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 Blood samples were withdrawn through a jugular vein cannula at 5, 15, 30, 45, 60, 90, and 120 min after bladder instillation (1).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

[1] Supplied by Hoffmann-La Roche B.V.

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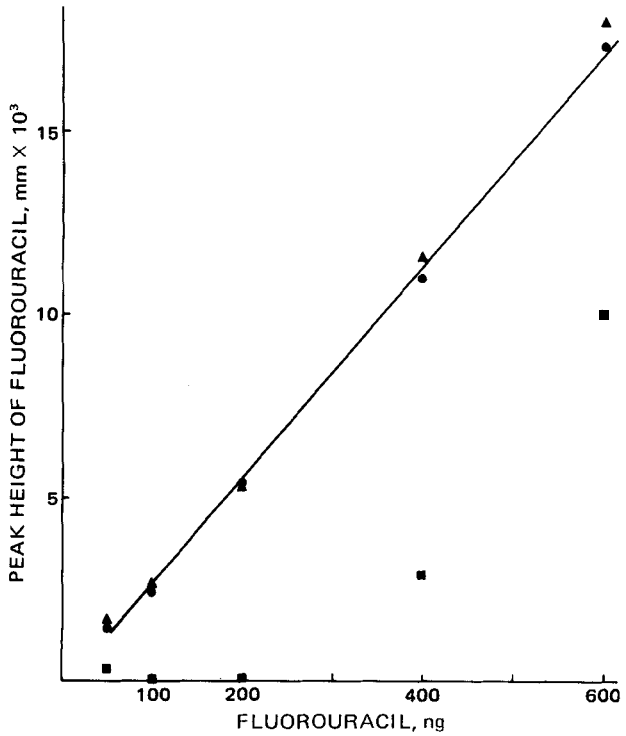

Figure 1—*Recovery of fluorouracil after evaporation of the test solution and reconstitution in plastic (▲) and glass (■) containers as compared to the initial solution (●).*

now enriched with 6-^{3}H-fluorouracil[2], thus permitting a radioactivity control of the GLC results. The recoveries of fluorouracil from glass and plastic vials obtained by the radiochemical determination were essentially the same as in Fig. 1.

The experiments were also extended to other materials. Silanized glass vials, several types of plastic (polyethylene and polypropylene) vials, and small Eppendorf tubes were investigated for their adhesive behavior. Adsorption was observed for glass, whereas fair to almost complete recoveries were found for silanized glass and plastic tubes.

Thus, a simple step in procedures used in the extraction of fluorouracil can produce a substantial loss of the compound in glass vials. We ascribe the observed loss to adsorption on the glass surface. So far, we have no indication that fluorouracil in solution also adheres to glass. However, we try to avoid any contact of fluorouracil with nondeactivated glass surfaces.

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Problems with Iodometric Assay of Penicillin V Benzathine

Keyphrases □ Penicillin V benzathine—iodometric analysis, interference by benzathine molecule □ Benzathine molecule—interference in iodometric analysis of penicillin V benzathine □ Iodometry—analysis, penicillin V benzathine, interference by benzathine molecule □ Antibacterials—penicillin V benzathine, iodometric analysis, interference by benzathine molecule

To the Editor:

Although antibiotics have traditionally been assayed by microbiological techniques, other chemical procedures are now used because of their advantages of speed, precision, and economy. The iodometric method for penicillins (1) is based on the observation that certain products of the inactivation of penicillin, but not the pure active substance, show a marked consumption of iodine.

Two identical samples are taken from a solution of penicillin. One is inactivated with alkali (or penicillinase) and then neutralized with acid. The same amount of iodine is added to both samples and, after a time, the excess iodine is back-titrated with thiosulfate. The difference in the consumption of iodine is a function of the amount of penicillin present.

In the original method, the pH of the iodine reaction solution was about 2. Ortenblad (2), however, found that the method did not always give reproducible values. At pH 4.5, the method was more accurate in the presence of iodine-absorbing impurities and more reproducible since the blank and the inactivated solutions are buffered at the same pH (2). However, with penicillin G procaine, the procaine interfered with the determination at a pH higher than 4.6. This modified procedure is the basis of the present European Pharmacopoeia (3) methods for penicillins G and V.

In 1959, Weiss (4) reviewed the factors affecting the reproducibility of the iodometric method, particularly interference from other antibiotics and excipients used in combination with penicillin. This interference is significant only in the blank determination part of the assay where it is evidenced by iodine absorption. In these cases, reducing the pH of the blank solution to below 2.0 almost completely eliminated the problem. Nevertheless, the present Code of Federal Regulations (CFR) procedure (5), which has recently become the conclusive assay for penicillins G and V and their salts (6), uses an iodine solution of about pH 4.

In the iodometric assay, a sample and reference standard of the same chemical species are normally analyzed under parallel conditions, and this method is probably one of the most rapid, accurate, and specific chemical tests available for a biologically active compound. With the benzathine