Plasma tHcy remained essentially stable for 24 h in the absence of MTX. Only a minor increase of 12.7% ± 9.3% (1.1 ± 0.8 μmol/L) was observed 8 h after the injection, a diurnal change that is in the same range as previously reported in healthy individuals (18). In contrast, there was a variable but substantial increase in tHcy in patients after MTX infusion, reaching a maximum of 49.3% ± 61.6% (3.0 ± 2.4 μmol/L) after 12 h, demonstrating the antifolate effect of MTX. Similar effects on plasma tHcy have been reported previously (10, 19, 20).

In the absence of MTX, the plasma T1/2 was 2.6 ± 0.5 h (k = 0.27 ± 0.03), corresponding to a clearance of 78 ± 10 mL/min. Plasma tHcy kinetics showed no consistent changes in response to HD-MTX. The mean differences in T1/2 and clearance between the two occasions were 13.3% ± 17.5% and −4.1% ± 10.4%, respectively (P > 0.05; Fig. 1 and Table 1).

From these data, the following conclusions can be made: (a) the plasma tHcy kinetics are not affected by HD-MTX and thereby folate status, as previously demonstrated by Hcy loading in folate-deficient subjects (11); and (b) the kinetics are similar albeit slightly more rapid than observed during Hcy loading (T1/2 = 2.6 vs 3.7 h; P = 0.008) (12). These results verify that peroral Hcy loading is an adequate procedure for the assessment of Hcy turnover in plasma.

This work was supported by grants from the Norwegian Council on Cardiovascular Disease. We greatly appreciate the technical assistance of Elfrid Blomdal, Gry Kvalheim, Else Leirnes, and Wenche Breyholz.
genes in many tissues and cell types. Recovery of DNA fragments, however, from silver-stained nondenaturing polyacrylamide gel is difficult. Numerous techniques for extracting DNA from the polyacrylamide gel have been reported, including using untreated dried polyacrylamide flakes (1), leeching with water (2), excision and boiling in 1× PCR buffer (3), and heating overnight in 0.5 mol/L ammonium acetate (4). The potential problems with these techniques are that inhibitors can be carried over into the PCR reaction and that inconsistent preparation can lead to poor re-amplification. In addition, most of these techniques require excessive thermal cycling to re-amplify the fragment. We developed a technique in which the DNA is electroeluted out of the acrylamide onto a DEAE membrane. With this technique, multiple samples can be processed and purified simultaneously, leading to reproducible and consistent re-amplification reactions.

Cytogenetically normal amniocytes were grown in duplicate in Chang’s in situ media (Irvine Scientific). Messenger RNA was isolated with an oligo dT/streptavidin magnetic bead capture system (Promega Corp.) (5) and converted to cDNA using MMLV reverse transcriptase and a degenerative oligo dT primer (5'-TTTTTTTTTTT TGG). For each DDA analysis, the following 20-μL reaction was set up: 1× PCR buffer with 1.5 mmol/L MgCl₂, 20 μmol/L each dNTP, 0.5 μmol/L oligo dT primer, 0.25 μmol/L upstream primer (5'-CTGCTTGAAG, 5'-GTGC CAGTC, 5'-GATCCGATTC, and 5'-AAACCTGGTC), 30 ng of cDNA, and 0.625 unit of Taq DNA polymerase (Perkin-Elmer) (6). Forty thermal cycles of 95°C, 30 s; 40°C, 1 min; and 72°C, 1 min were used. The DDA products were ethanol-precipitated, rehydrated in water, size-separated through a 5% nondenaturing polyacrylamide gel, and visualized by silver staining (7).

The DDA amplification products were excised from the dried polyacrylamide with sterile water and a clean scalpel. The excised acrylamide fragments were placed into the wells of a 2% agarose gel (Life Technologies) and flattened against the edge nearest the cathode. The wells were carefully filled with cooled 2% molten agarose. Once the agarose was set, DEAE membranes (Schleicher & Schuell), cut into 0.5 × 0.75-cm pieces, were inserted into the gel ~0.5 cm below each well. A narrow weighing spatula was used to facilitate the insertion of the dry DEAE membrane. The DNA was electrophoresed at 10 V/cm for 90 min. The DEAE membranes were removed and placed into microfuge tubes containing 500 μL of low-salt NET buffer (0.15 mol/L NaCl, 0.1 mmol/L EDTA, and 20 mmol/L Tris, pH 8.0). The low-salt NET buffer was replaced with 50 μL of the high-salt NET buffer (1 mol/L NaCl, 0.1 mmol/L EDTA, and 20 mmol/L Tris, pH 8.0) and incubated at 65°C for 30 min to elute the DNA. The DNA was then precipitated with 2.5 volumes of ethanol and rehydrated in 15 μL of water. Five microliters of the eluted DNA was amplified using the same conditions as described above. Amplified fragments were ligated into a T-vector (Promega) and sequenced.

This technique was used to purify DDA fragments excised from dried nondenaturing polyacrylamide gels.

Fig. 1 shows the amplification of four purified DDA fragments. A single band is present in each lane. With this technique, DNA ranging in size from 100 to 700 bp was precipitated in sufficient quantity for several re-amplification reactions. In addition, because a 2% agarose gel in Tris-borate-EDTA was used, multiple samples could be processed without the use of Tris-acetate-EDTA, low-melt agarose, specialized pipet tips, or electrophoresis chambers (8). Numerous authors have shown that DNA can be purified by elution onto DEAE membranes through agarose (9,10), and Dretzen et al. (11) showed that DNA could be eluted onto DEAE membranes directly from polyacrylamide gels. Electroeluting DNA directly out of large polyacrylamide gels, however, is not practical because these gels are very thin and dried.

We have shown here that DNA can be efficiently eluted out of acrylamide and through agarose onto a DEAE membrane, using standard laboratory protocols and equipment. With this technique, numerous samples can be consistently and reliably processed, producing inhibitor-free DNA. This technique should aid in the cloning and sequencing of DDA products.

Ramsey Foundation Grant N590 supported this work.

References
Prostate-specific Antigen Expression in Neoplastic Human Myeloid Cell Lines, Ferdinando Mannello,1* Francesca Papa,2 and Giancarlo Gazzanelli 1

Prostate-specific antigen (PSA) is a 33-kDa serine protease produced by prostatic epithelial cells under the influence of the androgen receptor (1). Although PSA previously was thought to be a prostatic tissue-specific protein not produced by prostatic epithelial cells under the influence of the androgen receptor, it is considered to be present in many nonprostatic tissues (2, 3). PSA expression in both physiological and pathological conditions is not organ- or sex-specific; however, it is steroid hormone-mediated (4). In fact, several cell lines (from breast, ovary, prostate, and lung cancer) showed specific PSA production modulated by steroid hormones (3, 5, 6). Recently, nested PCR has shown PSA messenger RNA in a variety of non-prostate cells, including hematological cell lines (7, 8), nondiseased peripheral blood (9, 10), and bone marrow specimens (11, 12). The finding of PSA expression within cells that are outside of the prostate is of great clinical utility in establishing the nonspecificity of PSA as an indicator of micrometastatic malignant disease (13–15).

To establish the presence of PSA immunoreactivity in nondiseased human peripheral blood unfractionated leukocytes (PBLs) and in leukemic cell lines, we undertook the present study on PSA content and immunoreactivity in blood samples from 10 healthy female and male volunteers, ages 25–42 years (mean, 33 ± 4 years). After collection, the blood samples were enriched in unfractionated mononuclear cells by centrifugation at 500g for 20 min at 4°C on Ficoll-Hypaque (Pharmacia). Erythrocyte-depleted blood samples were prepared and counted after treatment with a lysing solution (Coulter Electronics). Sera from PSA-negative healthy control females were used as the negative control.

The leukemic cell lines HL-60 (promyelocytic), K562 (erythroleukemia), and U937 (premonocytic), all myeloid cell lines (from breast, ovary, prostate, and lung cancer), were maintained in RPMI supplemented with 100 mL/L heat-inactivated fetal calf serum (Irvine Scientific), 2 mmol/L glutamine, 100 000 units/L penicillin, and 100 mg/L streptomycin (Seromed, Biochrom KG) in a 5% CO2 humidified incubator at 37°C. The prostate carcinoma cell line LNCaP was grown in RPMI medium supplemented with 50 g/L fetal calf serum in a separate CO2 humidified incubator at 37°C to avoid the possibility of cross-contamination. This cell line is known to produce PSA and was used as the positive control. The cell pellets were lysed for 30 min on ice with 1 mL of lysis buffer containing 50 mmol/L Tris, pH 7.5, 150 mmol/L sodium chloride, 5 mmol/L EDTA, 10 g/L nonidet NP-40 surfactant, 1 mmol/L phenylmethylsulfonyl fluoride, and 1 mg/L each of aprotoni and leupeptin as proteinase inhibitors (16). The suspensions were frozen at −80°C and thawed at 37°C three times and sonicated for 5 cycles at 40 W of output for 30 s on ice (Heat-System Ultrasonic Inc.). Cell debris was pelleted at 9000g at 4°C for 30 min, and the supernatants were collected and immediately assayed for PSA and total protein concentration.

The total protein content was determined with either the Coomassie G-250 or the bichinonic methods (with commercially available kits from Bio-Rad Laboratories and Pierce Chemical Co., respectively). Total and free PSA concentrations were measured by microparticle enzyme immunoassay (16, 17), using a monoclonal mouse anti-human PSA antibody (AxSYM® from Abbott Laboratories). The detection limits of the assay were 0.02 µg/L for total PSA and 0.01 µg/L for free PSA. To exclude the possibility of matrix artifacts, PBLs and leukemic cell extracts were serially diluted in PSA-negative female serum and reanalyzed for the response linearity. The analytical recovery of at least two concentrations of purified PSA added to the leukemic cellular extracts (3.5 and 7.0 µg/L) was tested (18).

All the assays performed on the cellular extracts were carried out in replicates of three in at least six independent experiments.

Reagents and equipment for Western blotting were purchased from Bio-Rad Laboratories. Our protocols were followed throughout, using the primary anti-human PSA monoclonal mouse antibody from Dako (16). Results, expressed as mean ± SD, were considered to be statistically significant when P < 0.01. All statistical analyses were performed with the StatView, Ver. 4.1, package (Abacus Concepts Inc.) on a Macintosh Power PC (Apple Computer).

The present work was carried out in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 1983.

The prostate cancer cell line LNCaP produces and secretes PSA in the culture medium; in our conditions, the

Clinical Chemistry 44, No. 9, 1998