Radioimmunoassay Measurement of Prostaglandins E, A, and F in Human Plasma

BERNARD M. JAFFE, HAROLD R. BEHRMAN, and CHARLES W. PARKER

From the Departments of Surgery and Medicine, Washington University, St. Louis, Missouri 63110 and the Department of Physiology, Harvard University, Boston, Massachusetts 02115

ABSTRACT The details of a radioimmunoassay capable of measuring as 5 pg of prostaglandin A, E, and F (PGA, PGE, and PGF) in human and rat plasma are described. Plasma samples are extracted (with 4000 cpm [^{3}H] PGE_1 added for calculation of recovery) with an organic solvent system at an apparent pH of 5.8 and then chromatographed on silicic acid columns with increasing concentrations of methanol to separate PGA, PGE, and PGF. Each chromatographed sample is measured by radioimmunoassay, using the homologous antibody and tritiated marker. 40 normal individuals had mean plasma concentrations of PGA, PGE, and PGF of 1062±107 pg/ml, 385±30 pg/ml, and 141±15 pg/ml, respectively. Elevated PGE levels were measured in the plasma of patients with medullary carcinoma of the thyroid, carcinoid, and neuroblastoma. Treatment of rats with indomethacin decreased serum PGE levels by 67%. The radioimmunoassay appears to be of considerable experimental as well as clinical interest.

INTRODUCTION

Despite the recent increase in pharmacologic data suggesting that prostaglandins have a broad spectrum of actions, it has been difficult to document that these 20 carbon fatty acids possess important physiologic activities. The limiting problem has been the lack of suitable methods for measuring concentrations of endogenous prostaglandins. Spectrophotometric measurements (1–3) are relatively specific and simple, but are sensitive only in the low microgram range. Techniques which combine gas chromatography and mass spectroscopy (4, 5) are specific and are very useful in the measurement of products of prostaglandin degradation; the sensitivity of these methods, however, is limited to the high picogram range and time requirements make them inappropriate for measurement of large numbers of samples. Bioassay systems have been utilized in the past (6–11), but they have serious limitations in sensitivity and specificity and obviously depend on biological responses.

We have previously described a radioimmunoassay capable of measuring subpicomole amounts of prostaglandins (12, 13). Despite the availability of these antibodies, there were two major difficulties which had to be solved before the immunoassay could be applied to the measurement of prostaglandins in tissue and plasma: (*a*) in equilibrium dialysis, an average of 30% of prostaglandin E_1 (PGE_1)[1] 0.1 to 100 ng/ml is bound to human serum albumin (HSA), 10 mg/ml; this extensive binding of prostaglandins to albumin and other proteins diminishes immunoassay sensitivity, and (*b*) the significant degree of immunological cross-reactivity among the various prostaglandins and their antisera make quantitation of the individual prostaglandins difficult. This report is concerned with the solution of these problems. Protein binding has been eliminated by utilizing an organic solvent extraction procedure that separates prostaglandins from plasma proteins. Problems related to immunological cross-reactivity have been circumvented by chromatographic separation of prostaglandins into three major groups, PGE, PGA, and PGF (14–16) followed by utilization of separate antibodies to PGE, PGA, and PGF. Using these modifications, plasma PGA, PGE, and PGF levels have been determined in 40 normal adults and several patients with widespread malignancy.

METHODS

Extraction of prostaglandins from plasma. Blood specimens (see below) obtained between 12:00 a.m. and 2:00

[1] *Abbreviations used in this paper:* GPBS, gelatin phosphate-buffered saline; HSA, human serum albumin; PBS, phosphate-buffered saline; PGE, PGA, and PGF, prostaglandin E, A, and F; POPOP, 1,4-bis[2-(5-phenyloxazole)] benzene; PPO, 2,5-diphenyloxazole.

Received for publication 1 August 1972 and in revised form 13 October 1972.

p.m., unless otherwise specified, were collected in plastic syringes, kept at 4°C, and centrifuged within 1 h after collection to separate the plasma. 1 ml plasma samples were placed in conical glass graduated centrifuge tubes and 13 pg (4,000 cpm) of [^{3}H]PGE$_1$ (New England Nuclear Corp., Boston, Mass., 87 C/mM) in 0.05 ml were added. The plasma was extracted with 3.0 ml of petroleum ether to remove neutral lipids. The aqueous layer was then exposed to 3.0 ml of 3:3:1 ethyl acetate: isopropanol: 0.1 M HCl, apparent pH 5.8, and vortexed, and a mixture of 2.0 ml of ethyl acetate and 3.0 ml of water was added. After further mixing, the two phases were separated by centrifugation (2,000 rpm for 5 min at ambient temperatures). 3 ml of the 3.5 ml organic phase were removed by aspiration and dried in air at 55°C.

Chromatographic separation of prostaglandins. Silicic acid slurry (Sigma Chemical Co., St. Louis, Mo., 100 mesh) was made up to 0.25 g/ml in 60:40 benzene: ethyl acetate (solvent 1). After adding 2.0 ml of slurry to each column (10 ml disposable pipettes with glass wool inserted at their tips), the resin was washed with 5.0 ml of solvent 2 (benzene: ethyl acetate: methanol, 60:40:20) and 1.5 ml of solvent 1. Sample residues were vortexed in 0.2 ml of solvent 3 (benzene: ethyl acetate: methanol, 60:40:10) and then 0.8 ml of solvent 1. The combined organic extracts were applied to the columns which were allowed to run dry. Prostaglandin fractions were obtained by developing the columns serially with 6 ml of solvent 1 (fraction I, PGA and PGB), 12 ml of solvent 4 (benzene: ethyl acetate: methanol 60:40:2) (fraction II, PGE), and 3 ml of solvent 2 (fraction III, PGF). The fractions were taken to dryness by evaporation in air at 55°C and dissolved in 1.0 ml of 0.15 M NaCl–0.01 M phosphate pH 7.54 containing gelatin (Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.) 1 mg/ml, gelatin phosphate buffered saline (GPBS). Samples of the aqueous fractions were dissolved in 0.5 ml NCS (Nuclear-Chicago Solubilizer, Nuclear Chicago Corp., Des Plaines, Ill.) and transferred to 10 ml of toluene scintillation solution for counting (Packard [Tricarb] Instrument Co., Inc., Downers Grove, Ill.) to permit calculation of recovery.

Antiprostaglandin antibodies. Antibodies to PGE$_1$, PGA$_1$, and PGF$_{2\alpha}$ were elicited by immunizing animals to prostaglandin-protein conjugates. The details of immunogen preparation and characterization, and the schedule of immunizing injections have been previously described (12, 13, 16). In the current radioimmunoassay system, each silicic acid column fraction is measured with the appropriate marker and antibody to the appropriate prostaglandin, i.e. for fraction I, [^{3}H]PGA$_1$, anti-PGA$_1$; fraction II, [^{3}H]-PGE$_1$, anti-PGE$_1$; and fraction III, [^{3}H]PGF$_{1\alpha}$, anti-PGF$_{2\alpha}$. The cross-reactivity of each antibody was characterized in the radioimmunoassay system by measuring the degree of inhibition of binding of marker caused by a wide range of concentrations of various prostaglandins as well as a large number of fatty acids, steroids, and fat-soluble vitamins.

Radioimmunoassay system. For each assay (i.e., PGE, PGA, and PGF), 0.05 ml each of appropriately diluted antibody in GPBS, 8,000–10,000 cpm of tritiated prostaglandin (New England Nuclear Corp., PGE$_1$, 87 C/mM, PGA$_1$, 81 C/mM, or PGF$_{1\alpha}$, 73 C/mM), and 1 mM EDTA are incubated for 1 h at 4°C with either unlabeled prostaglandins (15–4,000 pg) or the unknown sample as a silicic acid fraction in 0.1 ml of GPBS. Before separation of antibody-bound from unbound [^{3}H]PG, 0.1 ml of phosphate-buffered saline (PBS) containing 5 mg/ml gelatin is added to prevent nonspecific binding and stabilize the separation procedure (if added earlier, gelatinization prevents adequate reaction). Separation is accomplished by adding to the reaction vials 1.0 ml of dextran-coated charcoal (activated Norit A, 2.5 mg/ml: dextran T70, Pharmacia Fine Chemicals Inc., Piscataway, N. J. 0.25 mg/ml in PBS) (15–17). After a 10 min centrifugation at 4°C, 3,000 rpm, the supernatants (containing antibody bound, [^{3}H]PG) are immediately decanted into 10 ml of triton-containing toluene scintillation solution (18) (2,5-diphenyloxazole [PPO] 7 gm, 1,4-bis[2-(5-phenylorazolyl)]benzene [POPOP] 0.3 gm, toluene 667 ml, Triton X, Sigma Chemical Com., 333 ml). Radioactivity is counted in a Packard Tricarb liquid scintillation counter. When homologous marker, unlabeled PG, and antibody are incubated the calibration curves are all linear over the range of unlabeled PG added when they are plotted with the percent inhibition of binding on the ordinate and the logarithm of unlabeled prostaglandin added on the abscissa. PG concentrations in plasma are calculated by computer program, using the best fit of data to a straight line.

Plasma samples. Plasma samples for prostaglandin analyses were drawn from 40 healthy ambulatory young adult volunteers. For the first 15 patients, parallel samples were collected as blood without anticoagulant (serum), blood anticoagulated with EDTA (plasma), and blood drawn into siliconized syringes and transferred into heparinized, siliconized tubes (plasma); for the final 25 patients, only heparinized and siliconized glassware (plasma) was used. In addition, prostaglandin concentrations have been measured in two patients with medullary carcinoma of the thyroid (without diarrhea), two patients with carcinoid tumors, and one infant with a neuroblastoma and profound diarrhea. Concentrations of PGE in serum and kidney were measured in 10 rats treated with indomethacin, 10 mg/kg administered intraperitoneally the evening before and the day of sample collection.

RESULTS

Extraction, separation, and recovery. Silicic acid columns have been used to separate the major prostaglandin groups (14, 16). Using this scaled down silicic acid chromatographic procedure, it has been possible to obtain individual prostaglandin fractions in good yield (see below) beginning with only 1.0 ml of plasma. The chromatographic separation procedure was validated by adding [^{3}H]PGA$_1$, [^{3}H]PGE$_1$, and [^{3}H]PGF$_{1\alpha}$ to individual serum samples. After organic extraction and evaporation of the organic phase, the samples were chromatographed (Fig. 1). With extracts from serum containing [^{3}H]PGA$_1$ and [^{3}H]PGF$_{1\alpha}$, 97.6% of the recovered radioactivity was present in fractions I and III, respectively. With [^{3}H]PGE$_1$ extracts, 93.2% of the radioactivity recovered from the column was in fraction II. The slight decrease in recovery of PGE$_1$ radioactivity in fraction II may be at least in part due to PGE$_1$ conversion of PGA$_1$ during the extraction. As shown in Fig. 1, the recovered radioactivity in fraction I (the PGA fraction) was slightly greater than that for [^{3}H]PGE$_1$ chromatographed directly without prior extraction from serum. Judging from the results in

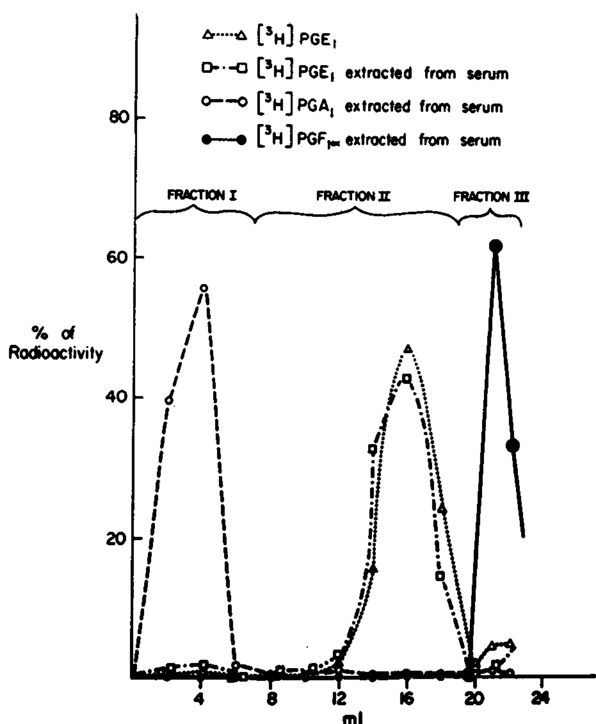

FIGURE 1 Verification of the silicic acid chromatographic separation. [^{3}H]PGA$_1$ 100,000 cpm (open circles), [^{3}H]-PGE$_1$ 100,000 cpm (open squares) and [^{3}H]PGF$_{1\alpha}$ 60,000 cpm (closed circles) were added to serum. Extraction was carried out as described in Methods. After evaporation of the organic phase, samples were chromatographed on silicic acid columns. Whereas ordinarily entire fractions are collected, in these experiments, 2.0 ml samples of fractions I and II and 1.0 ml samples of fraction III were collected. Samples were dried in air at 55°C and taken up to 1.0 ml in GPBS. 0.05 ml samples of each sample were dissolved in 0.5 ml NCS, transferred into 10 ml of toluene scintillation solution, and counted. For comparison, [^{3}H]PGE$_1$ was chromatographed without prior extraction from serum (open triangles).

this and seven additional experiments, however, the level of conversion does not exceed 5%.

After column chromatography, final recoveries of the tritiated prostaglandins added to plasma were consistent and reproducible. For the most recent 255 plasma extractions, recovery of [^{3}H]PGE$_1$ averaged 68.1±13.7% (mean ±1 SD). Recovery of [^{3}H]PGA$_1$ and [^{3}H]PGF$_1$ from the same samples likewise averaged 64.3±2.6% ($n = 3$) and 68.9±6.6% ($n = 12$), respectively. Since the recovery of all three major prostaglandin types is virtually identical, it is justified to add one radioactive label, calculate its recovery, and utilize this figure in calculating concentrations of the three prostaglandin groups. In our current method, we add [^{3}H]PGE$_1$ and utilize its recovery for correction of raw data to actual prostaglandin concentrations.

Antiprostaglandin antibodies. The cross-reactivity of antibodies to PGA$_1$ has been described previously (12, 13). Whereas 50 pg of PGA$_1$ causes 50% inhibition of the antibody binding of [^{3}H]PGA$_1$, comparable degrees of inhibition was achieved by 350 pg of PGA$_2$, 2,000 pg of PGE$_1$, 5,000 pg of PGE$_2$, and 25,000 pg of PGF$_{1\alpha}$. Used at a final concentration of 1:300,000, anti-PGA$_1$ antibodies bind 35% of added radioactive label.

Figs. 2 *a* and *b* compare the inhibition of anti-PGE$_1$-[^{3}H]PGE$_1$ binding by equimolar concentrations of PGE$_1$, PGA$_1$, and PGE$_2$. At concentrations which cause 50% inhibition of binding, the homologous ligand is 7–10 times as effective an inhibitor as either of these cross-reacting compounds. In this same system, PGE$_1$ is more than 1,000 times as effective as PGF$_{1\alpha}$ and PGF$_{2\alpha}$. Used at the final serum dilution of 1:6000, anti-PGE$_1$ antibody binds 52% of [^{3}H]PGE$_1$.

The specificity of the anti-PGF$_{2\alpha}$ antibodies has been described in detail (16). Although PGF$_{1\alpha}$ has a relative cross-reactivity of 0.453 (2.2 times the amount of PGF$_{1\alpha}$ is required to displace the same amount of marker as PGF$_{2\alpha}$), the corresponding values for PGE and PGA derivatives are less than 0.001. At the dilution used, 1:6,000, anti-F antibodies bind 40% of [^{3}H] PGF$_{1\alpha}$.

The major immediate plasma metabolites of prostaglandins are 15-keto derivatives (19), which cross-react to a negligible degree with antiprostaglandin antibodies ($P < 0.004$). Since these compounds circulate in concentration approximately 30 times that of the native compounds, (20) they do not appear to interfere with the radioimmunoassay system. Similarly, arachidonic acid, at the reported serum concentration, 7 μg/ml (21), does not interfere with the PGE radioimmunoassay. Only 2–3% of [^{3}H]arachidonic acid is recovered in any PG fraction. In addition, relative cross-reactivity of arachidonic acid with anti-PG antibodies is less than 0.00001. The combination of the extraction procedure and the very low degree of antibody cross-reactivity also excludes interference by linoleic and other free unsaturated fatty acids, steroid hormones, fat-soluble vitamins, and compounds that interact with the adenyl cyclase system.

We have found good agreement in data comparing PGE concentrations as measured by radioimmunoassay and by two bioassays, the cat blood pressure bioassay (11) and the organ superfusion bioassay technique (8). These observations further substantiate the ability of the radioimmunoassay to measure active prostaglandins.

Measurement of plasma prostaglandin concentrations. 15 pg of PGA$_1$, PGE$_1$, and PGF$_{1\alpha}$ caused 22%, 15%, and 20% inhibition, respectively, with the appropriate

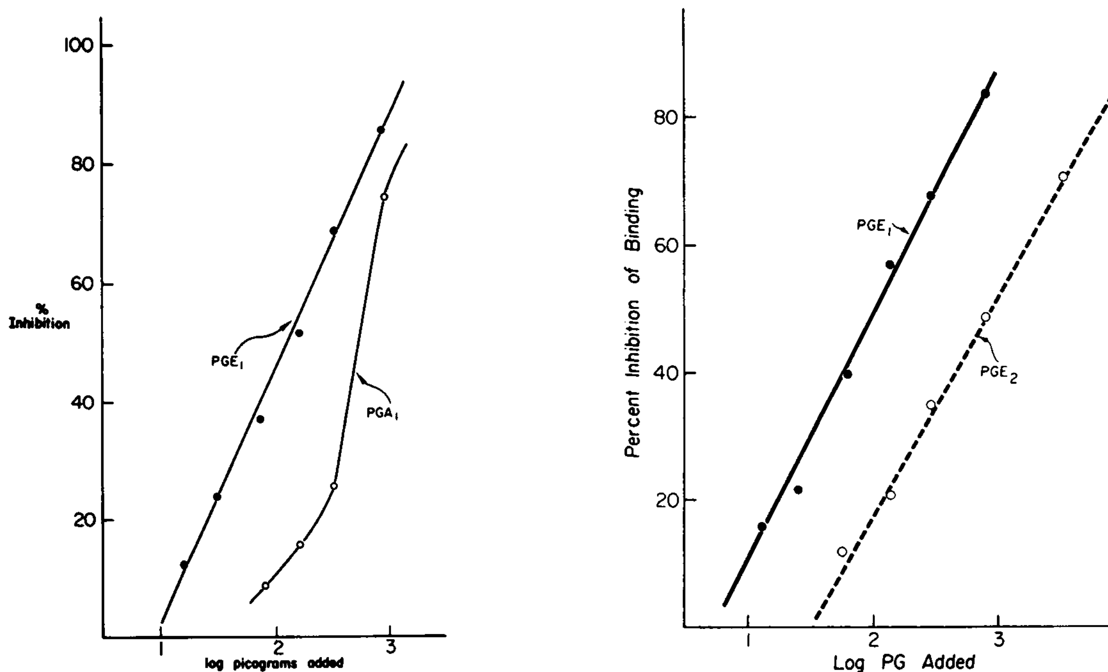

FIGURE 2 Specificity of anti-PGE_1 antibody. (*a*) Equimolar concentrations of PGE_1 and PGA_1 (15 to 800 pg) were incubated with 0.05 ml each of anti-PGE_1 1 mM EDTA, and [^{3}H]PGE_1 (20,000 cpm). (*b*) Similar experiment with equimolar concentrations of PGE_1 and PGE_2. After 1 h incubation, separation was accomplished using dextran-coated charcoal as in Methods. Percent inhibition of binding was calculated from $100 \times$ % bound without inhibitor $-$ % with inhibitor/% bound without inhibitor.

antibodies. Maximal sensitivity for the measurement of all three prostaglandins is 5 pg, i.e. 5 pg generally causes more than 10% inhibition of binding.

In order to verify the specificity and reliability of the radioimmunoassay, calibration curves were constructed comparing known concentrations of PGE_1 in GPBS and the curves achieved by extracting and chromatographing the same concentrations of PGE_1 added to serum. The results are plotted in Fig. 3. After correcting the "extracted" data for recovery, it is obvious that the two calibration curves are indistinguishable. In similar experiments linearity of measurement of PGA and PGF extracted from plasma was also established.

Before conducting studies of plasma prostaglandin levels, we compared the PGE concentrations in serum and plasma from the same individuals. Blood was obtained from 15 normal donors, and PGE concentrations measured in serum, plasma (EDTA), and plasma from samples drawn into heparinized, siliconized glassware. Without recovery corrections, mean PGE concentrations were serum, 161 ± 11 pg/ml (mean $\pm$ SEM; range 100–230 pg/ml); plasma, 168 ± 12 pg/ml (range 105–240 pg/ml), and plasma from siliconized glassware 166 ± 16 pg/ml (range 100–300 pg/ml), respectively. For this group of determinations, individual samples (serum, plasma, plasma from siliconized glassware) PGE concentrations varied an average of 16.3% from the mean for each individual patient. Similar results were obtained for PGF. Subsequent data were obtained using heparinized plasma. We have measured PGE levels in plasma separated at 30-min intervals after collection and have shown no alterations in plasma PGE as long as the interval between collection and separation of blood is no longer than 6 h.

Plasma levels of PGA, PGE, and PGF in 40 normal patients are listed in Table I. For the first 15 patients, radioactive prostaglandin was not added before extraction and recovery correction was based upon average recoveries at that time; for the remaining 25 patients, data was corrected based upon individual recovery of added [^{3}H]PGE_1. Circulating levels of prostaglandins were all in the picogram range. Concentrations of PGA were consistently higher than levels of PGE and PGF. Superficially, concentrations of all prostaglandin types appeared to parallel each other. Patients with PGE concentrations greater than 415 pg/ml (mean + 1 SEM) had PGA levels of 1300 ± 201 pg/ml and PGF levels of 177 ± 22; on the other hand, patients with PGE levels below 355 (mean $-$ 1 SEM) had plasma concentrations of PGA and PGF of 867 ± 133 and 89 ± 17 pg/ml, re-

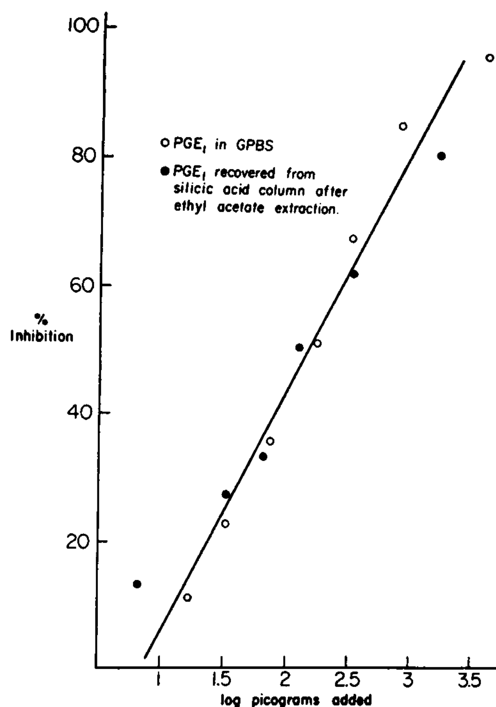

FIGURE 3 Verification of the PGE radioimmunoassay. PGE_1, 15 to 4,000 pg were added to 1.0 ml of serum, extracted, chromatographed, taken up to GPBS, and measured in the PGE radioimmunoassay system. After correcting for recovery (parallel experiments) and subtracting the initial prostaglandin concentration, the data was plotted (closed circles). The calibration curve in GPBS (without prior extraction from serum) is plotted for comparison.

spectively. However, correlation coefficients for data relating to PGE to PGA and PGE to PGF were only 0.272 and 0.304, respectively.

For estimation of normal levels of prostaglandins, all samples were drawn between 12:00 a.m. and 2:00 p.m. We have investigated in a preliminary way how constant levels of PGE are during the course of a day by drawing samples from two normal females at 9:00 a.m., 11:00 a.m., 2:00 p.m., and 5:00 p.m. For patient M. B., PGE levels were 908, 659, 512, and 401 pg/ml,

TABLE I
Normal Plasma Concentrations of Prostaglandins

	PGA	PGE	PGF
	pg/ml	*pg/ml*	*pg/ml*
Males (10)	1024±214	378±73	84±15
Females (16)	888±160	316±36	154±35
Mean	940±126	340±34	128±17
Females on oral contraceptives (14)	1285±197	478±55	162±19
Total, Mean±SEM	1062±107	385±30	141±15
Range	198−2612	139−1011	50−359

TABLE II
Plasma PGE Concentrations in Patients with Endocrine-Active Tumors

Patient	Age	Sex	Disease	PGE concentrations pg/ml*
1. L. C.	21	M	Medullary carcinoma of thyroid	2375
2. W. N.	40	M	Medullary carcinoma of thyroid	1000
3. M. J.	45	F	Carcinoid tumor with syndrome	3330
4. W. C.	54	M	Carcinoid tumor without syndrome	967
5. N. J.	5 mo.	F	Neuroblastoma with diarrhea	>10,000

* For the mean and range of PGE_1 concentrations in normal young adults, see Table I.

respectively; for the second patient, C. J., data were 830, 1,065, 1,071, 470 pg/ml, respectively. Thus, for these two patients, PGE levels varied considerably during the day but were higher in the morning than in the late afternoon.

Peripheral plasma concentrations of prostaglandins might be expected to be high in patients with medullary carcinoma of the thyroid (22) and associated malignancies (23, 24). Concentrations of PGE in the plasma of patients with these malignancies are listed in Table II. Neither patient with medullary carcinoma of the thyroid had associated diarrhea but both had widespread metastatic carcinoma and in addition, had elevated levels of thyrocalcitonin in their sera (25). The first patient with a carcinoid tumor (patient M. J.) had a primary lesion in the jejunum and carcinoid syndrome manifested primarily by flushing and accompanied by mild elevation of urinary 5-hydroxy indole acetic acid; the second patient in this group (W. C.) had an enormous carcinoid tumor of the rectum with extensive hepatic involvement but no symptoms of carcinoid syndrome. The last patient (N. J.) was an infant girl who died of a neuroblastoma and was very symptomatic with profound diarrhea. In patients 1, 3, and 5, concentrations of PGE were elevated above the range of any of the 40 normal subjects studied; levels in patients 2 and 4 were at the upper limits of "normal." One patient with a pheochromocytoma had normal PGE levels both before and after resection of the tumor.

One way of validating the specificity of the prostaglandin measurements is to study the effect of inhibitors of PGE synthesis on serum and tissue PGE concentrations. Under urethane anesthesia, the left kidneys of 10

control and 10 indomethacin treated rats were clamped for 4½ h; 5 min after release of the clamps, the rats were exsanguinated. Renal (right and left) and serum concentrations of PGE were measured. Indomethacin, a potent inhibitor of prostaglandin synthesis (26–28), lowered PGE concentrations in rat serum and kidney 67.0 and more than 98% (Table III) (29). In indomethacin treated animals, measurable amounts of PGE were noted in only 2/10 sera and 3/20 kidneys examined by comparison with 6/8 and 19/19, respectively from control animals.

DISCUSSION

Production of antibodies to $PGF_{2\alpha}$ suitable for radioimmunoassay has been reported by several other investigators (30–36). These antibodies have variable but significant degrees of cross-reactivity with $PGF_{1\alpha}$, but minimal degrees of cross-reactivity for PGE, PGA, and PGB compounds. Consequently, they can be used to assay $PGF_{2\alpha}$ and recently results of studies measuring endogenous $PGF_{2\alpha}$ concentrations have appeared in the literature (16, 37–39). In general, antibodies obtained by other investigators in response to immunization with PGE (30, 31, 36) and PGA-protein conjugates have not demonstrated the same group-specificity (30, 31, 36). We have previously shown that by taking precautions to to avoid prostaglandin degradation and by immunization of multiple animals, it is possible to obtain anti-E and anti-A antibodies with considerable group specificity (12, 13). Each of the antibodies demonstrates specificity for both the cyclopentane ring (and hence distinguish between PGE, PGA, and PGF compounds) as well as the aliphatic side chains (and hence can distinguish between PGA_1 and PGA_2, etc.). Using the homologous antiserum and marker, as little as 5 pg of PGA and PGE can be measured. Despite the relative specificity of the antisera we believe it is highly desirable to eliminate possible uncertainty by including an extraction-chromotographic separation procedure in the preparation of samples for measurement. The method for prostaglandin extraction and chromatographic separation described in this paper results in good recovery, is reproducible, can be easily applied to the measurement of tissue prostaglandin concentrations, and does not convert significant amounts of PGE to PGA. The extraction procedure should also eliminate possible difficulties from enzymes in plasma capable of degrading prostaglandins. In serum incubated at 37°C, a slow conversion of PGA and PGB takes place, apparently due to the presence of prostaglandin A isomerase (40). After removal of the proteins present in serum by extraction, this conversion should no longer be possible.

While the combined immunoassay-chromatographic separation procedure eliminates any problems created

TABLE III

Indonethacin Inhibition of PGE Synthesis

	Control	Indomethacin-Treated
Renal PGE_1 (pg/g)		
Mean±SEM	1118±377	21±12
Number of samples	19	20
Number immeasurably low	0	17
Serum PGE_1 (pg/ml)		
Mean±SEM	243±73	83±66
Number of samples	8	10
Number immeasurably low	2	8

Rats were exsanguinated and serum and kidneys were immediately extracted into the organic solvent system and chromatographed in silicic acid. The difference between renal concentrations of PGE in control and indomethacin-treated animals was statistically significant ($P < 0.02$). In the indomethacin-treated animals one high serum concentration (650 pg/ml) prevents the serum differences from being statistically different.

by group cross-reactivity it is not yet possible to clearly distinguish between PGE_1 and PGE_2, and PGA_1 and PGA_2, and $PGF_{2\alpha}$ and $PGF_{1\alpha}$. However, since antisera routinely exhibit specificity for aliphatic side chains, once all of the individual antisera are available, parallel determinations and the use of simultaneous equations should permit accurate measurement of individual prostaglandins as well as prostaglandin groups.

The major pathway of PGE and PGF metabolism is enzymatic conversion of the hydroxyl group at the 15 position to a largely inactive keto derivative (19, 41–43) predominantly in the lung (44–46). The existing data indicates that this degradation occurs very rapidly with conversion of the majority of PGE in one passage through the lungs. Most of the enzymatically inactivated prostaglandin is returned to the plasma for further metabolism or excretion. Since 15-keto prostaglandins are likely to circulate in significant concentrations, the fact that 15-keto prostaglandin derivatives do not cross-react immunologically to any significant degree is essential to the accuracy of PGE and PGF measurements. Similar enzymatic oxidation does not appear to occur with PGA compounds (47, 48) and this undoubtedly contributes to their relatively high concentrations in plasma by comparison with those of PGE and PGF. The dependency of blood PGE levels on continuous prostaglandin synthesis and release is further suggested by the results in indomethacin-treated rats where the drug produced a marked depression of serum and kidney PGE concentrations. The diminution of PGE under these circumstances can be explained by the ability of indomethacin to block the conversion of prostaglandin precursors to prostaglandins.

The fall in measurable PGE in association with indomethacin treatment provides important substantiation of the specificity of the assay for prostaglandin.

In contrast to the very low circulating levels of PGE induced by inhibition of the prostaglandin synthesis, it seems highly probable that the very high PGE concentrations in the plasmas of three of five patients with specific malignancies represent active secretion of prostaglandins by the tumors. Prostaglandin release by a variety of cell lines in tissue culture has been described previously from this laboratory (49, 50). The presence of large amounts of prostaglandins in human medullary carcinoma tissue has been suggested on the basis of bioassay measurements of tumor and serum extracts from patients with this condition (22). Our immunochemical measurements provide direct substantiation for the occurrence of increased circulating prostaglandin concentrations in association with certain tumors.

Elevated levels of circulating PGE have also been noted in two additional situations. In rats made hypertensive by ligating the branches to the lower pole of the left kidney (and not in control rats) concentrations of PGE in the ischemic kidneys were significantly higher than in the contralateral kidneys. (1962±383 vs. 1205±282 pg/g). Animals with surgically-induced renal ischemic also had a doubling in serum PGE levels (29), (1583±298 vs. 737±267 pg/ml). In addition, in studies of 32 human females we have noted elevations of plasma PGE during labor and in cord blood, but normal concentrations both during pregnancy and 24 h after delivery.[3] Finally although previous reports suggested that PGE levels in aqueous humor might be elevated in patients with primary open angle glaucoma (51), we have been unable to demonstrate such a difference (52).

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of Dr. M. Parker and the Washington University Health Service in the collection of normal patient specimens, Doctors L. O'Neal, V. Loeb, J. Ternberg, and C. Costello for permission to include their patients in the study, D. Collier and C. Johnson for their capable technical assistance, Doctors G. Marshall and P. Needleman for their assistance in the rat indomethacin experiment.

This work was supported in part by U. S. Public Health Service Training Grant no GM 381 and National Institutes of Health Research Grant no. A 104646. Some of the patients with malignancies were admitted to Washington University Clinical Research Center and supported by the National Institutes of Health Grant no. RR 00036.

REFERENCES

1. Anderson, N. H. 1969. Dehydration of prostaglandins: study by spectroscopic method. *J. Lipid Res.* **10**: 320.
2. Shaw, J. E., and P. W. Ramwell. 1969. Separation, identification, and estimation of prostaglandins. *Methods Biochem. Anal.* **17**: 325.
3. Bygdeman, M., and B. Samuelsson. 1964. Quantitative determination of prostaglandins in human semen. *Clin. Chim. Acta.* **10**: 566.
4. Axen, U., K. Green, D. Hörlin, and B. Samuelsson. 1971. Mass spectrometric determination of picomole amounts of prostaglandins E_2 and $F_{2\alpha}$ using synthetic deuterium labeled carriers. *Biochem. Biophys. Res. Commun.* **45**: 519.
5. Thompson, C. J., M. Los, and E. W. Horton. 1970. The separation, identification, and estimation of prostaglandins in nanogram quantities by combined gas chromatography-mass spectrometry. *Life Sci.* **9**: 983.
6. Bergström, S., L. A. Carlson, and J. R. Weeks. 1968. The prostaglandins: a family of biologically active lipids. *Pharmacol. Rev.* **20**: 1.
7. Karim, S. M. M. 1967. The identification of prostaglandins in human umbilical cord. *Br. J. Pharmacol. Chemother.* **29**: 230.
8. Vane, J. R. 1969. The release and fate of vaso-active hormones in the circulation. *Br. J. Pharmacol.* **35**: 209.
9. Eakins, K. E., S. M. M. Karim, and J. D. Miller. 1970. Antagonism of some smooth muscle actions of prostaglandins by polyphloretin phosphate. *Br. J. Pharmacol.* **39**: 556.
10. Lee, J. B., R. B. Hickler, C. A. Saravis, and G. W. Thorn. 1963. Sustained depressor effect of renal medullary extract in the normotensive rat. *Circ. Res.* **13**: 359.
11. Kannegiesser, H., and J. B. Lee. 1971. Difference in hemodynamic response to prostaglandins A and E. *Nature (Lond.).* **229**: 498.
12. Jaffe, B. M., J. Smith, W. T. Newton, and C. W. Parker. 1971. Radioimmunoassay for prostaglandins. *Science (Wash. D. C.).* **171**: 494.
13. Jaffe, B. M., and C. W. Parker. Radioimmunoassay for prostaglandins. *In* Radioassay in Clinical Medicine. R. Donati and W. T. Newton, editors. Charles C. Thomas, New York. In press.
14. Jaffe, B. M., and C. W. Parker. 1972. Extraction of PGE from human serum for radioimmunoassay. *In* Report of Third Conference on Prostaglandins in Fertility Control. S. Bergstrom, K. Green, and B. Samuelsson, editors. Karolinska Institutet, Stockholm. 69.
15. Caldwell, B. V., S. Burstein, W. A. Brock, and L. Speroff. 1971. Radioimmunoassay of the F prostaglandins. *J. Clin. Endocrinol. Metab.* **33**: 171.
16. Orczyk, G. P., and H. R. Behrman. 1972. Ovulation blockade by asprinin or indomethacin—in vivo evidence for a role of prostaglandin in gonodatrophin secretion. *Prostaglandins.* **1**: 3.
17. Herbert, V., K. Lau, C. W. Gottlieb, and S. J. Bleicher: 1965. Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol. Metab.* **25**: 1375.
18. Behrman, H. R., K. Yoshinaga, H. Wyman, and R. O. Greep. 1971. Effects of prostaglandin on ovarian steroid secretion and biosynthesis during pregnancy. *Am. J. Physiol.* **221**: 189.
19. Änggård, E., and B. Samuelsson. 1964. Prostaglandins and related factors. Metabolism of prostaglandin E_1 in guinea pig lung: the structures of two metabolites. *J. Biol. Chem.* **239**: 4097.
20. Green, K., F. Beguin, M. Bydeman, M. Toppozoda, and N. Wiquist. 1972. Analysis of prostaglandin $F_{2\alpha}$ and metabolites following intravenous intraammotic and

[3] Hertelendy, F., R. Woods, and B. M. Jaffe. Prostaglandin E levels in peripheral blood during labor. Submitted for publication.

vaginal administration. *In* Report of Third Conference on Prostaglandins in Fertility Control. S. Bergstrom, K. Green, and B. Samuelsson, editors. Karolinska Institute, Stockholm. 18.

21. Shrade, W., R. Biegler, and E. Böhle. 1961. Fatty-acid distribution in the lipid fractions of healthy persons of different age, patients with atherosclerosis, and patients with idiopathic hyperlipidaemia. *J. Altheroscler. Res.* **1**: 47.
22. Williams, E. D., S. M. M. Karim, and M. Sandler. 1968. Prostaglandin secretion by medullary carcinoma of the thyroid. A possible cause of the associated diarrhaea. *Lancet.* 1: 22.
23. Oates, J. A., and T. C. Butler. 1967. Pharmacologic and endocrine aspects of carcinoid syndrome. *Adv. Pharmacol.* **5**: 109.
24. Sipple, J. H. 1961. The association of pheochromocytoma with carcinoma of the thyroid gland. *Am. J. Med.* **31**: 163.
25. Block, M. A., C. E. Jackson, and A. H. Tashjian, Jr. 1972. Medullary thyroid carcinoma detected by serum calcitonin assay. *Arch. Surg.* **104**: 579.
26. Vane, J. R. 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat. New Biol.* **231**: 232.
27. Smith, J. B., and A. L. Willis. 1971. Aspirin selectively inhibits prostaglandin production in human platelets. *Nat. New Biol.* **231**: 235.
28. Ferreira, S. H., S. Moncada, and J. R. Vane. 1971. Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nat. New Biol.* **231**: 237.
29. Jaffe, B. M., C. W. Parker, G. R. Marshall, and P. Needleman. 1972. The renal concentrations of prostaglandin E in acute and chronic renal ischemia. *Biochem. Biophys. Res. Commun.* **49**: 799.
30. Levine, L., and H. Van Vunakis. 1970. Antigenic activity of prostaglandins. *Biochem. Biophys. Res. Commun.* **41**: 1171.
31. Levine, L., R. M. Gutierrez-Cernosek, and H. Van Vunakis. 1971. Specificities of prostaglandins B_1, $F_{1\alpha}$, and $F_{2\alpha}$ antigen-antibody reactions. *J. Biol. Chem.* **246**: 6782.
32. Kirton, K. T., J. C. Cornette, and K. L. Barr. 1972. Characterization of antibody to prostaglandin $F_{2\alpha}$. *Biochem. Biophys. Res. Commun.* **47**: 903.
33. Caldwell, B. V. 1972. Some practical considerations in the radioimmunoassay of the F prostaglandins. *In* Report of Third Conference on Prostaglandins in Fertility Control. S. Bergstrom, K. Green, and B. Samuelsson, editors. Karolinska, Institutet, Stockholm. 83.
34. Jubiz, W., and J. Frailey. 1971. Generation of antibodies to prostaglandins E_1 and $F_{2\alpha}$. *Clin. Res.* **19**: 127.
35. Jubiz, W., J. Frailey, and K. Bartholomew. 1972. Prostaglandins of the F group (PGF) : assessment by radioimmunoassay of their role in human physiology. *Clin. Res.* **20**: 178. (Abstr.)
36. Yu, S-C, L. Chang, and G. Burke. 1972. Thyrotropin increases prostaglandin levels in isolated thyroid cells. *J. Clin. Invest.* **51**: 1038.
37. Gutierrez-Cernosek, R. M., J. Zuckerman, and L. Levine. 1972. Prostaglandin $F_{2\alpha}$ levels in sera during human pregnancy. *Prostaglandins.* **1**: 331.
38. Gutierrez-Cernosek, R. M., L. M. Morrill, and L. Levine. 1972. Prostaglandin $F_{2\alpha}$ levels in peripheral sera of man. *Prostaglandins.* **1**: 71.
39. Caldwell, B. V., S. A. Tillson, W. A. Brock, and L. Speroff. 1972. The effects of exogenous progesterones and estradiol on prostaglandin F levels in ovariectomized ewes. *Prostaglandins.* **1**: 217.
40. Polet, H., and L. Levine. 1971. Serum prostaglandin A_1 isomerase. *Biochem. Biophys. Res. Commun.* **45**: 1169.
41. Pace-Asciak, C., K. Morawska, and L. S. Wolfe. 1970. Metabolism of prostaglandin $F_{1\alpha}$ by the rat stomach. *Biochim. Biophys. Acta.* **218**: 288.
42. Hamberg, M., and B. Samuelsson. 1971. On the metabolism of prostaglandins E_1 and E_2 in man. *J. Biol. Chem.* **246**: 6713.
43. Änggård, E., K. Green, and B. Samuelsson. 1965. Synthesis of tritium labeled prostaglandin E_2 and studies on its metabolism in guinea pig lung. *J. Biol. Chem.* **240**: 1932.
44. Änggård, E., and B. Samuelsson. 1965. Biosynthesis of prostaglandins from arachidonic acid in guinea pig lung. *J. Biol. Chem.* **240**: 3518.
45. Ferreira, S. H., and J. R. Vane. 1967. Prostaglandins: their disappearance from and release into the circulation. *Nature (Lond.).* **216**: 868.
46. Piper, P. J., J. R. Vane, J. H. Wyllie. 1970. Inactivation of prostaglandins by the lungs. *Nature (Lond.).* **225**: 600.
47. McGiff, J. C., N. A. Terragno, J. C. Strand, J. B. Lee, A. J. Lonigro, and K. K. F. Ng. 1969. Selective passage of prostaglandins across the lungs. *Nature (Lond.).* **223**: 742.
48. Horton, E. W., and R. L. Jones. 1969. Prostaglandins A_1, A_2, and 19-hydroxy A_1; their actions on smooth muscle and their inactivation on passage through the pulmonary and hepatic portal vascular beds. *Br. J. Pharmacol.* **37**: 705.
49. Jaffe, B. M., C. W. Parker, and G. W. Philpott. 1971. Immunochemical measurement of prostaglandin or prostaglandin-like activity from normal and neoplastic tissues in culture. *Surg. Forum.* **22**: 90.
50. Jaffe, B. M., C. W. Parker, and G. W. Philpott. 1972. Prostaglandin release by human cells in vitro. *In* Prostaglandins in Cellular Biology and the Inflammatory Process. P. W. Ramwell and B. B. Phariss, editors. Plenum Publishing Corp., New York. 207.
51. Wyllie, A. M., and J. H. Wyllie. 1971. Prostaglandins and glaucoma. *Br. Med. J.* **3**: 615.
52. Podos, S. M., B. M. Jaffe, and B. Becker. 1972. Prostaglandins and glaucoma. *Br. Med. J.* **4**: 232.