

Figure 2—Serum ibuprofen levels after administration of a 150-mg dose of a suppository of three different bases and one oral preparation. Key: □, theobroma oil; ○, polyethylene glycol 1540; ●, fatty acids; and ■, suspension.

jector⁵, a UV (254 nm) absorbance detector, and a strip-chart recorder. The deproteinated plasma samples were chromatographed at room temperature on a microparticulate (μ Bondapak C₁₈) reversed-phase HPLC column (4 mm \times 30 cm) with an eluting mobile phase of acetonitrile—0.1 M acetic acid (55:45 v/v). The flow rate was adjusted to 1 ml/min with an inlet pressure of \sim 1500 psi. The chart speed was 0.2 cm/min. The ratio of the peak height of ibuprofen to that of the internal standard was used to calculate the ibuprofen concentration, based on a calibration curve prepared from spiked plasma samples.

Calculations—Peak height ratios were obtained by dividing the peak height of ibuprofen by the peak height of the internal standard. Calibration curves from known ibuprofen concentrations in plasma were prepared by plotting the peak height ratios versus the ibuprofen concentration, expressed as micrograms per milliliter of plasma. Values of

unknown concentrations of ibuprofen in plasma samples were read directly from the graph.

RESULTS AND DISCUSSION

Typical chromatograms of blank rabbit plasma (A), blank rabbit plasma spiked with ibuprofen (B), blank rabbit plasma spiked with ibuprofen and internal standard (C), and plasma collected 60 min after administration of 150 mg of ibuprofen in a suppository or oral preparation and spiked with the internal standard (D) are shown in Fig. 1. Ibuprofen and the internal standard were well resolved and eluted with retention times of 8.5 and 5.5 min, respectively. No interference from metabolites was detected under these experimental conditions.

Table I illustrates recoveries of ibuprofen from plasma spiked with 0.1–50 μ g/ml. The standard curve is the average of five determinations; the regression line slope was calculated to be 0.128 with a standard correlation matrix of 0.999, indicating excellent linearity.

The plasma ibuprofen time courses for four rabbits in a bioavailability study are shown in Fig. 2. The AUC values for the four formulations were 89.77, 74.38, 96.5, and 128.94 μ g/ml/hr for 150 mg of ibuprofen in polyethylene glycol 1540, theobroma oil, esterified fatty acids (C₁₀–C₁₈)⁷, and an oral suspension, respectively.

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⁷ Witepsol H-15, Kay-Fries Chemicals, Montvale, NJ 07645.

Binding of Prostaglandins E₁ (Alprostadil), E₂ (Dinoprostone), F_{1 α} , and F_{2 α} (Dinoprost) to Human Serum Proteins

JOSEPH JUDIS

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Abstract □ Prostaglandins E₁ (alprostadil), E₂ (dinoprostone), F_{1 α} , and F_{2 α} (dinoprost) were characterized in terms of binding to human serum, albumin, γ -globulin II, β -globulin III, α -globulin IV-1, and α -globulin IV-4. By using equilibrium dialysis and tritium-labeled ligands, the percent binding for all four ligands was found to decrease in the following order: human serum, albumin, and α -globulin IV-4. For the other three proteins, the order was not consistent with the four ligands, and <10% binding was observed. In general, prostaglandins E₁ and E₂ showed a higher percent binding for all fractions than prostaglandins F_{1 α} and F_{2 α} . All four ligands can be characterized as showing significant binding to human serum, albumin, and α -globulin IV-4.

Keyphrases □ Prostaglandins—binding to human serum proteins, equilibrium dialysis of radiolabeled prostaglandins □ Protein binding—prostaglandins E₁ (alprostadil), E₂ (dinoprostone), F_{1 α} , and F_{2 α} (dinoprost) to human serum proteins, equilibrium dialysis of radiolabeled prostaglandins □ Binding, protein—binding of prostaglandins E₁ (alprostadil), E₂ (dinoprostone), F_{1 α} , and F_{2 α} (dinoprost) to human serum proteins, equilibrium dialysis of radiolabeled prostaglandins □ Equilibrium dialysis—of radiolabeled prostaglandins E₁ (alprostadil), E₂ (dinoprostone), F_{1 α} , and F_{2 α} (dinoprost) bound to human serum proteins

Since the discovery and description of the prostaglandins, a vast literature has evolved concerning their physiological and pharmacological actions (1). As circulating hormones, these compounds have different rates of dis-

appearance from the circulation after injection. Presumably, the differences reflect differences in metabolic degradation rates (2, 3). The availability of a compound for metabolic degradation could be related to whether or not

Table I—Binding of [³H]Prostaglandins E₁, E₂, F_{1α}, and F_{2α} to Human Serum Proteins^a

Serum Protein		Percent of Prostaglandin Bound ^b			
Fraction	Concentration, mg/ml	E ₁	E ₂	F _{1α}	F _{2α}
Albumin	40.0	81.40 (0.39)	71.00 (2.57)	63.3 (1.48)	42.4 (0.92)
α-Globulin IV-1	1.0	12.5 (0.46)	8.96 (0.92)	6.86 (0.29)	5.13 (0.52)
α-Globulin IV-4	5.0	55.00 (0.59)	26.90 (0.58)	31.0 (0.52)	24.10 (2.12)
β-Globulin III	7.0	5.04 (0.21)	9.13 (0.64)	3.17 (0.32)	2.92 (0.15)
γ-Globulin II	11.0	6.17 (0.43)	7.12 (0.38)	1.67 (0.12)	0
Human serum	—	92.60 (0.09)	80.10 (0.47)	76.4 (2.56)	70.9 (1.29)

^a Each system contained a total, in moles, of the following: prostaglandin E₁, 2.823 × 10⁻⁸; prostaglandin E₂, 2.84 × 10⁻⁸; prostaglandin F_{1α}, 2.82 × 10⁻⁸; and prostaglandin F_{2α}, 2.83 × 10⁻⁸. ^b Values in parentheses are standard deviations. Data were obtained using equilibrium dialysis method.

the compound is bound to a serum protein and the tightness of the binding (4). Prostaglandins bind to human plasma proteins, and some bind specifically to human serum albumin (2, 5–8).

Interest in serum protein binding capabilities of prostaglandins has centered on the quantitative analytical techniques in which prostaglandin must be quantitatively extractable from serum (8) and on factors involved in their physicochemical state as circulating hormones (2); knowledge of the latter is basic to the investigation of the pharmacological and pharmacokinetic properties of prostaglandins. Therefore, the percent binding of prostaglandins E₁ (alprostadiol), E₂ (dinoprostone), F_{1α}, and F_{2α} (dinoprost) to human serum proteins was examined.

EXPERIMENTAL

Methods—Protein binding was measured using a previously described technique (9). Equilibrium dialysis was used to determine whether binding occurred between a ligand and a specific protein and the percent of ligand bound to the protein. Values reported are the averages of at least three replicates. This concentration range was selected to approximate the range of higher concentrations of prostaglandin E₁ used by Unger (8) in albumin binding studies. Radioactivity was determined in a liquid scintillation system¹.

Materials—[5,6-³H(N)]Prostaglandin E₁², [5,6,8,11,12,14,15-³H(N)]prostaglandin E₂², [5,6-³H(N)]prostaglandin F_{1α}³, and [9-³H]-prostaglandin F_{2α}³ had specific activities of 89.5, 117, 40, and 15 Ci/mmole, respectively. Crystalline human serum albumin and other human

serum proteins were used as received⁴. A xylene-based phosphor solution⁵ was used in all radioactivity determinations, and all other chemicals were reagent grade. Radiopurity was rechecked prior to use utilizing the methods indicated by the suppliers. A radiopurity of 99% was the minimum acceptable.

RESULTS AND DISCUSSION

The extent to which each prostaglandin was bound to whole human serum and several human serum proteins or fractions is tabulated in Table I. All four prostaglandins were bound to a significant extent (>70% binding) to whole human serum, and prostaglandins E₁ and E₂ were bound to a higher percentage than prostaglandins F_{1α} and F_{2α}. This finding is in agreement with previous studies (5, 6) in which the affinity of prostaglandins for serum proteins decreased with an increase in the number of polar groups in the prostaglandin molecule.

The serum protein to which binding was greatest for all four compounds was albumin. All prostaglandins showed the next highest affinity for α-globulin IV-4 and a relatively low affinity for the other serum protein fractions. α-Globulin IV-4 contains corticosteroid binding globulin (10); although the latter protein cannot necessarily be implicated in the binding of prostaglandins to α-globulin IV-4, the nonpolar nature of corticosteroids and prostaglandins suggests its possible involvement. Evidence for a prostaglandin A₁ binding protein in human plasma other than albumin has been presented (11).

There was a significant difference in the affinity of the E prostaglandins for human serum albumin compared to the F prostaglandins. With all four prostaglandins, the concentration of bound ligand increased in relation to the total ligand concentration in the system (Fig. 1).

Under the study conditions, prostaglandin instability was not a factor as demonstrated by Karim *et al.* (12). Membrane binding was determined in all experiments and was uniformly <5%. At least 95% of the counts per minute added could always be accounted for in the sum of the counts per minute in both sides of an equilibrium cell, indicating no significant binding to the plexiglass.

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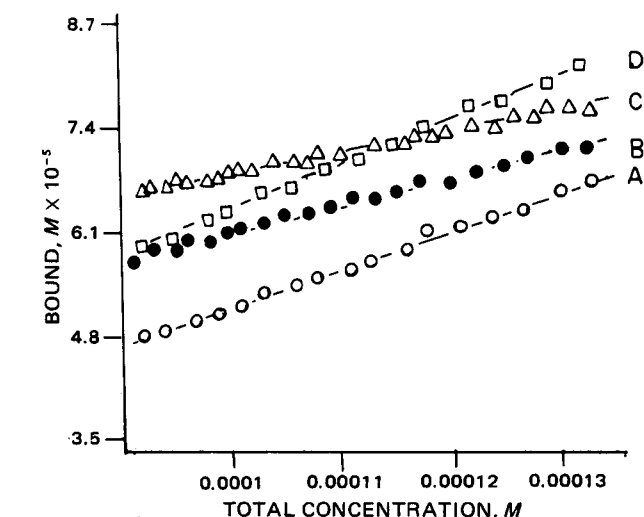


Figure 1—Total concentration of ligand in the system versus bound concentration. Albumin concentration was 5.797 × 10⁻⁴ mole (40 mg/ml). Key: A, prostaglandin F_{2α}; B, prostaglandin F_{1α}; C, prostaglandin E₁; and D, prostaglandin E₂.

¹ Beckman model LS-133.

² New England Nuclear Corp.

³ Amersham Corp.

⁴ ICN Life Sciences Group.

⁵ PCS, Amersham Corp.