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# Immunofluorometric assay of prostaglandin D synthase in human tissue extracts and fluids

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**A two-site sandwich-type assay for human prostaglandin D (PGD) synthase (β-trace) was developed with two monoclonal antibodies and using time-resolved fluorometry as the detection technique. The assay is precise (CVs <10%), accurate, and highly specific for PGD synthase and has a detection limit of 0.05 μg/L. Using this assay, we measured PGD synthase concentrations in serum, urine, amniotic fluid, cerebrospinal fluid (CSF), seminal plasma, breast cyst fluid, breast discharge fluid, breast milk, and breast tumor extracts. The highest concentrations were found in CSF. We identified proteolytic degradation of PGD synthase in amniotic fluid. Fetal tissues contained various amounts of the enzyme, with the highest values being found in brain and heart. In placental extracts, PGD synthase content was greatest at 11–28 weeks of gestation—in accordance with the concentrations measured in amniotic fluids for this gestational period. We conclude that PGD synthase is ubiquitous and is present in many fluids and tissues of adults and fetuses. This first quantitative and sensitive assay of PGD synthase should facilitate expansion of knowledge on this enzyme and possibly will have applications for diagnosis and monitoring of human diseases.**

INDEXING TERMS: prostaglandins • amniotic fluid • cerebrospinal fluid • β-trace protein • pregnancy • fetal status • breast milk • urine • seminal plasma • breast cyst fluid • enzyme activity • lipocalins

Prostaglandins, metabolites of arachidonic acid, have a wide variety of biological functions *[1]*. Prostaglandin $D_2$ ($PGD_2$) is a potent inhibitor of platelet aggregation in vitro and is involved in smooth muscle contraction and relaxation *[2]*.[5] $PGD_2$ has been implicated in a variety of functions of the central nervous system (CNS), including synaptic transmission *[3]*, hypothalamic control of temperature *[4]*, recovery from seizures *[5, 6]*, and release of lutropin *[7]*. $PGD_2$ is also implicated in sedation and sleep induction *[8, 9]*. Hayaishi et al. *[10–12]* have provided strong evidence that $PGD_2$ is involved in sleep induction in rats and monkeys.

Prostaglandin D synthase (prostaglandin-$H_2$ D-isomerase, EC 5.3.99.2; PGD synthase) catalyzes the conversion of $PGH_2$ to $PGD_2$ *[13]*. This enzyme is responsible for the biosynthesis of $PGD_2$ in the brain and is localized in the CNS *[14]*. Postnatally, the cellular location of the enzyme changes, and the enzyme is now thought to play important roles in both maturation and maintenance of the CNS.

Nagata et al. *[15]*, White et al. *[16]*, Urade et al. *[17]*, and Igarashi et al. *[18]* have cloned the human and rat PGD synthase genes and have shown that PGD synthase belongs to a superfamily of secretory proteins called lipocalins. Lipocalins share a common feature for binding and transport of small hydrophobic molecules *[19]*. Among the 26 members of this superfamily, PGD synthase shows the greatest homology with 24p3 oncogene product protein, suggesting a close evolutionary relationship between these two proteins *[18]*.

We have recently found that PGD synthase is expressed in various tissues and is present in numerous fluids, including normal breast tissue *[20]*, breast tumors *[21]*, breast cyst fluid *[21, 22]*, milk of lactating women *[21, 23]*, and amniotic fluid *[21, 24]*. At present, PGD synthase can be measured by semiquantitative Western blot analysis *[21]*.

Quantitative and sensitive methods for measuring PGD synthase are essential. In this study, we developed a highly sensitive and specific assay for PGD synthase by using two monoclonal antibodies against the enzyme and detecting terbium chelates labels with time-resolved fluorescence spectroscopy. This assay can be used for measuring PGD synthase in various human tissues and fluids.

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[5] Nonstandard abbreviations: $PGD_2$, prostaglandin $D_2$; PGD synthase, prostaglandin D synthase; SA-ALP, streptavidin–alkaline phosphatase conjugate; DFP, diflunisal phosphate; BSA, bovine serum albumin; and CSF, cerebrospinal fluid.
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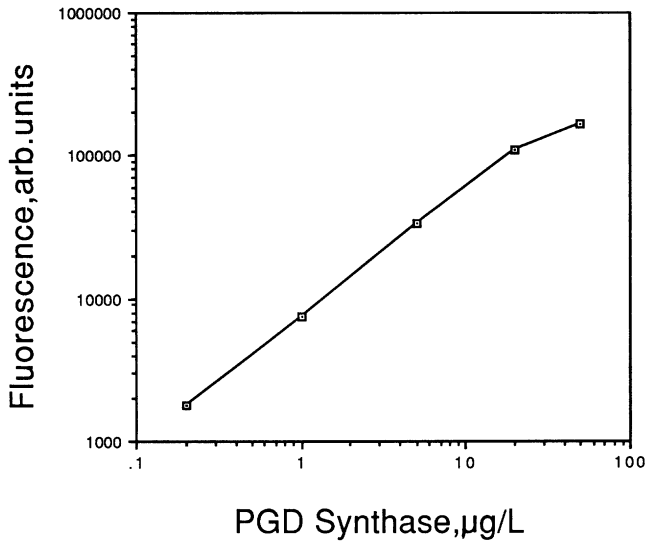


Fig. 1. Calibration curve of the PGD synthase assay.

The background signal, obtained with the zero calibrator, was ~1000 arbitrary fluorescence units and was subtracted from all other measurements.

## Materials and Methods

### REAGENTS

The diflunisal phosphate ester (DFP) was synthesized in our laboratory from diflunisal (Sigma Chemical Co., St. Louis MO) starting material. The stock solutions of DFP we used were 0.01 mol/L in 0.1 mol/L NaOH and were stable at 4 °C for at least 1 month. Alkaline phosphatase-labeled streptavidin (SA-ALP) was obtained from Jackson Immunoresearch (West Grove, PA) as a 1 g/L solution. Working SA-ALP solution was prepared by diluting the stock solution 20 000-fold in a bovine serum albumin (BSA) diluent described below. White, opaque, 12-well polystyrene microtiter strips were obtained from Dynatech Labs. (Alexandria, VA). The substrate buffer was Tris buffer (0.1 mol/L, pH 9.1) containing 0.1 mol of NaCl and 1 mmol of $MgCl_2$ per liter. The substrate working solution (DFP, 1 mmol/L in substrate buffer) was prepared just before use by diluting the DFP stock solution 10-fold in the substrate buffer. The SA-ALP diluent was a 60 g/L solution of BSA in 50 mmol/L Tris buffer, pH 7.40, containing 0.5 g of sodium azide per liter. The wash solution was prepared by dissolving 9 g of NaCl and 0.5 g of polyoxyethylenesorbitan monolaurate (Tween 20) in 1 L of 10 mmol/L Tris buffer, pH 7.40. The developing solution was prepared as described elsewhere *[25]*. The assay buffer is 50 mmol/L Tris buffer, pH 7.40, containing 60 g of BSA, 0.5 g of sodium azide, 100 mL of normal goat serum, 50 mL of normal mouse serum, and 0.5 g of Tween 20 per liter.

PGD synthase standards, 0, 0.2, 1, 5, 20 and 50 µg/L, were prepared in the SA-ALP diluent by using recombinant PGD synthase produced in BHK-21 cells as described elsewhere *[26]*. The recombinant enzyme was further purified by immunoaffinity chromatography and was a gift from H. Conradt (Department of Gene Regulation and Differentiation, Braunschweig, Germany).

### CLINICAL SAMPLES

The following clinical samples were used to examine the presence of PGD synthase: cerebrospinal fluid (CSF), male and female human serum, serum from pregnant women at various gestational ages, serum from patients with prostate cancer, amniotic fluid at various gestational stages, human urine, milk of lactating women, breast discharge fluid, breast tumor cytosolic extracts (prepared as previously described *[27]*), and seminal plasma. All samples were tested at various dilutions to establish optimal measuring conditions for each.

### INSTRUMENTATION

For measuring liquid-phase $Tb^{3+}$ fluorescence in white microtiter wells, we used the CyberFluor 615 Immunoanalyzer, a time-resolved fluorometer (Nordion International, Kanata, ON). More details are given elsewhere *[25]*.

### PROCEDURES

*Monoclonal anti-PGD synthase antibodies.* Five mouse monoclonal antibodies against PGD synthase were provided by Maruha Corp. (Tsukuba, Japan). Stock solutions were adjusted to 1 g/L in phosphate-buffered saline (pH 7.5). The specificity of the five clones for PGD synthase was confirmed by Western blot analysis of CSF, in which each clone recognized a single protein

**Table 1. Precision profile of the PGD synthase assay determined with 8 control samples.**

| | Within-run[b] | | | Day-to-day[c] | | |
|---|---|---|---|---|---|---|
| Control sample matrix[a] | Mean | SD | CV, % | Mean | SD | CV, % |
| BSA | 0.93 | 0.07 | 7.5 | 1.05 | 0.10 | 9.5 |
| BSA | 4.65 | 0.20 | 4.3 | 4.80 | 0.36 | 7.6 |
| BSA | 19.2 | 1.45 | 7.6 | 18.8 | 1.00 | 5.4 |
| Horse serum (HS) | 0.99 | 0.05 | 5.3 | 1.18 | 0.11 | 9.3 |
| HS | 10.8 | 0.86 | 8.0 | 13.2 | 1.2 | 9.1 |
| HS diluted 20-fold | 2.8 | 0.25 | 9.0 | 3.1 | 0.30 | 9.7 |
| HS diluted 20-fold | 11.4 | 0.57 | 5.0 | 9.9 | 1.0 | 10.0 |
| Amniotic fluid diluted 500-fold | 5.0 | 0.32 | 6.3 | 4.5 | 0.50 | 11.1 |

[a] To BSA and horse serum (HS) we added recombinant PGD synthase. Diluted matrices (amniotic fluid and HS) were diluted in 60 g/L BSA diluent.

[b] Mean and SD of 12 replicates are in µg/L. Concentrations shown are those after dilution.

[c] 10 different runs over 1 week; concentrations in µg/L.

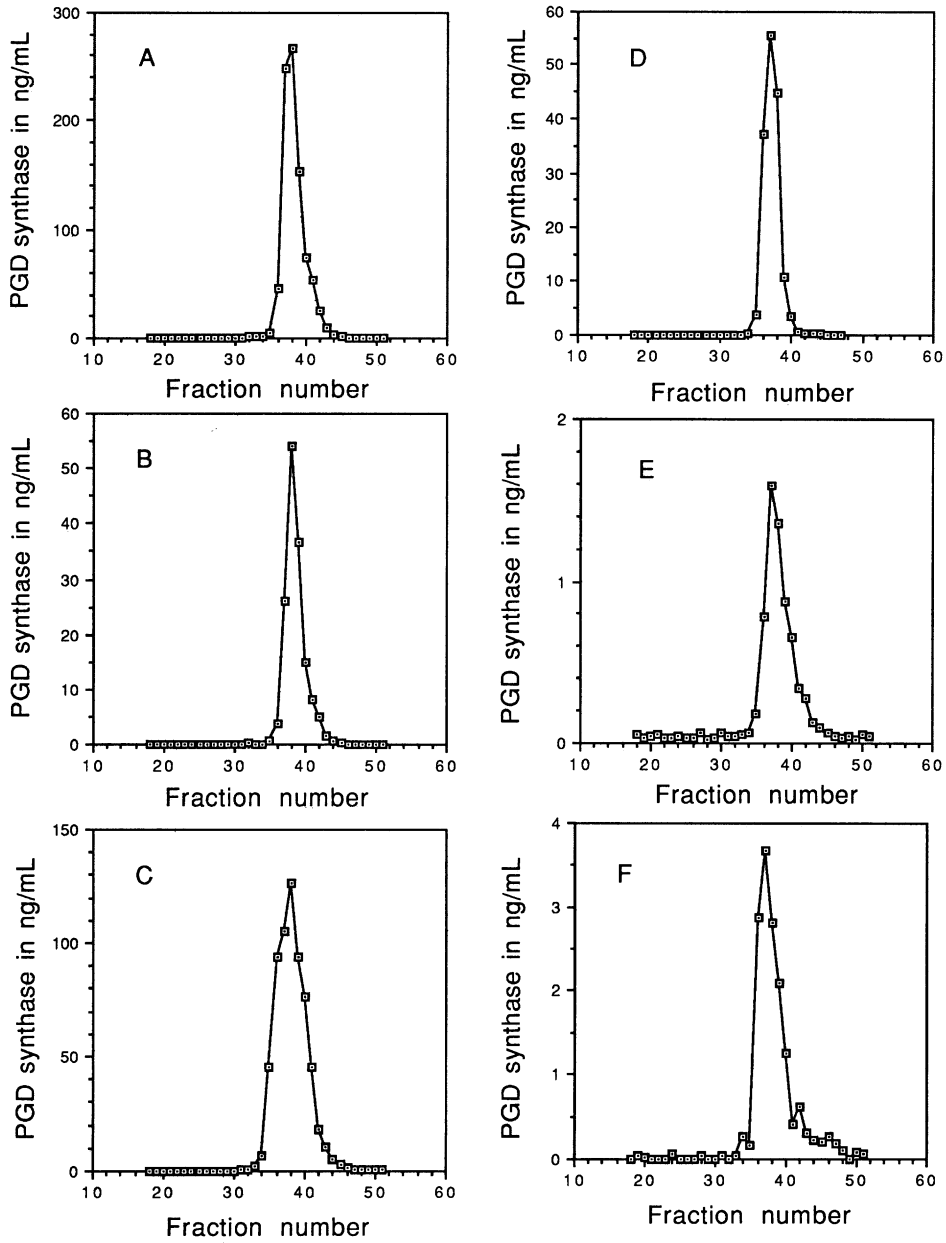


Fig. 2. HPLC elution profiles of the PGD synthase immunoreactivity in various samples: (*A*) amniotic fluid, (*B*) CSF, (*C*) seminal plasma, (*D*) urine, (*E*) breast tumor cytosol, and (*F*) breast discharge fluid.

In all cases, we obtained a single peak of the immunoreactivity corresponding to 27 kDa. Human serum gave similar results (data not shown).

at the same position as that of the purified enzyme, with a molecular mass of ~27 kDa (data not shown). The specificity of the antibodies was further confirmed by the immunofluorometric procedure described later.

*Coating of microtiter plates with clone 1B7 monoclonal antibody.* Microtiter wells were coated by incubating overnight with the 1B7 antibody (500 ng/well) in 100 μL of 50 mmol/L Tris buffer, pH 7.80. The wells were then washed six times with the wash solution and blocked for 1 h with 200 μL of the SA-ALP diluent per well. After another six washes with wash solution, the wells were ready to use.

*Biotinylation of clone 10A3 monoclonal antibody.* The 10A3 antibody was biotinylated with sulfosuccinimidyl 6-(biotinamido) hexanoate (NHS-LC-Biotin; Pierce Chemical Co., Rockford, IL) as previously described *[28, 29]*. The concentration of the stock biotinylated antibody was 0.5 g/L.

*Assay.* Pipet 50 μL of standards or samples (appropriately diluted in the SA-ALP diluent, see below) into coated microtiter wells and add 50 μL of the biotinylated antibody solution diluted 1000-fold in assay buffer. Incubate with mechanical shaking for 2 h at room temperature and then wash six times. To each well add 100 μL of SA-ALP conjugate diluted 20 000-fold in the

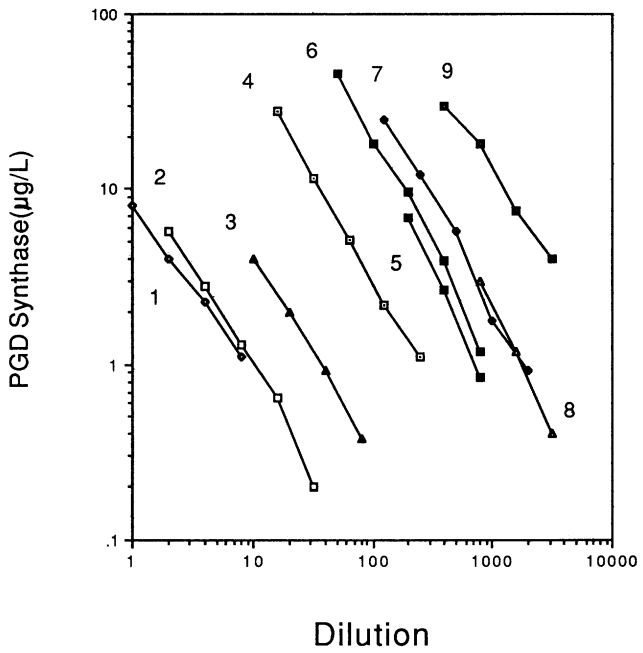


Fig. 3. Dilution curves of various human body fluids analyzed for PGD synthase by the immunofluorometric assay.

In all cases, near-linear curves were obtained. The diluent was 60 g/L BSA solution. Diluted fluids were (*1*) breast tumor extract, (*2*) milk from a lactating woman, (*3*) breast cyst fluid, (*4*) human serum, (*5*) seminal plasma, (*6*) human urine, (*7*) amniotic fluid, (*8*) breast nipple aspirate fluid, and (*9*) CSF.

SA-ALP diluent, incubate for 15 min as above, and wash six times. Add 100 µL of the 1 mmol/L working DFP substrate solution to each well and incubate for 10 min as above. Then, add 100 µL of the developing solution per well, mix by mechanical shaking for 1 min, and measure the fluorescence with the time-resolved fluorometer. The calibration curve and data reduction were carried out automatically by the CyberFluor 615 immunoanalyzer.

## Results

Five monoclonal antibodies were available for assay development. Each one of these antibodies was used for solid-phase coating and also was biotinylated and tested for detection in a sandwich-type, noncompetitive immunoassay configuration. Among the 20 possible combinations, the one selected (clone 1B7 for coating, biotinylated clone 10A3 for detection) gave the most sensitive assay. We have further optimized reagent dilutions and incubation times as described elsewhere *[30]*.

A typical calibration curve for the proposed optimized assay is shown in Fig. 1. The detection limit, defined as the concentration of PGD synthase corresponding to the fluorescence of the zero standard plus 2 SD of the zero standard fluorescence, was 0.05 µg/L. Within-run and between-run precision data are shown in Table 1. The hook effect was checked for PGD synthase concentrations as great as 7500 µg/L; all concentrations tested gave the result ">50 µg/L," suggesting no hook effect up to this concentration.

Assay specificity was checked with various tests. (*a*) The monoclonal antibodies used were tested on Western blots of CSF and gave single bands at the molecular mass of PGD synthase (~27 kDa). (*b*) The developed assay recognized PGD synthase purified from human brain as well as the recombinant protein produced in BHK-21 cells, which was immunopurified with different monoclonal antibodies *[26]*. (*c*) Tests of undiluted animal sera (from goat, horse, rat, calf, mouse) by the developed assay all gave negative results for PGD synthase. (*d*) In cross-reactivity experiments with purified prostate-specific antigen at concentrations up to 1000 µg/L, the present assay did not show any cross-reactivity (data not shown). We have previously shown

**Table 2. Analytical recovery of PGD synthase added to various fluids.**

| | PGD synthase, µg/L | | | |
|---|---|---|---|---|
| Sample matrix[a] | Initial | Added | Recovered | Recovery, % |
| Amniotic fluid, undiluted | 0.01 | 4.46 | 0.40 | 9 |
| | | 7.40 | 1.05 | 14 |
| Amniotic fluid, diluted 500-fold | 0.00 | 4.46 | 4.96 | 111 |
| | | 7.40 | 8.08 | 109 |
| Horse serum | 0.00 | 4.46 | 3.26 | 73 |
| | | 7.40 | 6.80 | 92 |
| Horse serum, diluted 100-fold | 0.00 | 4.46 | 4.35 | 98 |
| | | 7.46 | 7.44 | 100 |
| Breast tumor extract | 1.36 | 4.46 | 3.67 | 82 |
| | | 7.46 | 6.56 | 88 |
| Urine, diluted 100-fold | 15.15 | 4.46 | 3.33 | 75 |
| | | 7.46 | 5.93 | 79 |
| Serum, diluted 100-fold | 2.71 | 4.46 | 3.98 | 89 |
| | | 7.46 | 6.74 | 90 |
| Serum[b] | 221 | 625 | 560 | 90 |
| | | 1200 | 1010 | 84 |

[a] Dilutions were made in a 60 g/L BSA solution (the SA-ALP diluent).

[b] PGD synthase was added to serum; both the unaltered and the supplemented sera were then diluted 100-fold before analysis.

**Table 3. Analysis of PGD synthase in various fluids.**

| Sample | Optimal dilution, n-fold | PGD synthase conc., μg/L | | | |
|---|---|---|---|---|---|
| | | Range | Mean (SD) | Median | n |
| Serum | | | | | |
| All males | 100 | 114–436 | 222 (72) | 208 | 33 |
| Prostate cancer males | 100 | 148–329 | 215 (60) | 206 | 11 |
| Non-prostate-cancer males | 100 | 137–436 | 251 (77) | 254 | 16 |
| Nonpregnant females | 100 | 243–502 | 353 (81) | 332 | 12 |
| Pregnant females[a] | 100 | 110–345 | 234 (73) | 244 | 12 |
| Urine | | | | | |
| Normal males | 100 | 638–1679 | 1054 (418) | 1003 | 6 |
| Normal nonpregnant females | 100 | 70–1547 | 552 (534) | 387 | 6 |
| Amniotic fluid[b] | 500 | 105–2310 | 812 (718) | 492 | 12 |
| Cerebrospinal fluid | 1000 | 6784–26 490 | 14 854 (6893) | 12 915 | 12 |
| Seminal plasma | 500 | 1360 | — | — | 1 |
| Breast cyst fluid | 10 | 15–43 | — | — | 2 |
| Breast discharge fluid[c] | 250 | 100–3000 | — | — | 2 |
| Breast milk[d] | Undild. | 0.6–12 | 4.9 (4.3) | 3.9 | 5 |
| Breast tumor extracts[e] | Undild. | 1–8 | 2.7 (2.1) | 2.0 | 12 |

[a] Gestational weeks 12–22.
[b] Gestational weeks 11–22.
[c] Obtained with capillary glass tubes after mechanical pressure of breast; ~5 μL collected per patient.
[d] Obtained from lactating women ~1 week postpregnancy.
[e] Prepared as described elsewhere[27] for steroid hormone receptor analysis.

that polyclonal, but not monoclonal, PSA antibodies cross-react with PGD synthase in Western blots [21, 24]. (*e*) We fractionated the PGD synthase immunoreactivity by gel-filtration chromatography and analyzed all the fractions with the developed assay. For all samples—CSF, human male and female serum, amniotic fluid, breast tumor extract, seminal plasma, milk of lactating women, breast cyst fluid, and breast discharge fluid—we obtained a single immunoreactive peak at an elution position of 27 kDa (Fig. 2).

In dilution experiments (Fig. 3), near-linear dilution curves were obtained with all samples tested, when the samples were diluted at a starting concentration of <50 μg/L (the upper concentration of the calibration curve). For example, the amniotic fluid tested was diluted from 125-fold (to bring the concentration to <50 μg/L) to 2000-fold with 50 mmol/L Tris buffer, pH 7.80, containing 60 g/L BSA.

Recovery experiments were performed by adding PGD synthase to various sample matrices (Table 2). In many cases, we supplemented diluted matrices because the initial concentration in these fluids was >50 μg/L (our upper calibrator concentration). The recovery was acceptable in all cases, except for one notable exception of one amniotic fluid with no measurable PGD synthase. The recovery in this sample was 9–14%, whereas the recovery for the same sample after 500-fold dilution was 109–111%. Subsequent studies, as described below, showed that this fluid contained endogenous proteolytic activity that completely degraded the PGD synthase. When testing another, less sensitive version of this assay, we also found that undiluted amniotic fluids could not be measured accurately but required at least 20-fold dilution; this suggests that PGD synthase interacts and binds with some components of amniotic fluid (probably lipids).

We further analyzed various fluids and extracts to determine their PGD synthase concentration (Table 3). The highest concentration was found in CSF, followed by (in order) urine, amniotic fluid, seminal plasma, and breast discharge fluid. Small but significant amounts of PGD synthase were also detected in serum and other fluids. The data are not sufficient for detailed clinical conclusions; however, we can make the following observations ($r$ = Pearson correlation coefficient): (*a*) There are no differences in serum concentrations of PGD synthase between men with and men without prostate cancer ($t$-test; $P = 0.22$). (*b*) PGD synthase concentration in serum appears to correlate positively with age in men (linear regression, $r = 0.57$, $P = 0.02$) and negatively in women (linear regression, $r = -0.54$, $P = 0.07$). (*c*) The serum concentration of PGD synthase in pregnant women is lower than in nonpregnant women (paired $t$-test, $P = 0.003$, n = 12 pairs). (*d*) Among pregnant women, we found no correlation between serum PGD synthase concentration and gestational age (linear regression, $r = 0.07$, $P = 0.82$). (*e*) Amniotic fluid PGD synthase concentration increases between gestational weeks 11 and 22 (linear regression, $r = 0.69$, $P = 0.014$).

Among the few amniotic fluids tested, one was unique in having no detectable PGD synthase concentration. We postulated that this fluid may contain proteases that degrade PGD synthase. To study this possibility, we examined the stability of PGD synthase in this fluid (after addition of the enzyme) and in other fluids (Table 4). PGD synthase was stable for at least 24 h at 37 °C in CSF, urine, serum, and BSA solution, whereas both

**Table 4. Stability of PGD synthase in various fluids.**

| Fluid | Enzyme added | PGD synthase, μg/L: At addition (zero time) | After 24 h at 37 °C | After 10 days at 37 °C |
|---|---|---|---|---|
| Amniotic fluid[a] | Recombinant (685 μg/L) | 685 | 0 | 0 |
| | From human brain (28 μg/L) | 28 | 0 | 0 |
| | From CSF (1007 μg/L) | 1007 | 0 | 0 |
| CSF | –[b] | 11 730 | 13 990 | 8423 |
| Urine | –[b] | 1508 | 1551 | 570 |
| Serum | –[b] | 180 | 211 | 208 |
| Dilution buffer (BSA) | Recombinant (685 μg/L) | 685 | 845 | 749 |

[a] This is the amniotic fluid with proteolytic activity and low recovery (see Table 2).
[b] No addition; concentration represents endogenous PGD synthase.

native and recombinant PGD synthases were completely degraded in the amniotic fluid under question. PGD synthase remained stable in serum and in BSA solution for as long as 10 days at 37 °C but declined by ~30% and 60% in CSF and urine, respectively.

We prepared cytosolic extracts of various fetal tissues obtained from an aborted fetus with trisomy 21 and of placentas obtained from aborted fetuses at various gestational ages. The extracts were analyzed for PGD synthase and for total protein. The results are shown in Table 5 for fetal tissues and Table 6 for placentas. Among all fetal tissues tested, PGD synthase content was greatest in the brain and ~10-fold lower in the fetal heart. The other tissues contained comparatively less amounts of the enzyme. In placental tissue, PGD activity was low until gestational week 9, increased at gestational weeks 11–28, and declined slowly until term. A similar pattern for PGD synthase concentration was seen in the amniotic fluid (already described).

We obtained fetal blood and the corresponding amniotic fluid from two fetuses and compared the concentrations in the two fluids. In fetus A, the PGD synthase concentration in amniotic fluid was 2000 μg/L; the corresponding serum concentration was 600 μg/L (ratio 3.3). In fetus B, the amniotic fluid concentration was 1900 μg/L and the serum concentration was 750 μg/L (ratio 2.5). Measurement of another five fetal sera that became available showed PGD synthase concentrations of 300, 500, 450, 400, and 350 μg/L. These values are similar to those for PGD synthase in sera from men and women (Table 3).

## Discussion

PGD synthase was first discovered in human CSF in 1961 by Clausen *[31]*; it was named β-trace protein. Subsequently, Olsson developed a polyclonal antibody and semiquantitative radial immunodiffusion assays for β-trace and studied its distribution in the brain and various body fluids *[32–36]*. However, no contemporary immunological assay has been developed for this protein, given the lack of monoclonal antibodies and pure protein stocks. After the PGD synthase gene was cloned *[16–18]*, it became apparent that PGD synthase and β-trace are the same proteins. The recombinant protein was produced, and monoclonal antibodies were developed. The present method is based on monoclonal antibodies raised against recombinant PGD synthase and use of a highly sensitive detection technique, enzymatically amplified time-resolved fluorometry *[25]*. The glycosylation differences between recombinant PGD synthase and native PGD synthase in biological fluids have not as yet been examined.

The optimized assay has a detection limit of 0.05 μg/L and a dynamic range extending to 50 μg/L. The assay is highly specific for PGD synthase: It does not recognize any other proteins in various animal sera, and it does recognize a single protein of molecular mass 27 kDa in all fluids and extracts tested. As shown, assay validation through precision, recovery, and dilution experiments gave satisfactory results.

**Table 5. PGD synthase content of extracts of various fetal tissues.**

| Fetal tissue[a] | PGD synthase, ng/mg[b] |
|---|---|
| Brain | 1510 |
| Heart | 150 |
| Cartilage | 16 |
| Skin | 5 |
| Kidney | 4 |
| Lung | 3 |
| Testis | 2 |
| Liver | 1.5 |

[a] Obtained from an 18-week aborted fetus with trisomy 21.
[b] Nanograms of PGD synthase per milligram of total protein.

**Table 6. PGD synthase content of placentas of various gestational ages.**

| Weeks of gestation | PGD synthase, ng/mg[a] |
|---|---|
| 7 | 2.8 |
| 7–8 | 1.1 |
| 8 | 3.0 |
| 9 | 2.0 |
| 11 | 6.0 |
| 28 | 6.0 |
| 31 | 3.1 |
| 32 | 4.2 |
| 35 | 4.0 |
| 39 | 3.3 |
| 40 | 2.7 |

[a] As in Table 5.

PGD synthase was found in male and female serum and urine, amniotic fluid, CSF, seminal plasma, breast cyst fluid, breast discharge fluid, breast milk, and breast tumor extracts. We presented evidence that, at least in some amniotic fluids, the PGD synthase is rapidly proteolyzed. In fetal tissues, PGD synthase is present at highest concentrations in the brain, followed by the heart; other tissues contain considerably less. Placental extracts show the highest concentrations during gestational weeks 11–28, which paralleled the peak values seen in amniotic fluid. These data highly suggest that the source of PGD synthase in amniotic fluid is the placenta. In fetal blood, the PGD synthase concentrations are similar to those found in adults.

The measurement of PGD synthase has not as yet found clinical applications, given that the already described assays are semiquantitative and relatively insensitive. The new assay developed in this study is 100- to 1000-fold more sensitive and has excellent specificity and analytical performance. We are now in the process of evaluating whether the measurement of PGD synthase has value in diseases of the brain or other organs or in malignant diseases, including breast cancer. Additionally, we are examining its measurement in seminal plasma for evaluation of infertility and its measurement in amniotic fluid and maternal serum for evaluation of fetal status during pregnancy. We anticipate that this assay will help further knowledge of PGD synthase and its role in human pathobiology.

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