Hydrochlorothiazide Interference with Urinary Estriol Determination

Arthur F. Rosenthal and Marie R. Tomson

The commonly used diuretic, hydrochlorothiazide, interferes with the determination of urinary estriol, by destroying the latter during acid hydrolysis. Urine specimens from pregnant patients taking this diuretic may therefore show falsely low estriol values.

Additional Keyphrases acid and enzymatic hydrolysis of estriol conjugates • pregnancy urine • chlorothiazide • acetazolamide • low urinary estriol excretion • placental function assessment

Determination of 24-h excretion of urinary estriol has become an important means of assessing placental function during the third trimester of pregnancy. Among the common causes of risk for which estriol excretions are serially monitored are pre-eclamptic states involving some degree of renal insufficiency, for which various diuretics are frequently prescribed. We have observed what is apparently an instance of true chemical interference with estriol determinations by one commonly used diuretic, hydrochlorothiazide ("Hydrodiuril," Merck Sharp & Dohme; "Esidrix," Ciba).

Possible drug interference was suggested when we found very low estriol values after normal amounts in a patient who subsequently delivered a normal child free of any evident placental abnormality. Hydrochlorothiazide had been administered to this woman just before the apparent precipitous drop in estriol excretion.

Procedure and Results

Effects on pregnancy urine. In our laboratory, estriol is routinely analyzed in pregnancy urine by a gas chromatographic method involving a single ether extraction after acid hydrolysis (1). The most likely point of interference seemed, a priori, to be at the hydrolysis step, in analogy with the well-known effect of glucose (2). To test this possibility three widely used diuretics—chlorothiazide, hydrochlorothiazide, and acetazolamide ("Diamox," Lederle)—were added to pregnancy urine and the recovery of the estriol was determined. Initially, these materials were added as the appropriate pharmaceutical preparation, to test the effects of other components as well as of the active ingredient. The amounts used per 24-h specimen were based on the following dosages of active ingredient (a) chlorothiazide (50 mg/ml oral suspension), 500 mg; (b) sodium acetazolamide (100 mg/ml parenteral preparation), 500 mg; and (c) hydrochlorothiazide (50 mg oral tablet), 50 mg. Recovery of estriol from the urine was: with added chlorothiazide, 100%; with hydrochlorothiazide, 0%; with acetazolamide, 100%.

We then examined the effects of pure hydrochlorothiazide (from Merck Sharp & Dohme) on urinary estriol determinations. This compound is essentially insoluble in cold neutral solutions or in acidic aqueous solutions such as urine diluted with hydrochloric acid (6 mol/liter) for hydrolysis of estriol conjugates (1). The drug is also difficultly soluble when such solutions are heated. Nevertheless, to avoid any effects of organic solvents, various amounts of solid hydrochlorothiazide were added to pregnancy urines, which were then acid-hydrolyzed and analyzed for estriol (Table 1).

It has been our impression that estriol can be only partially recovered from the urine of patients who have taken hydrochlorothiazide when the estriol conjugates

<table>
<thead>
<tr>
<th>Table 1. Estriol Found in Urine of Two Patients, before and after Addition of Hydrochlorothiazide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrochlorothiazide added, mg/5 ml urine</td>
</tr>
<tr>
<td>------------------------------------------</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
</tr>
<tr>
<td>1.0</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>0.2</td>
</tr>
<tr>
<td>1.0</td>
</tr>
</tbody>
</table>

From the Department of Laboratories, The Long Island Jewish Medical Center, New Hyde Park, N. Y. 11040.
Received Dec. 27, 1971; accepted Jan. 17, 1972.
are hydrolyzed enzymatically rather than by the use of hot acid. In order to test this directly, we added hydrochlorothiazide (1 mg) to a 5-ml aliquot of urine and acetate buffer (0.1 mol/liter, pH 4.5) was added followed by a β-glucuronidase-sulfatase preparation (containing 100,000 Fishman units and 10,000 Whitehead units; Calbiochem, San Diego, Calif. 92112). The mixture was shaken overnight at 37°C. Estriol was extracted and analyzed; recovery was 98% of the control value. Recovery of estriol was similar when either chlorothiazide or acetazolamide was added to urine. However, the thiazides were very poorly dissolved in the enzymatic hydrolysis mixture.

Effects on estriol standards. The above experiment strongly suggested, however, that hydrochlorothiazide exerts its interference only at the hydrolysis stage and that it does not affect the subsequent analytical steps. To test this directly, we added hydrochlorothiazide to estriol standards in male urine (1 mg/5 ml aliquot of urine), with and without acid hydrolysis, and the recovery of estriol determined (Table 2).

Effects in patients. Table 3 shows two examples that are typical, in our experience, of the effects of hydrochlorothiazide administration on the recovery of estriol from the urine of pregnant patients. Little or no additional estriol was recovered when the urines of patients receiving hydrochlorothiazide were treated with ammonium sulfate or were enzymatically hydrolyzed.

Discussion

These results indicate a severe negative chemical interference by hydrochlorothiazide with the determination of urinary estriol, the estriol being destroyed at the acid hydrolysis stage. As compared with the similar effect of glucose (2), hydrochlorothiazide interferes much more. Curiously, the very closely related chlorothiazide has no such effect.

The lack of effect of added hydrochlorothiazide on the enzymatic hydrolysis of urinary estriol, which is contrary to our limited experience with enzymatic hydrolysis of the urine of patients taking this drug, may be explained by the virtually complete insolubility of the diuretic in the enzymatic hydrolysis mixture. It is possible that hydrochlorothiazide as normally excreted by the kidney is solubilized in some way that allows some possible second effect of the drug in inhibiting enzymatic hydrolysis to be manifest. We have no unequivocal data confirming such inhibition, however, and merely suggest this as a possibility and advise caution in using enzymatic hydrolysis as a means of analyzing urinary estriol in patients taking hydrochlorothiazide.

The quantities of hydrochlorothiazide that are necessary to produce drastic effects on urinary estriol determination in vitro are about two to five times the usual dose given to patients. The greater effects of the drug in vivo are perhaps explainable by its slow solubility even in hot aqueous acid solutions (assuming the destruction of estriol to be a somewhat slow reaction) or by the above-mentioned hypothetical solubilization that increases the interfering effect of the naturally excreted drug. Hydrochlorothiazide is believed to be excreted primarily through the kidneys, with little or no metabolic alteration (3), so that most of the administered dose appears unchanged in the succeeding 24-h urine. A third possibility is that additional interfering effects may be obtained from other drugs sometimes concomitantly administered in the treatment of pre-eclamptic states.

Foster and Hochholzer (4) have shown the noninterference of a variety of drugs, including hydrochlorothiazide, on urinary estriol determination, but only in the sense that they produce no peaks near estriol during gas chromatography. With this we concur, and since these authors apparently did not test the effects of these drugs on the recovery of estriol taken through the acid hydrolysis, their results cannot be considered to conflict with ours in any way.

We conclude that patients should not have taken hydrochlorothiazide for at least 48 h before beginning a 24-h urine collection for estriol analysis. Neither ammonium sulfate precipitation nor (we believe) enzymatic hydrolysis is adequate to overcome interference caused by the diuretic with estriol determination.

References