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SEPARATION OF PROSTAGLANDIN E_1 FROM ITS MAJOR METABOLITES

APPLICATION OF THE TECHNIQUE TO MEASURE FIRST-PASS CLEARANCE OF PGE_1 IN THE PULMONARY AND CEREBRAL CIRCULATIONS OF THE ANESTHETIZED DOG

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Abstract—A technique for the separation of prostaglandin E_1 (PGE_1) metabolites from parent PGE_1 in large numbers of plasma samples is described. Radiolabeled metabolites were enzymatically synthesized from [^{3}H]PGE_1, using either the 100,000 *g* supernatant fraction from rabbit lung homogenate or perfused dog lung, and purified by thin-layer chromatography. Separation of pure 15-keto[^{3}H]PGE_1, 13,14-dihydro-15-keto[^{3}H]PGE_1 and 13,14-dihydro[^{3}H]PGE_1 from parent [^{3}H]PGE_1 was achieved on microcolumns of silica gel. All three metabolites were completely eluted from the column with 10 ml of 0.1% formic acid in ethyl acetate. Less than 5 per cent of applied [^{3}H]PGE_1 appeared in the metabolite fraction. [^{3}H]PGE_1 was subsequently eluted from the column with 1% formic acid in ethyl acetate. Identical results were obtained with radiolabeled metabolites and parent PGE_1 extracted from dog blood. Application of this method to indicator-dilution experiments designed to measure removal of [^{3}H]PGE_1 in dog lung and brain is reported. Pulmonary extraction and metabolism of [^{3}H]PGE_1 at the peak of indicator-dilution outflow curves were 84.1 ± 2.2 and 41.8 ± 9.3 per cent (mean ± S.D., N = 20) respectively. Brain extraction from duplicate runs in one animal averaged 22 per cent with less than 5 per cent metabolism of [^{3}H]PGE_1. These estimates agree well with previously reported data and thereby support the reliability and usefulness of this new method.

The lung can synthesize, take up, metabolize and release prostaglandins [1, 2]. Recently, we have applied indicator-dilution techniques to determine, *in vivo*, the pulmonary disposition of a variety of compounds, including 5-hydroxytryptamine and norepinephrine [3]. In order to use these methods to study the fate of injected prostaglandins during their passage through the lungs of intact animals, it is necessary to determine the concentrations of both the parent compounds and their metabolites in a large number of plasma samples. Since no suitable technique to achieve such separation existed, we developed the method described in this paper.

Several methods are presently available for the separation and quantitation of prostaglandins and prostaglandin metabolites in plasma, urine and tissue samples. Gas chromatography–mass spectrometry [4], high-performance liquid chromatography [5], and radioimmunoassay [6] have all been used successfully to measure prostaglandins, but they are impractical for routine separation of metabolites from parent prostaglandins. Thin-layer chromatography is capable of separating classes of prostaglandins (e.g. PGE, PGF, PGA, PGB) [7, 8] or parent prostaglandins from their metabolites [9, 10]. Although widely used [11, 12], this technique is somewhat cumbersome for use with large numbers of samples.

Column chromatography utilizing microcolumns of silica gel [13], acid-washed Florisil [14], silicic acid [15], or Sephadex LH-20 [16] have been used to separate classes of prostaglandins, but not their metabolites. Microcolumns of Sephadex LH-20 [17] were used recently to separate PGE_1 and $PGF_{1\alpha}$ from their metabolites. Although this latter method can separate metabolites and parent prostaglandins, the columns have relatively slow flow rates and thus application to multiple samples is very time consuming.

In this paper, we describe the separation and quantitation of [^{3}H]PGE_1 from its three major metabolites (15-keto[^{3}H]PGE_1; 13,14-dihydro-15-keto[^{3}H]-PGE_1; and 13,14-dihydro[^{3}H]PGE_1) on microcolumns of silica gel, which provide relatively fast flow rates and are therefore suitable for handling large numbers of samples. We also describe application of this separation method to indicator-dilution experiments designed to determine the extent of clearance and metabolism of [^{3}H]PGE_1 in lung and brain of the anesthetized dog.

MATERIALS AND METHODS

Preparation of [^{3}H]*PGE_1 metabolites.* The 15-keto and 13,14-dihydro metabolites of [^{3}H]PGE_1 were prepared from rabbit lung homogenate by modification of the procedure described by Moore and Hoult [18]. Rabbit lung was homogenized in 50 mM potassium phosphate buffer, pH 7.5, containing 1 mM Na_2EDTA and 1 mM dithiothreitol at 4° with

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an Ultra-Turrax homogenizer (4 × 5 sec). The homogenate was centrifuged at 100,000 *g* for 45 min at 4°. The cell-free supernatant fraction containing prostaglandin-metabolizing enzymes was either used immediately or stored at −20° for a maximum of 10 weeks. Protein was determined by the method of Lowry *et al.* [19].

15-Keto-[^{3}H]PGE_1 was prepared by incubation of [^{3}H]PGE_1 [18] with 1 ml of the 100,000 *g* supernatant fraction (approximately 10–12 mg protein) containing 5 mM NAD^+ at 37° for 60 min. After incubation, the reaction tube was placed on ice and the reaction mixture was acidified to pH 3 with HCl and extracted with 2 × 2 ml ethyl acetate. The extract was dried in a stream of air, and the residue was resuspended in 200 μl ethanol and applied to a thin-layer chromatography plate which was developed in chloroform–methanol–acetic acid (95:1:5) to a height of 15 cm. The band at an R_f value corresponding to cold marker 15-keto-PGE_1 was scraped off the plate and eluted with ethyl acetate, which was subsequently concentrated to a small volume and stored at −20°. The 13,14-dihydro[^{3}H]PGE_1 metabolite was prepared in a similar manner by incubation of 15-keto[^{3}H]PGE_1 with the high-speed supernatant fraction containing 5 mM NADH.

The 13,14-dihydro-15-keto[^{3}H]PGE_1 metabolite was prepared by recirculating perfusion of an isolated dog lung with [^{3}H]PGE_1 in 150 ml of Krebs-bicarbonate buffer (NaCl, 118.2 mM; KCl, 4.7 mM; $CaCl_2 \cdot 2H_2O$, 2.5 mM; KH_2PO_4, 1.2 mM; $MgSO_4 \cdot 7H_2O$, 1.2 mM; $NaHCO_3$, 26.2 mM; D-glucose, 11.1 mM; and $CaNa_2EDTA$, 0.03 mM). The solution was aerated with 95% O_2–5% CO_2, and the lung was perfused at a rate of 120 ml/min at 37° for 10 min. The entire perfusate was acidified to pH 3 with HCl and extracted with 2 × 75 ml of ethyl acetate. The extract was evaporated under vacuum to a small volume, and then evaporated to dryness under a stream of nitrogen. The residue was dissolved in a small volume of ethyl acetate, applied to a thin-layer chromatography plate and developed in chloroform–methanol–acetic acid (95:1:5) to a height of 15 cm. The band of radioactivity corresponding to cold marker 13,14-dihydro-15-keto-PGE_1 was scraped off, eluted with ethyl acetate, and stored at −20°.

Samples of all metabolites prepared in this manner, as well as [^{3}H]PGE_1, were re-chromatographed for identification and purity checks. These thin-layer chromatograms were developed as above and each plate was scraped in either 0.5 or 1 cm sections into plastic minivials. Instagel was added to the vials, and radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer.

Column chromatography. Glass microcolumns (11 cm × 0.5 cm i.d.) fitted on top with a reservoir and at the base with a fritted glass disk and stopcock were used for all column chromatography. A silica gel slurry in ethyl acetate was poured into the column to a final height of 11 cm, representing approximately 1.6 g of silica gel. An aliquot of [^{3}H]PGE_1 or [^{3}H]metabolite was prepared for application to the column by evaporation to dryness under a stream of air and was either resuspended in 100 μl of ethyl acetate or extracted from dog blood as described below for indicator-dilution experiments. The [^{3}H]prostaglandin was then applied to the column in 100 μl of ethyl acetate that was allowed to flow into the silica gel and was followed by a 900-μl ethyl acetate wash. This first milliliter of ethyl acetate was collected as a single fraction and contained no radioactivity. All [^{3}H]prostaglandins were then eluted from the column by one of two methods: (1) 5 ml ethyl acetate followed by 40 ml of 0.1% formic acid in ethyl acetate applied to the column in 0.5 ml aliquots and collected as 0.5 ml fractions; or (2) 10 ml of 0.1% formic acid in ethyl acetate applied to the column as a single addition, followed by 10 ml of 1% formic acid in ethyl acetate, and eluates collected as 0.5 ml fractions with a drop-counter fraction collector or, in indicator-dilution experiments, as two 10 ml fractions. The 0.5 ml column eluate fractions were collected in plastic minivials, to which was added 4 ml Instagel, and radioactivity was counted in a Packard Tri-Carb scintillation spectrometer. Aliquots of pure [^{3}H]PGE_1 or [^{3}H]metabolites used to calculate recoveries were treated in a similar fashion to assure uniformity of counting efficiencies. The 10 ml column eluates were collected in large plastic vials and allowed to evaporate to dryness overnight in a fume hood. Ten milliliters of Econofluor was added to each vial and radioactivity was determined.

The final column chromatographic procedure chosen for use in indicator-dilution experiments is shown in Fig. 1.

Indicator-dilution technique. Experiments were performed on eleven heparinized dogs (200 units/kg) under sodium pentobarbital (30 mg/kg) anesthesia. Animals were paralyzed with gallamine triethiodide (2 mg/kg), intubated, and ventilated with a positive pressure respirator. A femoral vein was cannulated for infusion of fluids and supplemental drugs. Ten animals were catheterized for estimates of pulmonary prostaglandin extraction. A Swan-Ganz catheter (Edwards Laboratories, Inc., Santa Ana, CA) was advanced from a femoral vein into the pulmonary artery to monitor pulmonary arterial and capillary

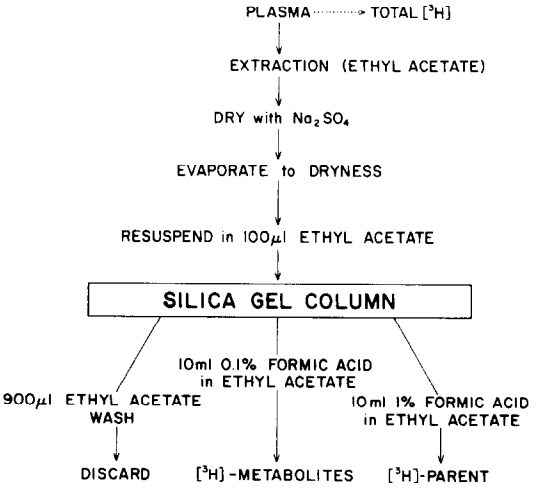

Fig. 1. Procedure for separation of [^{3}H]PGE_1 metabolites from [^{3}H]PGE_1 in plasma samples using silica gel microcolumns. See text for details.

wedge pressure, and a catheter was advanced from the femoral artery to monitor systemic arterial pressure. The respective catheters were connected to pressure transducers (Statham, Hato Rey, P.R.) for continuous monitoring of blood pressure. The heart was exposed through a median sternotomy and an electromagnetic flowprobe (Gould-Statham, Hato Rey, P.R.) was placed around the ascending aorta to continuously monitor aortic flow. A catheter was advanced into the superior vena cava from the external jugular vein for injection of the tracer substances. An apical catheter was inserted into the left ventricle for withdrawal of blood samples. Arterial blood gases were routinely measured and were maintained within physiologic limits (pH: 7.451 ± 0.060; arterial carbon dioxide tension: 33.7 ± 5.4 mm Hg; arterial oxygen tension: 130.8 ± 42.7 mm Hg; hematocrit 0.40 ± 0.06; N = 10, mean ± S.D.). In a separate closed-chest animal, the cerebral circulation was isolated by the cerebral venous outflow technique recently described by Pitt *et al.* [20]. Briefly, the confluence of the sagittal, straight and lateral sinuses was cannulated and the lateral sinuses were occluded with bonewax. Blood was diverted from the confluence of the sinuses to a femoral vein by an extracorporeal circuit consisting of an in-line electromagnetic flowprobe (Gould-Statham), reservoir with electric switch and level detector and peristaltic pump. Tracers were injected into a non-occlusive catheter advanced from a common carotid artery into the internal carotid artery. Blood was sampled from a sidearm in the venous outflow tubing.

The indicator-dilution technique to estimate pulmonary clearance of metabolizable tracers has been described in detail elsewhere [3]. Briefly, an aliquot of [^{3}H]PGE_1 in ethanol was added to a plastic tube and evaporated to dryness under nitrogen. It was then resuspended in a solution of indocyanine green and an aliquot was injected into the superior vena cava or carotid artery. In the cerebral circulation experiment, trace amounts of iodinated serum albumin were also present in the injectate. The amount of prostaglandin injected was always less than 100 pmoles. The tracers were injected as a bolus into the arterial supply of the organ to be studied and, simultaneously, blood was withdrawn from either the left ventricle (60 ml/min) or the cerebral venous outflow (15 ml/min) with the aid of a peristaltic pump (Cole Parmer Instrument Co., Chicago, IL). The concentration of cardiogreen in the effluent samples was determined by means of a flow cell cuvette and densitometer (Gilford IR-103, Oberlin, OH), and the output of the pump was connected to an escargot-type fraction collector (Gilson Medical Electronics, Inc., Middleton, WI) equipped with 13 × 100 mm tubes filled with 1 ml of chilled isotonic saline. Tubes were inverted several times and then centrifuged at 900 *g* for 15 min (Sorvall GLC-2B, Dupont Instruments, Newton, CT). Total radioactivity in an 0.35 ml aliquot of each supernatant fraction was determined after addition of an equal amount of H_2O_2 and 5 ml of Instagel by means of liquid scintillation spectrometry (Beckman LS-7000, Beckman Instruments, Irvine, CA). A second 0.35 ml aliquot was mildly acidified (pH 3.0 to 3.5) with 0.2 N HCl and extracted in 2 ml of ethyl acetate. The tubes were centrifuged for 15 min at 900 *g* and the organic fraction was transferred to polyethylene tubes and stored overnight at −20°. In the cerebral circulation experiment, radioactivity in a third 0.35 ml aliquot was directly measured in a Beckman 4000-Gamma Counter (Beckman Instruments). In addition to the total and metabolite tubes, two or three additional tubes containing blood, saline and a 10 μl aliquot of the injectate were processed with the run and used to measure total radioactivity injected.

The following day, the ethyl acetate extracts were dried with 100 mg Na_2SO_4 and then evaporated under a stream of air. Each sample was resuspended in ethyl acetate and applied to glass microcolumns filled with silica gel as described above. A 10 ml fraction of 0.1% formic acid in ethyl acetate was collected in scintillation vials for each sample and evaporated to dryness in a fume hood. The samples were reconstituted in 10 ml of Econofluor and radioactivity was determined in a liquid scintillation counter (Fig. 1).

The data were corrected for efficiency and expressed as disintegrations per minute (dpm). Fractional concentrations of isotopes in each sample were determined by dividing dpm/ml by total dpm in the injectate. Fractional concentrations of cardiogreen were determined at 1 sec intervals from the concentration curve record of the densitometer. The densitometer was calibrated several times during the experiment with known amounts of cardiogreen in blood.

The following parameters were calculated as previously described [3]:

$$\text{Mean transit time} = \text{appearance time} + [\int_0^t t \cdot C(t)dt]/[\int_0^t C(t)dt]$$

$$\text{Blood flow} = \text{cardiogreen injected}/[\int_0^t C(t)dt]$$

$$\text{Volume of distribution} = (\text{mean transit time}) \times (\text{blood flow})$$

where $C(t)$ refers to time-dependent fractional concentrations of tracer.

Data were treated according to Crone [21]. Extraction, E, was computed at the peak of the reference curve as:

$$E = \frac{C_R - C_D}{C_R} \times 100\%$$

where C_R is the fractional concentration of the non-diffusable, intravascular reference substance (cardiogreen or ^{125}I-albumin) and C_D is the fractional concentration of the metabolized test substance, [^{3}H]PGE_1. This method was used to evaluate pulmonary extraction of [^{3}H]PGE_1 (in duplicate) in ten animals. Cerebral extraction of [^{3}H]PGE_1 was estimated in duplicate in one animal.

Materials. [5,6(n)-^{3}H]Prostaglandin E_1 (sp. act. 50.3 Ci/mmole) was purchased from the Amersham Corp., Arlington Heights, IL, and was purified on silicic acid columns [15] at regular intervals; [^{125}I]albumin (sp. act. 1.61 mCi/mg) was obtained from the New England Nuclear Corp., Boston, MA; and indocyanine green (cardiogreen) from Hynson, Westcott & Dunning, Baltimore, MD. NAD^+ and NADH were obtained from the Sigma Chemical Co., St. Louis, MO; silica gel 60 (0.063

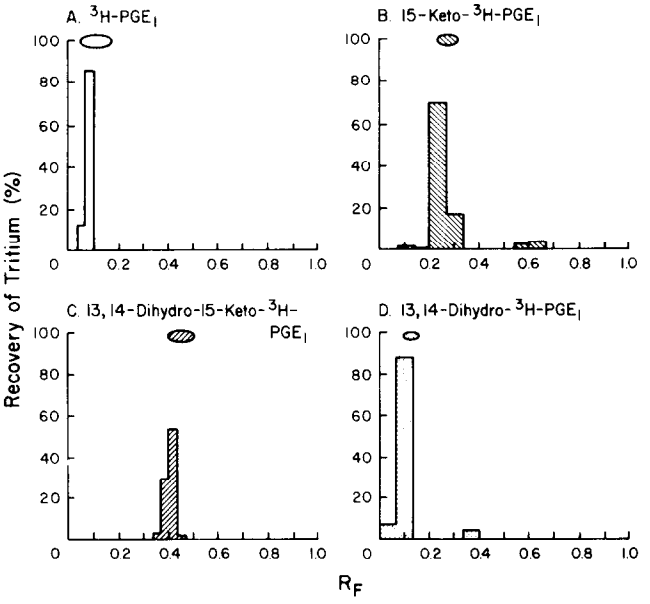

Fig. 2. Thin-layer chromatograms of [^{3}H]PGE$_1$ and [^{3}H]metabolites developed in chloroform–methanol–acetic acid (95:1:5) to a height of 15 cm. Each plate was scraped in 0.5 or 1 cm sections and radioactivity was determined by liquid scintillation counting. The ordinate represents the per cent of total tritium recovered from plate. Above each radiochromatogram is the location of unlabeled reference prostaglandin.

to 0.200 mm, 70–230 mesh ASTM) and precoated silica gel 60 glass-backed thin-layer chromatography plates (0.25 mm thick) from E. Merck, Darmstadt, West Germany; ethyl acetate (ChromAr) from Mallinkrodt, Paris, KY; and formic acid (90%) (certified ACS) from the Fisher Scientific Co., Pittsburgh, PA. Instagel was obtained from the Packard Instrument Co., Downer's Grove, IL, and Econofluor from the New England Nuclear Corp. Reference standards of PGE$_1$, 15-keto-PGE$_1$, 13,14-dihydro-15-keto-PGE$_1$ and 13,14-dihydro-PGE$_1$ were donated by Dr. John E. Pike, Upjohn Co., Kalamazoo, MI.

RESULTS

Synthesis of [^{3}H]*PGE$_1$ metabolites.* Three metabolites of [^{3}H]PGE$_1$ (15-keto[^{3}H]PGE$_1$; 13,14-dihydro-15-keto[^{3}H]PGE$_1$; and 13,14-dihydro-[^{3}H]PGE$_1$) were prepared as described in Materials and Methods. The purified radiolabeled metabolites, as well as [^{3}H]PGE$_1$, were applied to thin-layer chromatography plates and developed in chloroform–methanol–acetic acid (95:1:5). Each radiolabeled prostaglandin co-chromatographed with its respective reference standard (visualized in an iodine chamber) shown above the bar graph of radioactivity for each chromatogram in Fig. 2. The R_f values of radiolabeled prostaglandins were 0.083 for [^{3}H]PGE$_1$, 0.25 for 15-keto[^{3}H]PGE$_1$, 0.40 for 13,14-dihydro-15-keto[^{3}H]PGE$_1$ and 0.10 for 13,14-dihydro[^{3}H]PGE$_1$ and are similar to reported values [9]. The [^{3}H]PGA/B fraction from silicic acid column purification of [^{3}H]PGE$_1$ had an R_f of 0.52 in the same solvent system.

Column chromatography. Elution or [^{3}H]PGE$_1$ and its metabolites from silica gel columns with 0.5 ml volumes of eluant (5 ml ethyl acetate followed by 40 ml of 0.1% formic acid in ethyl acetate) exhibited the patterns shown in Fig. 3 (A–F). No radioactivity was eluted with ethyl acetate; therefore, fraction one represents the first 0.5 ml fraction from the 0.1% formic acid eluant. Individual columns (Fig. 3, A–D) showed that 15-keto[^{3}H]PGE$_1$ and 13,14-dihydro-15-keto[^{3}H]PGE$_1$ were eluted primarily in fractions 10 and 11, whereas 13,14-dihydro[^{3}H]PGE$_1$ was eluted between fractions 10 and 30. [^{3}H]PGE$_1$ is eluted from fractions 30 to 70. The composite figure of these individual columns (Fig. 3E) shows that the [^{3}H]PGE$_1$ metabolites were completely separable from [^{3}H]PGE$_1$ and that 13,14-dihydro[^{3}H]PGE$_1$ was partially separated from the 15-keto- and 13,14-dihydro-15-keto[^{3}H]PGE$_1$ metabolites. When the three [^{3}H]metabolites and [^{3}H]PGE$_1$ were applied together to the same column, the elution pattern was nearly identical to the composite pattern (compare Fig. 3E and 3F).

Elution of prostaglandins from the columns by application of 0.5 ml elution volumes is impractical for large numbers of samples. Therefore, elution volumes of 10 ml of 0.1% formic acid in ethyl acetate followed by 10 ml of 1% formic acid in ethyl acetate were applied to the columns and eluant was collected in 0.5 ml fractions. One per cent formic acid was used to decrease the total volume of solvent required to elute [^{3}H]PGE$_1$. The results with pure [^{3}H]PGE$_1$ metabolites or [^{3}H]PGE$_1$ are shown in Fig. 4 (A–E). To these columns, [^{3}H]prostaglandin was applied in 100 μl of ethyl acetate, followed by a 900 μl wash. The eluate was collected as a single fraction, contained no radioactivity, and was discarded. Fraction one represents the first 0.5 ml fraction from the 0.1% formic acid eluant. The elution patterns were similar to those in Fig. 3, except that 13,14-dihydro[^{3}H]PGE$_1$ was eluted in a smaller volume and was not separated

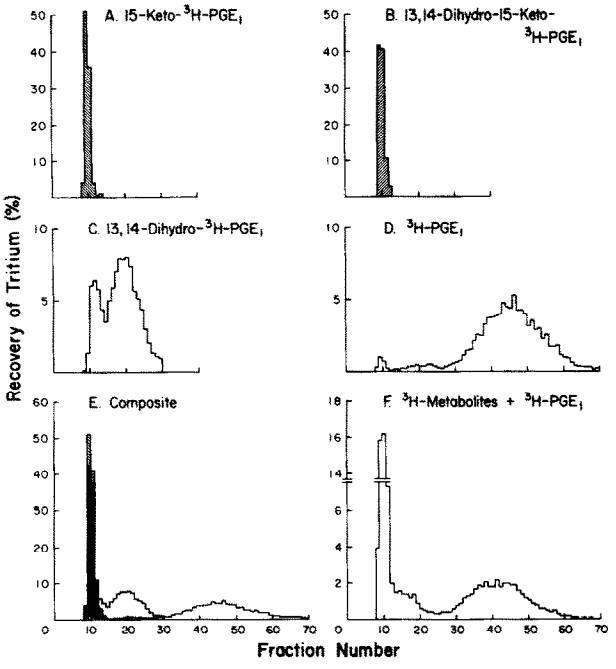

Fig. 3. Elution patterns of [^{3}H]PGE_1 and [^{3}H]metabolites on silica gel microcolumns with 0.5 ml volumes of 0.1% formic acid in ethyl acetate and collected in 0.5 ml fractions. Columns A–D represent elution of each metabolite or parent; E represents a composite diagram of A–D; F represents the elution pattern of the three metabolites and parent applied together to the same column. The ordinate represents the per cent of total tritium eluted from the column. In columns C and D the ordinate scale has been expanded to show the elution patterns more clearly.

from the 15-keto- and 13,14-dihydro-15-keto[^{3}H]PGE_1 metabolites. Most importantly, all three metabolites were completely separated from [^{3}H]PGE_1. Less than 10 per cent of the [^{3}H]PGE_1 eluted from the column appeared in the 0.1% formic acid eluate, while greater than 90 per cent was eluted with 10 ml of 1% formic acid in ethyl acetate (Fig. 4D). A composite of these individual column eluates clearly shows the separation of [^{3}H]PGE_1 metabolites from [^{3}H]PGE_1 (Fig. 4E). When each [^{3}H]metabolite or [^{3}H]PGE_1 was individually extracted from 1 ml of dog blood according to the protocol for experiments *in vivo* (see indicator-dilution experiments in Materials and Methods), applied to a column in ethyl acetate and eluted with 10 ml eluant volumes (as above), identical elution patterns were obtained (Fig. 5, A–E). [^{125}I]Albumin and cardiogreen, which are present in blood samples in the indicator-dilution experiments, did not interfere with extraction from plasma or elution from the column.

The three [^{3}H]metabolites applied together to the same column, in either pure form or extracted after addition to dog blood, were entirely eluted with 10 ml of 0.1% formic acid in ethyl acetate (Fig. 6, A and B). This was identical with the elution patterns obtained with individual columns (see composite diagrams, Figs. 4E and 5E). When [^{3}H]PGE_1 was combined with the three [^{3}H]metabolites and applied to the same column, in either pure form or extracted from dog blood, there was complete separation of [^{3}H]metabolites from [^{3}H]PGE_1 by elution with 10 ml of 0.1% formic acid in ethyl acetate followed by 10 ml of 1% formic acid in ethyl acetate (Fig. 6, C and D). This separation was identical to that obtained with individual columns (see composite diagrams, Figs. 4E and 5E). Flow rates were 0.5 to 0.6 ml/min for 10 ml of eluant to pass through the columns. Total elution time for metabolites was approximately 20–30 min, compared to several hours for Sephadex LH-20 columns [17] or t.l.c. development.

Recoveries of radioactivity in plasma from blood and in the ethyl acetate extract of plasma were virtually complete (>95 per cent). There was no loss of radioactivity associated with Na_2SO_4 drying; however, there was some loss of radioactivity upon evaporation of the extract to dryness and subsequent dissolution in ethyl acetate. Elution of each [^{3}H]metabolite (pure or extracted) was greater than 90 per cent of total radioactivity applied to the columns and appeared in only the 0.1% formic acid in ethyl acetate fraction. Elution of [^{3}H]PGE_1 from the

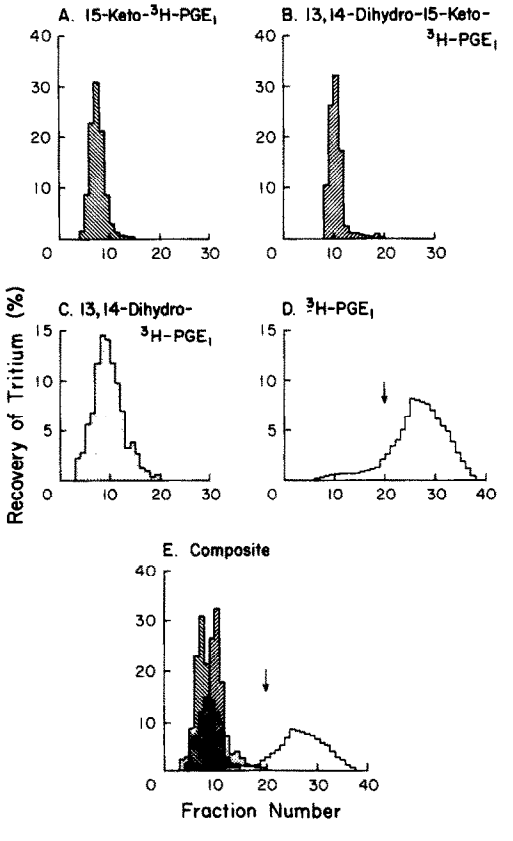

Fig. 4. Elution patterns of pure [^{3}H]PGE_1 and [^{3}H]metabolites on silica gel microcolumns. Columns A–C eluted with 10 ml of 0.1% formic acid in ethyl acetate; column D eluted in addition with 10 ml of 1% formic acid in ethyl acetate (at arrow); E represents a composite diagram of A–D. The ordinate represents the per cent recovery of total radioactivity eluted from column. In C and D the ordinate scale has been expanded to show the elution patterns more clearly. Fraction volume: 0.5 ml.

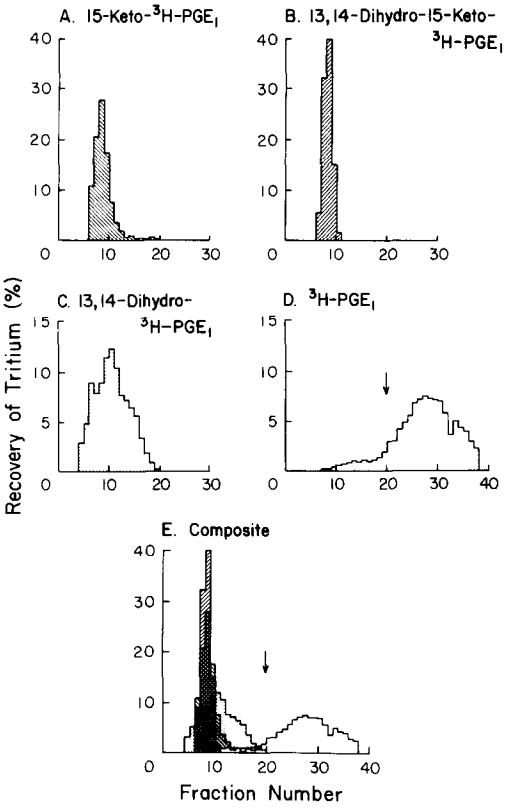

Fig. 5. Elution patterns of [³H]PGE₁ and [³H]metabolites extracted from dog blood on silica gel microcolumns. Columns A–C eluted with 10 ml of 0.1% formic acid in ethyl acetate; column D eluted in addition with 10 ml of 1% formic acid in ethyl acetate (at arrow); E represents a composite diagram of A–D. The ordinate represents the per cent of total tritium eluted from the column. In C and D the ordinate scale has been expanded to show the elution patterns more clearly. Fraction volume: 0.5 ml.

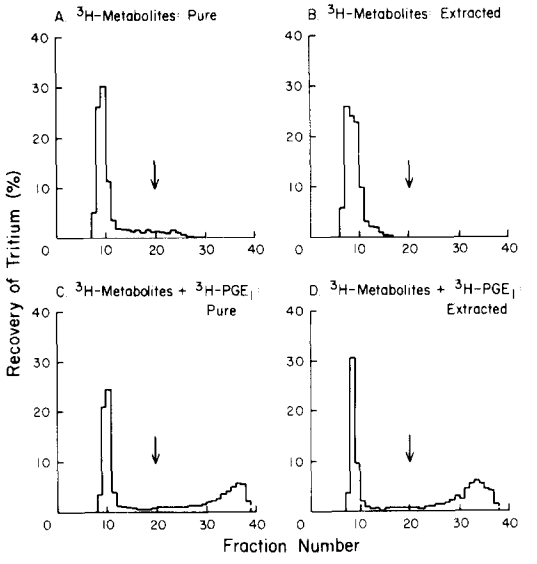

Fig. 6. Elution patterns of [³H]PGE₁ and [³H]metabolites in either pure form or extracted from dog blood applied together to one silica gel microcolumn. Columns were eluted with 10 ml of 0.1% formic acid in ethyl acetate followed by 10 ml of 1% formic acid in ethyl acetate (at arrow). The ordinate represents the per cent recovery of total tritium eluted from column. Fraction volume: 0.5 ml.

columns was incomplete; recoveries were approximately 70 per cent, of which 90 per cent appeared in the 1% formic acid eluant fraction. Therefore, overall recoveries for the entire procedure from plasma extraction through column elution were approximately 80 per cent for each [³H]metabolite and 55 per cent for [³H]PGE₁. Recoveries of radiolabeled metabolites added either individually or together to plasma, made on several occasions during the course of these studies, were consistently 80 per cent, enabling us to use this recovery value for metabolites in all experiments. Consequently, it was not necessary to determine recoveries in each experiment. The poor recovery of [³H]PGE₁ from the columns was not a factor since its concentration was estimated as the difference between total tritium and that associated with pulmonary metabolites (see Discussion).

Measurements of pulmonary and cerebral [³H]*PGE₁ extraction* in vivo. There were no significant pulmonary hemodynamic changes during the course of the *in vivo* lung experiments. Mean systemic arterial pressure was 114 ± 20 mm Hg; aortic blood flow averaged 1.79 ± 0.38 l/min; pulmonary arterial and capillary wedge pressures were 10.6 ± 3.0 and 3.8 ± 1.6 mm Hg, respectively (mean ± S.D., N = twenty observations in ten animals). Pulmonary vascular resistance (estimated by the ratio of pulmonary arterial minus capillary wedge pressure to aortic flow) was unchanged at 3.85 ± 2.03 mm $Hg \cdot l^{-1} \cdot min^{-1}$. The injection of the tracers had no significant effect on any of these parameters.

A typical example of an indicator-dilution experiment in dog lung is shown in Fig. 7. In Fig. 7A, the fractional concentrations of the intravascular marker, cardiogreen, and the diffusable tracer, [³H]PGE₁, are plotted at the time after injection when they appeared in effluent blood. The apparent volume of distribution of [³H]PGE₁ (blood flow × mean transit time, see Materials and Methods) was significantly greater than that of cardiogreen. The fractional concentration of [³H]PGE₁ is corrected for the appearance of its radiolabeled metabolites. The time and magnitude of [³H]PGE₁ metabolite appearance are shown in Fig. 7B. It can be seen that in this animal by the peak of the cardiogreen curve 50 per cent of the radioactivity in the total tube was eluted into the metabolite fraction of silica gel columns. The average per cent radioactivity appearing as metabolite at the peak of the cardiogreen curve in ten animals (two runs/animal) was 41.8 ± 9.3 per cent. Radioactivity associated with metabolite presumably is due to [³H]PGE₁ transported out of the vasculature, metabolized by cytoplasmic prostaglandin dehydrogenase and associated enzymes, and returned to the vascular space [22].

Several samples were assayed in duplicate on thin-layer chromatography plates and good agreement was obtained between estimates of relative metabolite present in the total tube by silica gel columns and these thin-layer chromatographic esti-

mates. Furthermore, duplicate samples of the 0.1% formic acid column eluants were evaporated to dryness, resuspended in ethanol, and applied to thin-layer chromatographic systems. In these samples, there was no radioactivity associated with $[^3H]PGE_1$, although there was a peak of radioactivity corresponding to the location of unlabeled 13,14-dihydro-15-keto-PGE_1. This observation confirms the qualitative efficacy of silica gel columns in separating parent from metabolite.

Pulmonary extraction of $[^3H]PGE_1$ in Fig. 7B was 85 per cent at the peak of the cardiogreen curve and was relatively constant before and after this peak. The shape of this curve is consistent with a linear transport model of a trace amount of $[^3H]PGE_1$ leaving the vascular space and either distributing in a large extravascular volume, or more likely, being metabolized [22]. The average pulmonary extraction for the first run in ten animals was 84.6 ± 5.6 per cent and was not significantly different (83.6 ± 4.8 per cent) when repeated a second time in these animals.

Cerebral extraction of $[^3H]PGE_1$ was studied in duplicate in one animal. The injectate itself did not affect cerebral hemodynamics, and total cerebral blood flow averaged 21 ml/min in this animal. An indicator-dilution curve for one run in this animal is shown in Fig. 8A. The upslope and the peak of the $[^3H]PGE_1$ curve were less than those of the intravascular marker, $[^{125}I]$albumin. In Fig. 8B, it can be seen that cerebral extraction of $[^3H]PGE_1$ was 28 per cent at the peak of the $[^{125}I]$albumin curve. The rapid downslope of the extraction curve (Fig. 8A) must represent back diffusion into the vascular space of unchanged $[^3H]PGE_1$ that was extracted in the earlier transit portion. Very little metabolism of $[^3H]PGE_1$ took place within one transit of the brain (<5 per cent near the end of the transit). In a second run in the same animal, cerebral extraction of $[^3H]PGE_1$ was 16 per cent at the peak of the $[^{125}I]$albumin curve.

DISCUSSION

The separation of prostaglandins and prostaglandin metabolites can be achieved by various methods presently available [4–17]. Our method is especially useful for separating major metabolites (as one fraction) from parent prostaglandin (in this case, PGE_1) by relatively simple and rapid silica gel column chromatography, which can be applied in processing large numbers of samples. The elution solvent for the columns was based on a mixture, used previously for thin-layer chromatographic separation of PGE_1 and its metabolites [9], which most clearly separated the metabolites from parent PGE_1 and required varying the content of only one solvent (formic acid) in the second (ethyl acetate). Other solvent systems, e.g. benzene–dioxane–acetic acid, require varying the ratio of three solvents and would not be expected to result in a clean separation, especially of 13,14-dihydro-PGE_1 from PGE_1. Indeed, this was the case when we used the latter solvent system on silica gel columns.

In the initial experiments in which 0.5 ml volumes of eluant were applied to the column and collected as individual fractions, there was partial separation of 13,14-dihydro$[^3H]PGE_1$ from 15-keto- and 13,14-dihydro-15-keto$[^3H]PGE_1$, as well as complete separation of the three $[^3H]$metabolites from $[^3H]PGE_1$ (Fig. 3E). However, this method was impractical for processing large numbers of samples and 40 ml of solvent was required to elute all $[^3H]$metabolites and $[^3H]PGE_1$. A single application

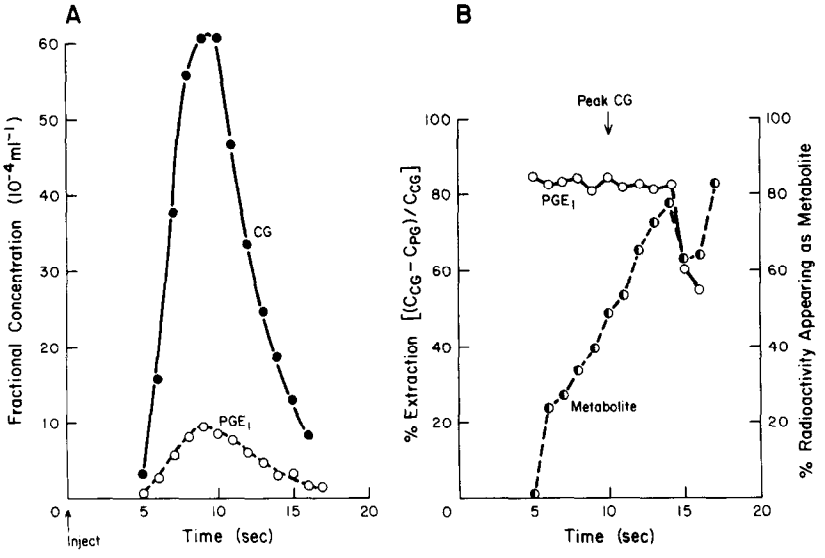

Fig. 7. Time–concentration and extraction ratio curves following injection of $[^3H]PGE_1$ into the superior vena cava of an intact anesthetized dog. In panel A, fractional concentrations of intravascular marker, cardiogreen, and diffusable tracer, $[^3H]PGE_1$, are plotted at time sampled after injection (arrow). In panel B, the extraction ratio (open circle) is plotted for each sample in panel A. In addition, the per cent of radioactivity appearing as metabolite (half-closed circle) in each sample is also shown.

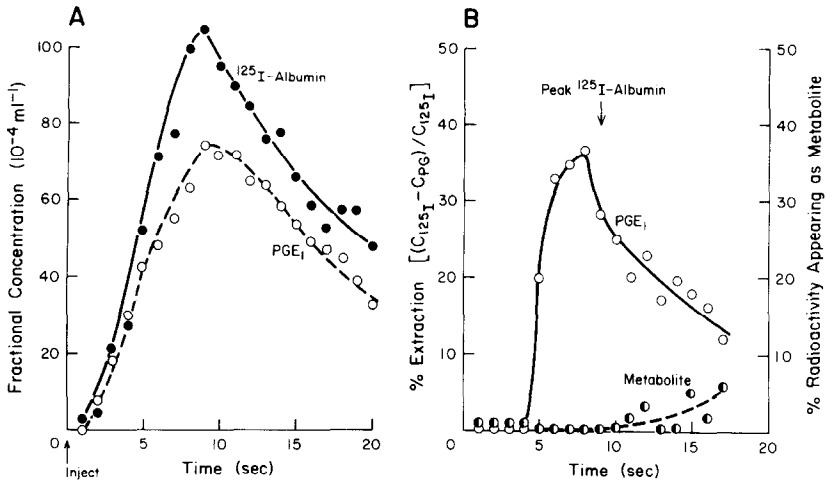

Fig. 8. Time–concentration and extraction ratio curves following injection of [^{3}H]PGE$_1$ into the internal carotid artery of an intact anesthetized dog. In panel A, fractional concentrations of intravascular marker, [^{125}I]albumin, and diffusable tracer, [^{3}H]PGE$_1$, are plotted at time sampled after injection (arrow). In panel B, the extraction ratio (open circle) is plotted for each sample of panel A. In addition, the per cent of radioactivity appearing as metabolite (half-closed circle) in each sample is also shown.

of 10 ml of eluant (0.1% formic acid in ethyl acetate) resulted in complete elution from the column of all three [^{3}H]metabolites. but only a small percentage (approximately 5 per cent) of [^{3}H]PGE$_1$, whether applied to columns in pure form or extracted from dog blood (Figs. 4E, 5E, 6C and 6D). This resulted in virtually complete separation of the [^{3}H]metabolites from parent [^{3}H]PGE$_1$. In the *in vivo* experiments reported here, or in other possible applications, it is unnecessary to elute parent [^{3}H]PGE$_1$ if a "total tube" (i.e. aliquot of plasma containing [^{3}H]PGE$_1$ plus [^{3}H]metabolites) is taken, since [^{3}H]PGE$_1$ can be determined as the difference of total-^{3}H minus metabolite-^{3}H. This is particularly useful since [^{3}H]PGE$_1$ is incompletely eluted from the column under these conditions. Complete elution would require larger volumes of solvent.

Indicator dilution has proven to be a useful technique to evaluate organ extraction of metabolizable substances such as prostaglandins [12] and biogenic amines [3, 23] in intact experimental animals and man. Application of a simplified technique to separate radioactivity associated with metabolite or parent ([^{3}H]PGE$_1$) considerably facilitates estimation of pulmonary PGE$_1$ clearance in clinical or experimental situations. Although we described experiments in open-chest animals, catheterization of any arterial site provides sufficient access to estimate pulmonary extraction. We have previously sampled from the brachial artery of man following bolus injections of radiolabeled 5-hydroxytryptamine into the venous circulation and reported reproducible and reliable estimates of human pulmonary clearance of serotonin [23].

Pulmonary extraction of [^{3}H]PGE$_1$ was 84.1 ± 2.2 per cent (mean ± S.D., $N = 20$) in the ten dogs of our study. This value is in good agreement with previous estimates of pulmonary extraction of radiolabeled PGE$_1$ in isolated perfused cat [24] and rabbit [25] lungs and of transpulmonary gradients of endogenous immunoreactive prostaglandin E [26] or following infusion of exogenous unlabeled PGE$_1$ in the venous circulation of intact dogs [27]. Furthermore, it is similar to that observed by indicator-dilution of tracer amounts of [^{3}H]PGE$_1$ in the isolated perfused cat lobe [12] and agrees well with our estimates of pulmonary extraction of [^{3}H]PGE$_1$ in cardiac surgical patients [28]. Reproducibility is also evident in the insignificant difference between measurements in the same animal. The rapid and extensive metabolism of [^{3}H]PGE$_1$ is also consistent with previous findings [12, 25, 28] and suggests that the uptake and conversion of [^{3}H]PGE$_1$ take place in a site accessible to the vascular space. Uptake of prostaglandin E$_1$ in isolated perfused rat lung has been shown to be a saturable energy dependent and perhaps active process [29]. Although prostaglandin E metabolizing enzymes have been associated with a cytoplasmic location, the cell type in which these processes take place remains unknown [1].

In contrast to lung, cerebral extraction and single pass metabolism of [^{3}H]PGE$_1$ were relatively low (Fig. 8B). Other workers have shown that PGE$_1$ probably does not cross cell membranes by simple diffusion and that a carrier-mediated system may facilitate PGE$_1$ transport across the blood–brain barrier following administration of PGE$_1$ into the cerebrospinal fluid of rats [30]. If a facilitated uptake system exists within the cerebral circulation, then perhaps its low surface area compared to lung makes apparent single pass uptake considerably less in the former. Regardless of the mechanism, comparison of lung and brain extraction of [^{3}H]PGE$_1$ by indicator-dilution and our chromatographic assay with previously reported data supports the reliability and usefulness of the approach described.

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