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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Table I—Plasma Concentrations (Nanograms per Milliliter) following *In Vivo* Instillation of Various Doses of Saccharin into the Urinary Bladder of the Rat

Minutes after Dosing	10 μg/kg		1 mg/kg		100 mg/kg	
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6
5	4.62	2.94	81.6	130.0	25,200	13,500
15	3.95	3.30	105.0	173.0	39,500	18,300
30	3.88	2.66	100.0	137.0	48,200	18,100
45	3.85	3.52	82.7	114.0	53,000	17,500
60	3.28	3.23	73.8	113.0	53,000	13,800
90	4.62	3.37	73.6	92.3	51,400	14,200
120	4.32	2.87	63.2	88.8	39,100	11,900
Fraction of dose in urinary bladder at 120 min	0.43	0.48	0.57	0.69	0.58	0.80

^{35}S-Saccharin sodium was instilled in the urinary bladder of pentobarbital-anesthetized male Sprague–Dawley rats. Three groups of two rats, 200–240 g, were administered 10-μg/kg or 1- or 100-mg/kg doses in equimolar sodium solutions (pH 6.5) of 1 ml/kg *via* a bladder cannula. Blood samples were withdrawn through a jugular vein cannula at 5, 15, 30, 45, 60, 90, and 120 min after bladder instillation (1). At 120 min, the urine–dose solution was removed from the urinary bladder. Plasma samples and urine–dose solutions were counted for ^{35}S-activity by liquid scintillation counting.

The 1-mg/kg dose is comparable to a single-dose ingestion of diet soft drink or one cup of coffee with two saccharin tablets (~70 mg). The 10-μg/kg and 100-mg/kg doses were chosen to study the possible dose dependence in saccharin pharmacokinetics in the rat.

Since saccharin is not metabolized in the rat (2), ^{35}S-activity measurements can be assumed to reflect only saccharin. The results of the bladder instillation experiments are presented in Table I. Saccharin was absorbed extensively from the urinary bladder of the rat. These data, in conjunction with data from previously reported oral absorption studies (3) and data following intravenous administration from continuing studies in this laboratory, indicate that the absorption from the bladder is slower than from the GI tract and that approximately 50% to equal amounts of the bladder dose ultimately will reach the systemic circulation when compared to the oral route. Preliminary bladder absorption studies also indicate that the extent of initial absorption may be dose and pH dependent in the rat; *i.e.*, greater amounts reach the systemic circulation at higher doses and at higher pH.

In summary, significant absorption of saccharin occurs from the urinary bladder in the rat. The continued high plasma concentrations following bladder instillation are probably, at least in part, due to the closed system created by inhibiting urination during the present experiment. However, significant reabsorption of saccharin would also decrease the elimination rate for the compound in much the same way as would enterohepatic cycling and probably accounts for the retention of saccharin in urinary bladder tissue (3). The use of the rat as an acceptable model for carcinogenicity studies with saccharin will be highly dependent on interspecies differences with respect to the absorption phenomenon.

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Adsorption of Fluorouracil on Glass Surfaces

Keyphrases □ Fluorouracil—adsorption on glass surfaces □ Adsorption—fluorouracil on glass surfaces □ Antineoplastic agents—fluorouracil, adsorption on glass surfaces

To the Editor:

The determination of the cytostatic drug fluorouracil in plasma has been described (1–6). The experimental procedures leading to the isolation of fluorouracil from biological fluids and the subsequent sensitive determination are rather complicated (5) and sometimes require expensive equipment (3, 4). In attempts to design a GLC assay of underivatized fluorouracil, we encountered a phenomenon that could be responsible for problems during its analysis: when standard solutions of fluorouracil in methanol in a glass vial were evaporated under nitrogen and redissolved in methanol in the same vial, less fluorouracil was found than in a corresponding amount of the original standard solution. This finding prompted us to study the possible adsorption of fluorouracil on glass surfaces using GLC as well as radioactive measurements.

Standard solutions of fluorouracil[1] in methanol (0.05–0.6 μg/μl) were prepared in closed plastic containers. Aliquots of 10 μl of these standard solutions (0.5, 1, 2, 4, and 6 μg of fluorouracil) were evaporated under nitrogen in glass and plastic vials. The residue was redissolved carefully in 100 μl of methanol; 10 μl of this solution was analyzed by GLC.

The absolute peak heights of the fluorouracil samples were compared with those of the standard solutions in the plastic containers (Fig. 1). An almost quantitative recovery was obtained from the plastic vials, whereas a significant loss of fluorouracil was observed in glass vials.

The experiments were repeated with standard solutions

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