It is simply an incidental finding in many unrelated conditions or in apparently normal individuals. As evidenced by our patients and documented in the literature (2, 6, 7) it is most commonly found in patients who are experiencing abdominal pain and who are often diagnosed as having pancreatitis. Macroamylase assays were not requested in any of our three patients; pancreatitis was a principle diagnosis being considered or treated in all of these patients.

Three types of macroamylasemia have been recognized. Type I, the classical form, is characterized by high serum and low urinary amylase activity in the presence of a normal rate of excretion of creatinine. In type II, urinary amylase is above normal and the proportion of macroamylase to normal amylase in the serum is less than in type I. Thus, the traditional criterion that above-normal urinary amylase activity excludes macroamylasemia is invalid. Type III macroamylasemia is associated with normal amylase activity in both the serum and the urine (4); it has no known clinical significance.

We conclude that assay for macroamylase by the PEG method of Levitt and Ellis (1) is indeed rapid, reproducible, and suitable for routine use in the laboratory. The results from this method compared favorably with those by gel-filtration chromatography or electrophoresis. PEG precipitation thus is our method of choice for routine screening for macroamylase in this community-hospital laboratory.

References
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Improved Assay of Unconjugated Estriol in Maternal Serum or Plasma by Adsorption and Liquid Chromatography with Fluorimetric Detection

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In this improved assay only a 500-μL sample is needed and a single assay can be done within 30 min, 10 samples within 90 minutes. The sample of serum or plasma is diluted 20-fold with water, and the estriol is adsorbed from it onto graphitized carbon black (Carbopack B, Supelco). After two washings the estriol is desorbed with chloroform/methanol (60/40 by vol), which then is evaporated. The residue is redissolved in 50 μL of water/acetonitrile, and 20 μL is injected into the chromatograph. Analytical recovery for estriol-supplemented serum or plasma averaged 98.6%. Day-to-day CVs ranged from 3.9% at 2 μg/L to 2.1% at 20 μg/L. The limit of sensitivity is 0.3 μg/L, which makes this procedure suitable for determination of estriol even in the first half of pregnancy. Our method is inexpensive, and shows that liquid chromatography can be used to determine estriol in pregnancy serum or plasma. It also is more sensitive and precise and requires less sample than other such methods.

Additional Keyphrases: monitoring pregnancy · estriol

Since Gurpide et al. (1) first elaborated an immunassay for determining estrogens in biological liquids, many radioimmunoassay (RIA) methods have been proposed for monitoring estriol in maternal serum, and this technique is currently the most widely used. Sensitivity, specificity, simplicity, and rapidity are features claimed for many RIAs. Specificity, however, is not so excellent as claimed. Sample purification by extraction with solvent or adsorbent before RIA improves accuracy, but at the expense of rapidity and simplicity (2, 3). Moreover, for clinical laboratories with a low demand for serum estriol assays the short shelf-life of the reagent makes these techniques uneconomical.

Only a few years ago the only valid alternative technique for measuring estriol concentrations in maternal serum was gas chromatography with use of a capillary column (4). This time-consuming technique involves many steps in sample preparation and requires highly specialized technicians.

"High-performance" liquid chromatography (HPLC) is increasingly becoming more reliable and practicable for routine laboratory assays. During the course of our experiments, two papers appeared reporting determination of unconjugated estriol in pregnancy serum by HPLC (5, 6).

We describe here a simple procedure involving HPLC with fluorimetric detection, which has a sensitivity comparable with that of RIA methods and requires only 500 μL of serum or plasma. The sample is purified by adsorption onto graphitized carbon black (Carbopack B) (7–9).

Materials and Method

Instrumentation

We used a Series 3B liquid chromatograph equipped with a Model 650 S LC fluorescence detector having a 20-μL flow cell and a Rheodyne Model 7125 injector with a 20-μL loop
Recovery

"Chromatography-grade" acetonitrile was obtained from Carlo Erba, Milano, Italy. All other solvents (Carlo Erba) were of analytical grade and were distilled in a glass system before use. Working phosphate buffer, pH 5.2, was prepared by diluting 10-fold a stock solution, which we prepared by dissolving 2.76 g of NaH2PO4 in 1 L of freshly distilled water and adjusting the pH to 5.2 with 0.1 mol/L KOH. The stock solution was stable at 4°C for at least two months.

Stock estriol standard (Sigma Chemical Co., St Louis, MO 63178) was dissolved in methanol to give a concentration of 1 g/L. This standard was diluted with methanol to give 1000, 500, 250, and 100 μg/L working standards. To prepare the estriol working plasma or serum standards, we evaporate 400 μL of the estriol working standards and reconstitute the residue in 20 mL of estriol-free fresh serum or plasma.

Carbopack B was kindly supplied by Supelco.

Procedure

Prepare the 3 × 0.6 cm column of Carbopack B (80-120 mesh) by pouring 0.25 g of this adsorbent into water, decanting any floating particles, and then introducing the suspension into a 15 × 0.6 cm glass column that has a small pledget of glass wool in the bottom. Pack the adsorbent by gently tapping the column while passing water through it. Through the column pass 500 μL of freshly collected serum or plasma previously diluted with 9.5 mL of water in a glass vial. Rinse the vial successively with two 2.5-mL portions of water and pass the rinsings through the column. Then wash the column with 5 mL of 3 mmol/L aqueous HCl, followed by 15 mL of methanol. Elute the estriol with chloroform/methanol (60/40 by vol) and collect 3 mL of the eluate, starting from the moment the eluting solution is applied to the column. Evaporate it under a stream of nitrogen, at 60°C, reconstitute the residue in 50 μL of the solution used as mobile phase, and inject 20 to 22 μL into the chromatograph. Calculate the estriol concentration of standard and patients’ samples by comparing the height of the peak produced by the estriol in the sample with that of a standard. The latter is prepared for chromatography by evaporating 10 μL of one of the estriol working standard solutions and reconstituting with 50 μL of the mobile phase. The response of the fluorimetric detector is linearly related to amounts of injected estriol within the range 0.08 to 30 ng.

Results and Discussion

Analytical Variables

Column variables. We observed that, in terms of estriol recovery and interferences, either plasma or serum can be used for this assay. EDTA was used as anticoagulant.

The 20-fold dilution of the sample is essential to quantitative recovery. Less estriol could be accounted for in the plasma effluent as the dilution was decreased, probably because of nonspecific protein–estriol interactions.

There was a dramatic loss of estriol when the acidic-washing step was omitted from the purification scheme. As recently reported (10), this can be ascribed to the formation of a chemical complex between anions and a Carbopack B surface chemical impurity. This complex is, however, readily broken by washing Carbopack B with an acidic solution.

We varied the flow rate at which the sample is percolated through the purification column by using Carbopack B of different particle sizes. About 15% of the estriol was unaccounted for in the plasma effluent when the flow rate was increased from 2.5–3.5 mL/min (80–120 mesh) to 5–6 mL/min (60–80 mesh). We believe that a high flow rate, together with the relatively large interstitial volume between Carbopack particles, partly hinders the desorption of estriol from proteins and the subsequent adsorption onto the Carbopack surface.

To make our HPLC procedure more suitable for routine use in terms of economy and speed, we evaluated the reusability of the Carbopack B column by doing repeated extractions of plasma or serum on the same column. After each extraction the column was restored with 3 mL of chloroform/methanol (60/40 by vol), 2 mL of methanol, and 2 mL of water, with care to avoid formation of large bubbles. After five such extractions the absolute analytical recovery of estriol was unchanged within the precision of the method, but the column permeability decreased.

When not in use, the Carbopack column was stored filled with water.

Recovery and precision. We assessed the analytical recovery of estriol and the within-run precision of the method by adding increasing amounts of authentic estriol to estriol-free pooled serum from men (Table 1) and assaying. The average recovery in the concentration range we considered was 98.6%. Each serum sample was assayed eight times during a month. The day-to-day CVs ranged from 2.1 to 20 μg/L to 3.9% at 2 μg/L.

Sensitivity. The limit of sensitivity (signal/noise ratio = 3) was 0.3 μg/L of serum. At such a concentration, the CV was 9.0%. Figure 1 shows typical chromatograms.

Specificity. Washing the Carbopack B column with 15 mL of methanol before the estriol is eluted effectively eliminates certain drugs such as barbiturates, commonly used antidepressants and anticonvulsants, quinidine, theophylline, salicylate, and procainamide. Estradiol—and to a lesser extent estrone—are partly co-eluted with estriol from Carbopack B. However, under the HPLC conditions selected, estradiol is eluted with a retention time of 12 min and estrone, at the concentrations of interest, is not detected by fluorimetry. Estriol conjugates are strongly retained, apparently by virtue of the Carbopack B surface chemical heterogeneities mentioned above, and are not eluted by the chloroform/methanol mixture.

Method Comparison

We compared results by our method with those by two previously reported methods (11, 12) in which extraction with Sep-Pak C18 cartridges is used and by a commercially available (Amersham) direct radioimmunoassay kit (13).

| Table 1. Results of Nine Replicate Analyses for Unconjugated Estriol Added to Pooled Serum from Men |
|-------------------------------------------------|------------------|-----------------|
| Added | Found (mean and SD) | Recovery, % |
| μg/L | | |
| 2.00 | 1.96 (0.07) | 98.0 | 94.3–102 |
| 5.00 | 4.95 (0.13) | 98.9 | 96.4–102 |
| 10.0 | 9.83 (0.22) | 98.3 | 96.1–101 |
| 20.0 | 19.8 (0.4) | 99.1 | 96.7–101 |
women in the last trimester of pregnancy agrees well with the average value of 9.06 µg/L measured by another HPLC method (6). As expected, the direct RIA method exhibited a positive bias, but the difference as measured by us was not as large as reported by Kabra et al. (5).

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References