mately was excreted. The remainder of the dose may be accounted for by other minor metabolites (*e.g.*, oxalic acid and ascorbate 2-sulfate) (10–12), or a portion of the dose may remain in the ascorbate body pool. Approximately 84% of the recovered vitamin was excreted in the form of ascorbic acid.

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Comparative Pharmacokinetics of Coumarin Anticoagulants XXXVII: Simultaneous *In Vivo* Displacement of Dicumarol from Serum Protein and Tissue Binding Sites by Tolbutamide in Rats

Keyphrases □ Dicumarol—*in vivo* binding to serum proteins and tissues, effect of tolbutamide in rats □ Tolbutamide—effect on *in vivo* binding of dicumarol to serum proteins and tissues in rats □ Binding, serum protein and tissue—dicumarol *in vivo*, effect of tolbutamide in rats □ Anticoagulants, coumarin—dicumarol, *in vivo* binding to serum proteins and tissues, effect of tolbutamide in rats □ Antidiabetic agents—tolbutamide, effect on *in vivo* binding of dicumarol to serum proteins and tissues in rats

To the Editor:

The ability of some drugs and endogenous substances to displace other drugs or endogenous substances from serum or plasma protein binding sites has been studied extensively. However, there is only very limited information on such displacement effects in various organs and tissues, probably because of the technical difficulties of such investigations, which must be carried out *in vivo* (1). Recently developed pharmacokinetic theory indicates that a full appreciation of the pharmacokinetic implications of changes in drug binding can only be obtained by assessing the magnitude of such changes in serum as well as in tissues (2). During a study of pharmacokinetic and pharmacodynamic interactions between dicumarol and tolbutamide, we have found evidence that tolbutamide administration causes a displacement of dicumarol not only from serum proteins but also from binding sites in the liver.

Thirty adult male Sprague–Dawley rats, 250–350 g, received ^{14}C-dicumarol, 8 mg/kg iv, and the pharmacokinetics of this drug were determined by methods described previously (3) (Experiment I). Twenty of these animals were matched into pairs with nearly identical total clearance values. One month later, these rats again received a dicumarol injection (Experiment II), but one member of each pair also received tolbutamide, 125 mg/kg ip at the time of dicumarol administration and 62.5 mg/kg every 3–4 hr thereafter while the other member received saline injections at these times. The animals were killed when serum dicumarol concentrations were expected, by extrapolation of earlier concentrations (determined during the experiment), to have declined to about 7 μg/ml. The liver was removed and blood was expressed from it; it was homogenized, and the homogenate was assayed for dicumarol.

Another six pairs of rats (not from the original group of 30) with nearly identical dicumarol clearance received only tolbutamide (125 mg/kg for the first dose and then 62.5 mg/kg every 3–4 hr for 30 hr) or saline, but no dicumarol, one month after Experiment I. They were killed 3 hr after the last dose of tolbutamide; plasma was obtained from blood withdrawn from the abdominal aorta and diluted 1:10 with pH 7.4 isotonic sodium phosphate buffer. The liver was excised and homogenized with three volumes of ice-cold phosphate buffer. ^{14}C-Dicumarol, 10 μg/ml final concentration, was added to both plasma and liver homogenate, and the samples were dialyzed at 25° against an equal volume of phosphate buffer to equilibrium (4).

The effects of tolbutamide on the pharmacokinetics of dicumarol when both drugs were administered *in vivo* are summarized in Table I. Intentionally, animals with widely different total clearance values were selected for the study (range in Experiment I: 3.33–16.8 ml/hr/kg), so standard deviations are reported only for the mean of the individual ratios of various parameter values. The average total clearance, the volume of distribution, and the β value of the control and tolbutamide groups were almost identical in Experiment I; these values were also nearly identical in Experiments I and II of the control group. These results reflect the excellent matching of the two groups and the reproducibility of the pharmacokinetic parameter values in repeated experiments.

Concomitant administration of tolbutamide caused a pronounced increase in the total clearance and β and a smaller, but still statistically significant, increase in the volume of distribution (Experiment II, tolbutamide group). The free fraction of dicumarol in diluted plasma (mean ± *SD*, $n = 6$) was 0.000754 ± 0.000156 for control animals and 0.00213 ± 0.00095 for tolbutamide-treated animals ($p < 0.01$). A method to determine protein binding of dicumarol in undiluted plasma was not available when these studies were done.

Since tolbutamide administration was acute, it is unlikely to have caused enzyme induction. This conclusion

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Table I—Effect of Tolbutamide on the Pharmacokinetics of Dicumarol in Rats [a]

Pharmacokinetic Parameter	Control Group			Tolbutamide Group		
	Experiment I	Experiment II	Ratio, II/I	Experiment I	Experiment II	Ratio, II/I
Total clearance, ml/hr/kg	8.60	8.46	1.04 ± 0.18[b]	8.17	18.7[c]	2.40 ± 0.49[c]
Apparent volume of distribution, ml/kg	133	129	0.979 ± 0.081	126	156[c]	1.27 ± 0.26[c]
β, hr^{-1}	0.0651	0.0648	1.06 ± 0.184	0.0649	0.118[c]	1.90 ± 0.32[c]
Concentration in serum/concentration in liver[d]	—	2.15 ± 0.42	—	—	1.90 ± 0.40[c]	—
Amount in liver/amount in body[d]	—	0.113 ± 0.040	—	—	0.0887 ± 0.027[c]	—

[a] All animals received dicumarol, 8 mg/kg iv. Experiment II was carried out 4 weeks after Experiment I, at which time the tolbutamide group also received tolbutamide, 125 mg/kg ip at zero time and 62.5 mg/kg every 3–4 hr thereafter while the control group received saline injections at these times. There were 10 rats in each group. [b] All ± values are ± *SD*. [c] $p < 0.005$ compared to the corresponding value in the control group. [d] The serum concentrations at the time of determination were 7.13 ± 1.08 and 6.95 ± 0.70 μg/ml for control and tolbutamide groups, respectively.

is confirmed by the fact that the liver weights were not increased (mean ± *SD*: 10.7 ± 1.6 g and 10.4 ± 1.2 g for control and tolbutamide-treated animals, respectively). The pronounced increase in dicumarol clearance caused by tolbutamide is consistent with the strong displacing effect of the latter on the former in plasma and with the previously demonstrated direct proportionality between dicumarol clearance and the free fraction in serum (3). This direct proportionality suggests a 2.4-fold increase of the dicumarol free fraction in plasma (since clearance increased by that magnitude), similar to the change in the free fraction observed *in vitro*.

The *in vivo* serum to liver concentration ratio of dicumarol was only slightly affected by tolbutamide treatment; it decreased by about 12% on the average (Table I). This finding indicates that tolbutamide administration resulted in a displacement of dicumarol not only from plasma protein but also (and to an almost similar extent) from hepatic tissues. Had there been no appreciable displacement of dicumarol from binding sites in the liver, the plasma or serum to liver concentration ratio of dicumarol should have decreased by about 60%.

Confirmation of the displacing effect of tolbutamide (and, possibly, its metabolites) on dicumarol in the liver was obtained from a partly *in vitro* experiment. Homogenates were prepared from the livers of control rats and tolbutamide-treated rats, and the binding of dicumarol in these homogenates was determined following the *in vitro* addition of dicumarol. The free fraction of dicumarol in these homogenates (mean ± *SD*, $n = 6$) was 0.0121 ± 0.0050 for control rats and 0.0283 ± 0.0110 for tolbutamide-treated animals ($p < 0.01$). Thus, *in vivo* administration of tolbutamide caused a pronounced displacement of dicumarol from hepatic binding sites upon *in vitro* addition of the latter. While presently used methods for the *in vitro* determination of drug binding to tissues have pronounced limitations (1), the liver homogenate data can serve as supportive evidence, particularly since the displacing agent was administered *in vivo*.

The *in vivo* pharmacokinetic study revealed that tolbutamide treatment caused an increase in the apparent volume of distribution of dicumarol as well as a significant decrease in the ratio of the amount of drug in the liver to the amount of drug in the total body (estimated as concentration in plasma times apparent volume of distribution). This effect suggests a shift of the anticoagulant from the plasma and liver to other tissues. One may infer, therefore, that the displacing effect of tolbutamide on dicumarol in these other tissues considered together (*i.e.*, as a weighted average) is less pronounced than in the plasma and liver.

Recently developed pharmacokinetic theory suggests that changes in tissue binding can be more important than changes in plasma protein binding as a cause of alterations in the biological half-life of drugs (2). In view of these theoretical considerations and the indications of drug displacement from tissue binding sites reported here, it is important that future pharmacokinetic studies of drug interactions, particularly in animals, be designed to include assessment of possible displacement of drugs from tissues.

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Absorption of Saccharin from Rat Urinary Bladder

Keyphrases □ Saccharin—absorption from urinary bladder, rats □ Absorption, urinary bladder—saccharin in rats □ Sweeteners—saccharin, absorption from urinary bladder, rats

To the Editor:

The renal excretion of drugs and chemicals has generally been considered an elimination route. The absorption or reabsorption of compounds from the urinary bladder has not been studied widely. I have been studying saccharin pharmacokinetics in the rat and have discovered unusual plasma and urinary profiles for the compound. During renal clearance studies, it became apparent that saccharin was cleared from the plasma more quickly when the urine was removed from the urinary bladder at 5-min intervals than when it was left for 60 min. This communication discusses this observation and describes the absorption (reabsorption) of saccharin from the urinary bladder of the rat.

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