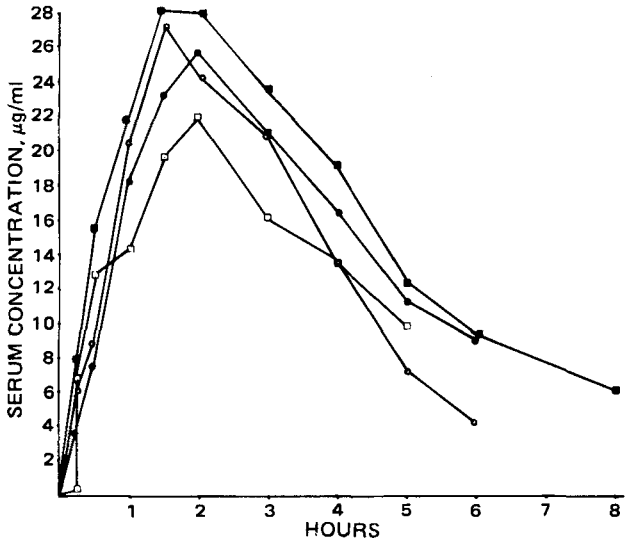

Figure 2—*Serum ibuprofen levels after administration of a 150-mg dose of a suppository of three different bases and one oral preparation. Key:* □, *theobroma oil;* ○, *polyethylene glycol 1540;* ●, *fatty acids; and* ■, *suspension.*

jector[5], a UV (254 nm) absorbance detector, and a strip-chart recorder. The deproteinated plasma samples were chromatographed at room temperature on a microparticulate (μBondapak C_{18}) reversed-phase HPLC column (4 mm × 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 ~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 μg/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 μg/ml/hr for 150 mg of ibuprofen in polyethylene glycol 1540, theobroma oil, esterified fatty acids (C_{10}–C_{18})[7], and an oral suspension, respectively.

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Binding of Prostaglandins E_1 (Alprostadil), E_2 (Dinoprostone), $F_{1\alpha}$, and $F_{2\alpha}$ (Dinoprost) to Human Serum Proteins

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Received October 20, 1978, from the *College of Pharmacy, University of Toledo, Toledo, OH 43606.* Accepted for publication January 13, 1981.

Abstract □ Prostaglandins E_1 (alprostadil), E_2 (dinoprostone), $F_{1\alpha}$, and $F_{2\alpha}$ (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 E_1 and E_2 showed a higher percent binding for all fractions than prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$. 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 E_1 (alprostadil), E_2 (dinoprostone), $F_{1\alpha}$, and $F_{2\alpha}$ (dinoprost) to human serum proteins, equilibrium dialysis of radiolabeled prostaglandins □ Binding, protein—binding of prostaglandins E_1 (alprostadil), E_2 (dinoprostone), $F_{1\alpha}$, and $F_{2\alpha}$ (dinoprost) to human serum proteins, equilibrium dialysis of radiolabeled prostaglandins □ Equilibrium dialysis—of radiolabeled prostaglandins E_1 (alprostadil), E_2 (dinoprostone), $F_{1\alpha}$, and $F_{2\alpha}$ (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 disappearance 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

0022-3549/81/0800-0945$01.00/0

Table I—Binding of [^{3}H]Prostaglandins E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$ to Human Serum Proteins [a]

Serum Protein		Percent of Prostaglandin Bound [b]			
Fraction	Concentration, mg/ml	E_1	E_2	$F_{1\alpha}$	$F_{2\alpha}$
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 E_1, 2.823×10^{-8}; prostaglandin E_2, 2.84×10^{-8}; prostaglandin $F_{1\alpha}$, 2.82×10^{-8}; and prostaglandin $F_{2\alpha}$, 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 E_1 (alprostadil), E_2 (dinoprostone), $F_{1\alpha}$, and $F_{2\alpha}$ (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 E_1 used by Unger (8) in albumin binding studies. Radioactivity was determined in a liquid scintillation system[1].

Materials—[5,6-^{3}H(N)]Prostaglandin E_1[2], [5,6,8,11,12,14,15-^{3}H(N)]prostaglandin E_2[2], [5,6-^{3}H(N)]prostaglandin $F_{1\alpha}$[3], and [9-^{3}H]-prostaglandin $F_{2\alpha}$[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 received[4]. A xylene-based phosphor solution[5] 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.

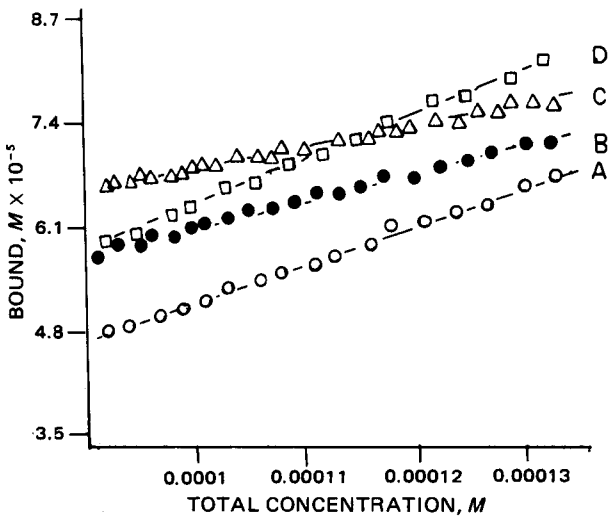

Figure 1—*Total concentration of ligand in the system* versus *bound concentration. Albumin concentration was 5.797 × 10^{-4} mole (40 mg/ml). Key: A, prostaglandin $F_{2\alpha}$; B, prostaglandin $F_{1\alpha}$; C, prostaglandin E_1; and D, prostaglandin E_2.*

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 E_1 and E_2 were bound to a higher percentage than prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$. 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 A_1 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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ACKNOWLEDGMENTS

The technical assistance of Mrs. Virginia Matusek is gratefully acknowledged.

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