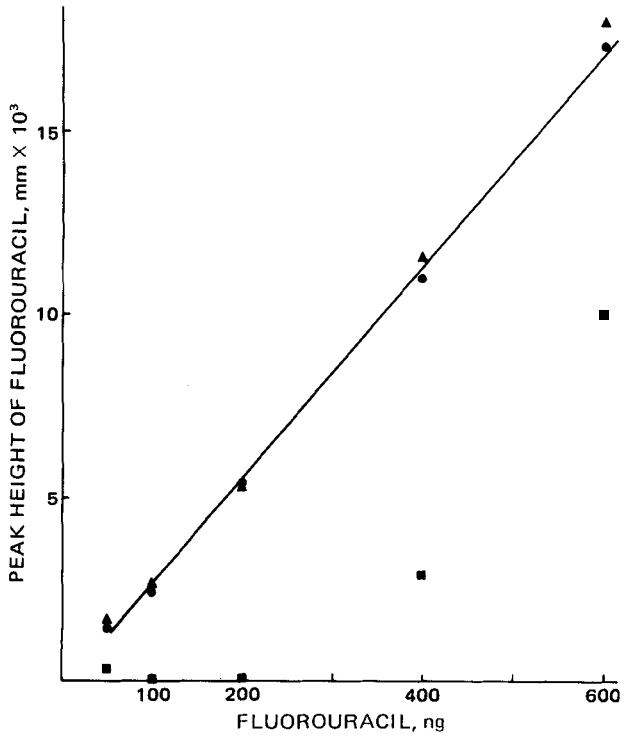

Figure 1—*Recovery of fluorouracil after evaporation of the test solution and reconstitution in plastic (▲) and glass (■) containers as compared to the initial solution (●).*

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

Table I—Analysis of Oral Suspensions

Manufacturer	Reagent	
	Set A	Set B
1	50.95[a]	54.16
2	49.97	54.15

[a] Expressed as milligrams of penicillin V per gram of oral suspension to avoid the necessity of a specific gravity determination.

Table II—Analysis of Synthetic Mixtures of Penicillin V and Benzathine

Benzathine Added, mg	Penicillin V, mg		Recovery[a], %
	Added	Found	
	Set A		
Control	101.24	101.24	100
20.7	98.90	93.70	94.7
41.4	98.83	89.02	90.1
82.7	102.17	84.94	83.1
	Set B		
Control	102.92	102.92	100
20.7	99.86	99.88	100.0
41.4	104.49	102.05	97.7
82.7	100.22	92.10	91.9

[a] Relative to assigned value of 100% for penicillin V control.

salts of penicillins V and G, however, these products are assayed against penicillin V free acid and penicillin G sodium standards, respectively. In developing a high-performance liquid chromatographic (HPLC) assay for the penicillin V content of all penicillin V acid and salt formulations, we doubted the accuracy of the results obtained by the official CFR iodometric method for the penicillin V benzathine pure drug substance and formulations. That the erroneous results were caused by interference of the benzathine portion of the molecule was shown by the following experiments.

Two sets of reagents (iodine and thiosulfate) were used. Set A was prepared according to the CFR (5), and Set B differed only in that the pH of the iodine solution was adjusted to 2.8 [as originally suggested by Weiss (4)] and the thiosulfate solution was stabilized with chloroform (1 ml/liter) instead of sodium carbonate. Two samples of penicillin V benzathine powder for oral suspension from different manufacturers were reconstituted according to the label instructions and were assayed against the USP penicillin V reference standard on the same day by the same analyst with both sets of reagents.

The results in Table I are averages of two determinations and show that the CFR reagents gave lower potency values for both products. A similar discrepancy was obtained when a sample of penicillin V benzathine pure drug substance was assayed. No difference was encountered, however, when penicillin V free acid was assayed with both sets of reagents. The addition of common excipients, propylparaben, methylparaben, sorbitol, and sodium benzoate, to the penicillin V free acid had no effect on results.

The analysis of synthetic mixtures of benzathine and penicillin V provided evidence that the interference was caused by benzathine. For each set of reagents, approximately 100 mg of penicillin V was dissolved in 2 ml of methanol in four 100-ml volumetric flasks. Aliquots of 0, 10, 20, or 40 ml of a solution of benzathine (2.07 mg/ml) in 1% phosphate buffer (pH 6.0) were then added, the solutions were diluted to volume with buffer, and 2-ml aliquots were determined iodometrically. The results (Table II) conclusively indicate that iodine reacts with benzathine in the blank titration, leading to inaccurate values not only with the CFR method but, to a lesser extent, with the modified iodine reagent as well. Penicillin V benzathine contains approximately 25% benzathine, and Table II indicates that one would expect results from the CFR iodometric method to be from 5 to 10% low. With the more acidic iodine reagent, this error is reduced to about 2%.

The reaction of the iodine with benzathine no doubt involves one or both of the secondary amines in the benzathine molecule. This reaction explains the reduced interference with the iodine reagent at lower pH since the amine is probably present as the hydrochloride salt instead of as the free base. A reaction product has been isolated, and work is continuing to determine its structure and the mechanism of the reaction.

These results indicate that the iodometric assay as presently described (5) is unacceptably inaccurate when applied to the analysis of the benzathine salts of penicillins G and V and that its use as a regulatory procedure should be reevaluated. Alternatives include lowering the pH of the blank solution with accompanying reproducibility problems (2, 7), adoption of more specific methods such as HPLC, and use of the benzathine salts of the penicillins as the reference standards.

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