In summary, the results of the present study demonstrated that EtG, but not EtS, is sensitive to bacterial hydrolysis, particularly when specimens are infected by E. coli, which is the most common source of UTIs. Given that UTIs caused by E. coli are among the most frequent bacterial infections encountered in clinical practice (13), this represents an obvious risk factor for false-negative and falsel low EtG results. Because EtG and EtS show similar windows of detection after alcohol consumption (3), it may therefore be advantageous and recommended to measure EtS instead of EtG for detection or confirmation of recent drinking, or at least to combine EtG with EtS analysis, which is possible by LC-MS (3, 23). In any case, ensuring that specimens are refrigerated or frozen and/or that sampling tubes contain fluoride preservatives to prevent bacterial growth is recommended practice.

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References
300 mg/24 h after 20 weeks of gestation (6). Early-onset preeclampsia was defined as having symptoms before 34 weeks of pregnancy, whereas late-onset was defined as having symptoms after 34 weeks of pregnancy (3, 4). Thirty-two maternal blood samples were drawn from normotensive pregnant women who all delivered healthy babies at term. These were matched by gestational age to the preeclampsia study group at the time of blood sampling. All pregnancies were singleton.

The plasma samples were separated by centrifugation and stored frozen as described previously (9). All samples were shipped by air freight on dry ice from Cape Town to Basel for analysis. Circulatory fetal DNA was quantified by a real-time PCR assay for the SRY gene located on the Y chromosome (6) (for full details, see Table 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol51/issue9/). Total circulatory DNA was quantified by a real-time PCR assay for the ubiquitous glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, which is present in all genomes (6) (see Table 1 in the online Data Supplement). Because circulatory maternal DNA makes up the bulk (>95%) of the circulatory DNA in a maternal plasma sample, this assay is indicative of the amount of circulatory maternal DNA (10). The concentrations of circulatory DNA are given in genome-equivalents per milliliter (GE/mL) of maternal plasma. The presence of circulatory placenta-derived fetal mRNA was assayed by a real-time reverse transcription-PCR assay for corticotropin-releasing hormone (CRH) mRNA transcripts as described previously (7, 9) (see Table 1 in the online Data Supplement). The concentrations of circulatory CRH mRNA are given in copies/mL of maternal plasma. The quality of total circulatory mRNA was assayed by a real-time reverse transcription-PCR assay for the ribosomal 18S gene according to the manufacturer’s instructions (Applied Biosystems; data not shown). Stringent anticontamination procedures were used throughout. No false-positive results were recorded.

The data were analyzed by SPSS® for Windows. Because our analysis indicated that the data did not follow a gaussian distribution, they were examined by the Mann–Whitney U-test for nonparametric data with $P < 0.05$ being regarded as statistically significant. The data concerning concentrations of circulatory DNA and mRNA are presented by box plots (Fig. 1).

The maternal characteristics and gestational age at time of sampling and delivery are listed in Table 1, with the cases with preeclampsia stratified into early- ($n = 23$) and late-onset forms ($n = 19$). It is noteworthy that many women affected by the early-onset form of preeclampsia delivered prematurely.

Our analysis of circulatory nucleic acid (DNA and mRNA) concentrations indicated that these were significantly higher in both preeclampsia study groups than in the matched control group (Table 1 and Fig. 1). A feature readily apparent in this analysis is that the extent of the increase of all 3 circulatory nucleic acids examined, fetal and maternal (total) DNA as well as fetal
CRH mRNA, was more pronounced in the cases with early-onset preeclampsia than in those with the late-onset form of the disorder (Table 1 and Fig. 1). Because increased release of these fetal and maternal circulatory nucleic acids in preeclampsia has been proposed to be attributable to some form of cell turnover, death, or damage (6, 11), their increased presence in cases with early-onset preeclampsia compared with those with the late-onset form suggests that the early form of preeclampsia may be associated with more cellular damage than the late form. In this context, it is worth noting that in our analysis of the 2 control groups, the pattern was reversed: the concentrations of circulatory fetal DNA and mRNA were higher in the control samples taken closer to term (>34 weeks of gestation) than in those taken at an earlier gestational age (<34 weeks of gestation). Therefore, if a correction is made for gestational age, then the difference observed between the early- and late-onset preeclampsia study groups may become even more pronounced.

From our data it is very clear that the amounts of circulatory fetal DNA and CRH mRNA in the preeclampsia study groups were significantly increased compared with the respective control groups. This is particularly true for the group with early-onset preeclampsia, where very little overlap was found to occur with the matched control group. In this manner, a cutoff value of 300 GE/mL of maternal plasma for circulatory fetal DNA and 300 copies/mL of maternal plasma for circulatory CRH mRNA could theoretically be used to distinguish between the study and control groups in our current data set.

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Clinical assessments of autonomic function often include tyramine (TYR) infusion. After uptake of TYR by sympa-thetic nerves via the cell membrane norepinephrine (NE) transporter and translocation of axoplasmonic TYR into vesicles, NE exits the vesicles. Some of the NE enters the extracellular fluid and occupies adrenoceptors on cardio-vascular smooth muscle cells, increasing blood pressure. A small proportion reaches the circulation, so that plasma NE concentrations increase (1–3).

Jacob et al. (4) reported an ~50-fold mean increase in plasma dopamine (DA) concentrations during intravenous TYR infusion, associated with “paradoxical” forearm vasodilation. We also noted high plasma DA concentrations in healthy volunteers during TYR infusion (5). When we assayed the catechol contents of the TYR infusate dispensed by our pharmacy [1 g/L (6.5 mmol/L)], we found that the infusate contained ~50 μmol/L DA, corresponding to 0.7% contamination (6). Whether during TYR infusion such contamination could actually raise plasma DA concentrations was unclear, as was whether, while stored as is customary in solution at 4 °C, TYR could be converted to DA. The present study examined these possibilities.

Solutions of TYR for infusion were prepared by the NIH Pharmaceutical Development Service, using TYR hydrochloride (Sigma) dissolved in sterile water, with pH ~9. The solutions were stored either in a refrigerator at 4 °C or in an ultra-low temperature freezer at ~70 °C for up to 9 months and then were assayed for catechol content, including DA.

Infusate and arterial plasma concentrations of catechols were assayed (7), and hemodynamics were assessed before and during intravenous TYR infusion [6.5 μmol (1 mg)/min] in a total of 34 adults who underwent TYR infusion as part of autonomic function testing. Of the 34 participants, 6 were healthy volunteers, 13 were patients with chronic orthostatic intolerance, 9 had neurogenic orthostatic hypotension, 5 had undergone thoracic sympathectomy, and 1 had an undiagnosed movement disorder. All gave informed written consent before participating in the study protocols, which were approved by the Intramural Research Board of the National Institute of Neurological Disorders and Stroke.

Blood samples were drawn after at least 15 min with the person supine. For arterial sampling, a brachial catheter was placed percutaneously after local anesthesia of the overlying skin. Arm venous blood was drawn through an indwelling intravenous catheter.

Forearm blood flow was measured by venous occlusion strain gauge plethysmography (Hokanson). The circulation of the hand was not excluded. Forearm vascular resistance was calculated from the ratio of mean arterial pressure to forearm blood flow. Blood pressure was monitored continuously, either via the arterial catheter or noninvasively by a finger oscillometric (Finometer or Portapres; TNO) or radial tonometric (Colin) device. Cardiac stroke volume was measured via impedance cardiography (Cardiodynamics), a noninvasive method we had validated previously (8).

Neurochemical and hemodynamic data were analyzed by linear regression and dependent-means t-tests. Values are reported as the mean (SE). A P value <0.05 defined statistical significance.

All of the 29 TYR infusates assayed after infusion into humans contained DA. During ~9 months of refrigerated (4 °C) storage of TYR solutions in the dark, DA in the infusate increased exponentially, from <25 to >600 nmol/L (r = 0.99; P < 0.0001; Fig. 1A). In contrast, the DA concentration in TYR infusate stored frozen at ~70 °C remained unchanged.

The neurochemical and hemodynamic results were unrelated to diagnosis. Below a DA concentration of 50 nmol/L in the infused TYR solution, there was no relationship between the increment in arterial plasma DA and expectant management at a secondary hospital in close association with a tertiary institution. BJOG 2005;112:84–8.


