increases in free fatty acid levels. In some cases, these levels can exceed 5 mmoles/liter. Moreover, the increasingly widespread use of intravenous fat emulsions probably is associated with significant variation in the serum concentrations of free fatty acids.

Schwartz *et al.* (13) recently demonstrated that variation in the concentration of oleic acid, a major component in the free fatty acids found in serum, can cause significant variations in the protein binding of salicylate. Similar findings concerning the effect of variation in free fatty acid concentrations on the protein binding of drugs were published previously (14–17).

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