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Interaction of Prostaglandins with Blood Plasma Proteins

COMPARATIVE BINDING OF PROSTAGLANDINS A_2, $F_{2\alpha}$ AND E_2 TO HUMAN PLASMA PROTEINS

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1. The binding of prostaglandin A_2 and prostaglandin $F_{2\alpha}$ to human plasma proteins was investigated by DEAE-Sephadex chromatography and polyacrylamide-gel electrophoresis. Both prostaglandins, when added to human plasma *in vitro*, were found to become bound mainly to plasma albumin. 2. The extent of binding of prostaglandins added to human plasma in low to moderate concentrations was found to be approx. 88, 73 and 58% for prostaglandins A_2, E_2 and $F_{2\alpha}$ respectively. The order of affinities for the binding of the three prostaglandins to albumin appear to be $A_2 > E_2 > F_{2\alpha}$. 3. The apparent association constants for the binding of these prostaglandins to human serum albumin were estimated to be approx. 4.8×10^4, 2.4×10^4 and 0.9×10^4 litre/mol for prostaglandins A_2, E_2 and $F_{2\alpha}$ respectively. The results are compared with previously reported association constants for the binding of long-chain fatty acids to both human and bovine albumins.

Radioactive prostaglandin E_1 administered intravenously to both rat and man was shown to disappear rapidly from the blood circulation, with recovery of some of the radioactivity in the urine and faeces (Samuelsson, 1964; Granström, 1967). The identification in rat urine of seven metabolities, after injection of prostaglandin E_2, and of five metabolites, after injection of prostaglandin $F_{2\alpha}$, has been reported by Green (1971*a,b*). The initial steps in the metabolism of intravenously administered prostaglandins in plasma have not been studied in detail. Jones (1970) reported the presence in cat plasma of a prostaglandin A_1 isomerase enzyme, which converts prostaglandin A_1 into B_1. Inactivation of the smooth-muscle activity of prostaglandins E_1, E_2 and $F_{2\alpha}$ in the lung of the cat and the dog has been reported (Ferreira & Vane, 1967). The process probably involves the conversion of these prostaglandins into the 15-oxo-13,14-dihydro or 13,14-dihydro derivatives, as was demonstrated for prostaglandin E_1 in guinea-pig lungs (Anggard & Samuelsson, 1966). On the other hand, prostaglandins A_1 and A_2 were shown not to be inactivated by passage through the lungs of the dog (McGiff *et al.*, 1969) and were suggested as possible circulating hormones (McGiff *et al.*, 1969; Horton & Jones, 1969).

In an effort to evaluate prostaglandins as possible circulating hormones, investigation of the physicochemical state of prostaglandins added to human plasma was undertaken. In a previous report (Raz, 1972) the binding of prostaglandin E_2 to human serum albumin has been established. This binding blocked the smooth-muscle activity of prostaglandin E_2 on the isolated gerbil colon *in vitro* but did not decrease the hypotensive effect of prostaglandin E_2 in the rat. The studies presented here are concerned with the binding of prostaglandins $F_{2\alpha}$ and A_2 to human plasma proteins and with some of the factors that affect the binding of prostaglandins $F_{2\alpha}$, A_2 and E_2 to human serum albumin.

Methods and Materials

Methods

Addition of prostaglandins to plasma and to solutions of human serum albumin. Prostaglandins dissolved in acetone were added to fresh human plasma or protein solutions as described previously (Raz, 1972). After the addition the mixture was equilibrated by gentle shaking for 6h at room temperature.

DEAE-Sephadex chromatography. Human plasma (55ml) containing radioactive prostaglandins was fractionated by DEAE-Sephadex (Pharmacia, Piscataway, N.J., U.S.A.) chromatography, with 0.05M-potassium phosphate buffer, pH 7.5, and a linear NaCl gradient for elution, as described in detail previously (Raz, 1972). Portions from the column effluent were analysed for content of radioactivity and protein.

Equilibrium dialysis. Equilibrium dialysis was used for the quantitative determination of the binding of prostaglandins to plasma and protein solutions. The studies were conducted in 0.05M-potassium phosphate buffer, pH 7.4, containing 7×10^{-4}M-EDTA. A mixture of a prostaglandin and protein solution was placed inside the dialysis bag and buffer was placed outside. Details of the procedure were given by Raz (1972). After dialysis, samples (0.5ml) from

both the 'inside' and 'outside' solutions were assayed for radioactivity. The percentage of prostaglandin bound to protein in the 'inside' solution after dialysis was calculated as follows:

100×[(c.p.m./5ml of 'inside' soln.)−(c.p.m./5ml of 'outside' soln.)/c.p.m./5ml of 'inside' soln.]

The concentration of prostaglandin in the 'inside' solution after dialysis was calculated as follows:

$$\frac{\text{Total prostaglandin added (ng) to 'inside' soln.}}{\text{Total c.p.m. recovered after dialysis}} \times \text{c.p.m./ml of 'inside' soln.}$$

The amount of prostaglandin bound to human serum albumin (ng of prostaglandin/mg) in the 'inside' solution after dialysis was calculated as:

$$\frac{\text{[Prostaglandin] (ng/ml of 'inside' soln. after dialysis)}}{\text{[Human serum albumin] (mg/ml of 'inside' soln.)}} \times \text{\% prostaglandin of bound to human serum albumin}$$

From this value, and taking the molecular weights of prostaglandins E_2, A_2 and $F_{2\alpha}$ as 341, 323 and 343 respectively and the molecular weight of human serum albumin as 69000, the molar ratio prostaglandin bound/human serum albumin could be calculated.

Other methods. Other methods used in the experiments described here, including concentration of protein fractions by ultrafiltration and polyacrylamide disc-gel electrophoresis, were described in detail previously (Raz, 1972). Radioactivity measurements were done in a Packard Tri-Carb liquid-scintillation spectrometer with diotol solution (Burdick and Jackson, Muskegan, Mich., U.S.A.) or Bray's (1960) solution. Quenching was corrected by adding tritiated toluene as internal standard to selected samples.

Materials

Tritium-labelled prostaglandin E_2 (specific radioactivity 1.2Ci/mmol) was prepared as described previously (Raz, 1972) from [^{3}H]arachidonic acid, which was obtained from New England Nuclear Corp. (Boston, Mass., U.S.A.). Mild hydrochloric acid treatment of the labelled prostaglandin E_2 afforded labelled prostaglandin A_2, which was purified by t.l.c. Prostaglandins E_2, A_2 and $F_{2\alpha}$ were kindly pro-

Table 1. *Binding of prostaglandins E_2, A_2 and $F_{2\alpha}$ to whole human plasma and to human serum albumin*

Radioactive prostaglandins were added to each of the following solutions: saline, human plasma, tenfold-diluted plasma, human serum albumin soln. (4mg/ml) and acid α_1-glycoprotein soln. (4mg/ml). The concentration of a given prostaglandin refers to the amount present in the protein solution placed inside the dialysis bag at the start of the equilibrium dialysis. Values given in the last column are results of duplicate experiments.

Prostaglandin	Solution	Concn. of prostaglandin (ng/ml)	Binding of prostaglandin (% bound to proteins)
E_2	Saline	20	0,0
E_2	Plasma	20	73,73
E_2	Diluted plasma	20	40,38
E_2	Human serum albumin	20	42,44
E_2	Acid α_1-glycoprotein	20	1,2
A_2	Saline	10	0,0
A_2	Plasma	10	87,89
A_2	Diluted plasma	10	75,72
A_2	Human serum albumin	10	75,77
A_2	Acid α_1-glycoprotein	10	0,2
$F_{2\alpha}$	Saline	120	0,0
$F_{2\alpha}$	Plasma	120	58,60
$F_{2\alpha}$	Diluted plasma	120	33,31
$F_{2\alpha}$	Human serum albumin	120	32,34
$F_{2\alpha}$	Acid α_1-glycoprotein	120	1,1

vided by Dr. John E. Pike of the Upjohn Co. (Kalamazoo, Mich., U.S.A.). Human serum albumin was obtained from Pentex Biochemicals (Kankakee, Ill., U.S.A.; lots 28 and 29). Acid α_1-glycoprotein was obtained as described previously (Raz & Goodman, 1969).

Fresh plasma was isolated by centrifugation of blood, which was withdrawn from healthy subjects into bags containing 0.2vol. of citric acid–sodium citrate–glucose anticoagulant buffer.

Results and Discussion

Equilibrium-dialysis studies on the binding of prostaglandins E_2, A_2 and $F_{2\alpha}$ to human plasma and to albumin solutions

In my previous publication (Raz, 1972) I reported on the binding of prostaglandin E_2 to human plasma *in vitro*. These studies were extended for prostaglandins A_2 and $F_{2\alpha}$.

Binding of prostaglandins E_2, A_2 and $F_{2\alpha}$ to human plasma, saline-diluted human plasma and to solutions of human serum albumin was evaluated by equilibrium dialysis. The results (Table 1) indicated that all three prostaglandins are capable of binding with human plasma and with human serum albumin, but not with acid α_1-glycoprotein, indicating the binding to be specific for certain plasma proteins, one of which is albumin. The results in Table 1 also indicate that the extent of binding in plasma diluted tenfold (approx. albumin concn. 4.5mg/ml) is almost identical with that observed in a solution of human serum albumin containing 4.0mg/ml, suggesting that albumin is quantitatively the major prostaglandin-binding protein in plasma. The differences between the three prostaglandins in the relative amounts of free and protein-bound forms in equilibrium indicate that there are differences in the binding affinities of these compounds towards plasma proteins, with the affinity of prostaglandin A_2 being the strongest and that of prostaglandin $F_{2\alpha}$ the weakest.

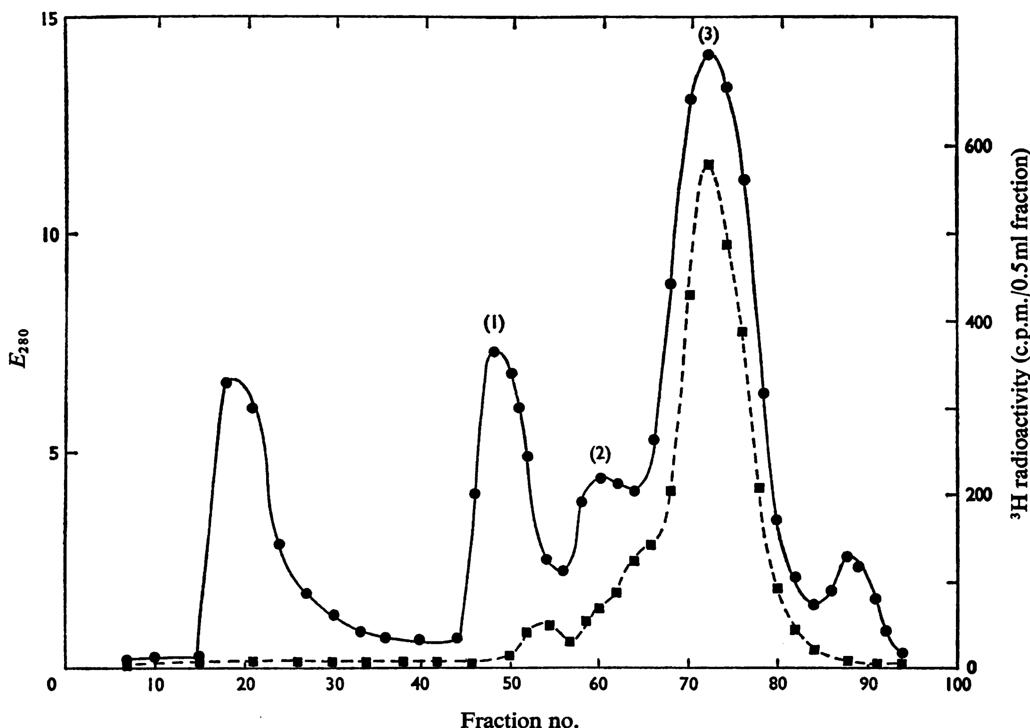

Fig. 1. *Chromatography of prostaglandin A_2-containing plasma on a column of DEAE-Sephadex*

Prostaglandin A_2 in acetone was added to 55ml of plasma and the plasma chromatographed as described in the Methods and Materials section. The fractions obtained were assayed for protein (E_{280}, ●) and for radioactivity (■). Peak 1, fractions 50–57; peak 2, fractions 58–65; peak 3, fractions 66–80.

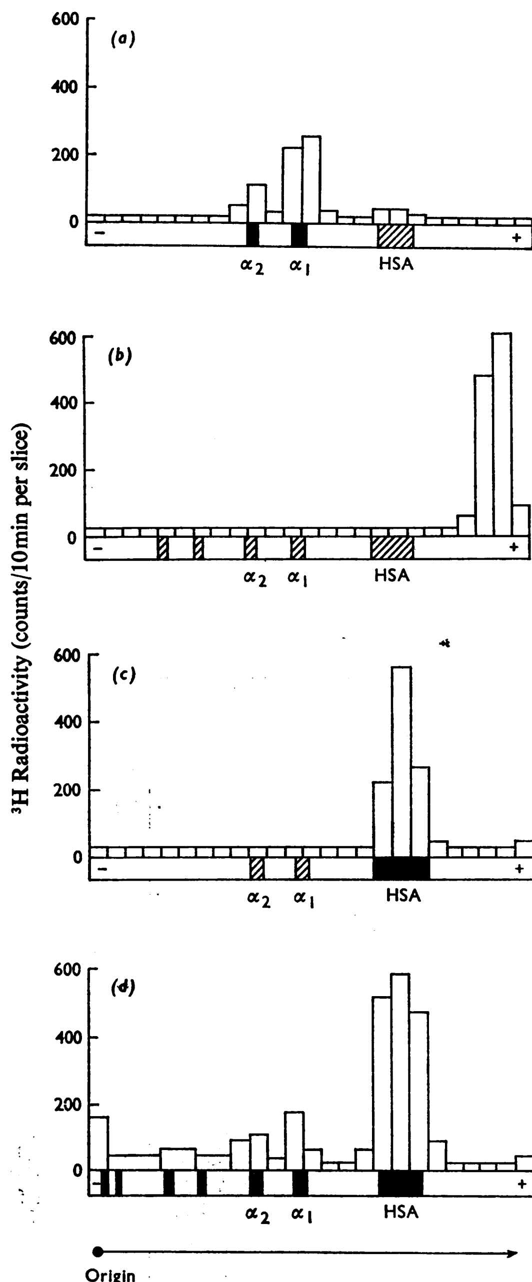

Fig. 2. *Disc-gel-electrophoresis pattern and radioactivity distribution of prostaglandin A_2 in the three protein peaks isolated after DEAE-Sephadex chromatography*

The peaks are shown in Fig. 1. (*a*) Peak 1; (*b*) peak 2; (*c*) peak 3; (*d*) whole plasma. HSA, Human serum albumin; α_1 and α_2, α_1- and α_2-protein.

Binding of prostaglandins A_2 and $F_{2\alpha}$ to human plasma determined by DEAE-Sephadex chromatography and polyacrylamide-gel electrophoresis

Radioactive prostaglandin A_2 or $F_{2\alpha}$ was added to plasma to a final concentration of 1 μg/ml, followed by DEAE-Sephadex chromatography. The fractions obtained were assayed for protein (E_{280}) and for radioactivity. The elution pattern for prostaglandin A_2 is given in Fig. 1. A major portion of the added prostaglandin became bound to the main protein peak (peak 3), with some radioactivity associated with two earlier peaks (peaks 1 and 2). Ultrafiltration of the three peaks, with an Amicon UM-10 membrane, suggested that the prostaglandin in peaks 1 and 3 is bound to proteins whereas peak 2 contains mainly the free prostaglandin. A similar elution pattern was obtained for prostaglandin $F_{2\alpha}$, except that a larger portion of the added prostaglandin remained unbound. This difference indicates the binding affinity of human serum albumin for prostaglandin A_2 to be greater than that for prostaglandin $F_{2\alpha}$, and further supports a similar conclusion stemming from the results in Table 1.

Analysis by polyacrylamide disc-gel electrophoresis of the radioactivity distribution of added prostaglandin A_2 between the various proteins in the three peaks (Fig. 2) revealed that the portion of prostaglandins present in peak 3 is indeed bound to albumin, the portion in peak 1 is bound to human serum albumin and to two α-proteins, whereas the portion isolated in peak 2 migrates on electrophoresis in a manner similar to the free prostaglandin (Raz, 1972). Similar patterns were obtained for the prostaglandin $F_{2\alpha}$. These results are quite similar to those obtained previously for prostaglandin E_2, and suggest a common binding site (or sites) for prostaglandins on the albumin molecule.

Effects of variations in the concentration of prostaglandins E_2 and $F_{2\alpha}$ on their binding to human serum albumin

To gain further knowledge of the binding of prostaglandins to human serum albumin, the effect of varying their concentrations on the binding was studied. The results are given in Fig. 3 and indicate that, within the range of concentrations of prostaglandins employed (approx. 1.4–50000 ng/ml), the percentage binding was essentially constant for each prostaglandin. Thus, at a fixed concentration of human serum albumin, the amount of albumin-bound prostaglandin is directly proportional to the total amount of prostaglandin in the medium. Furthermore, the data in Fig. 3 confirm the previous indication (Table 1) with regard to the relative binding affinities of the three prostaglandins, the order of affinity being $A_2 > E_2 > F_{2\alpha}$.

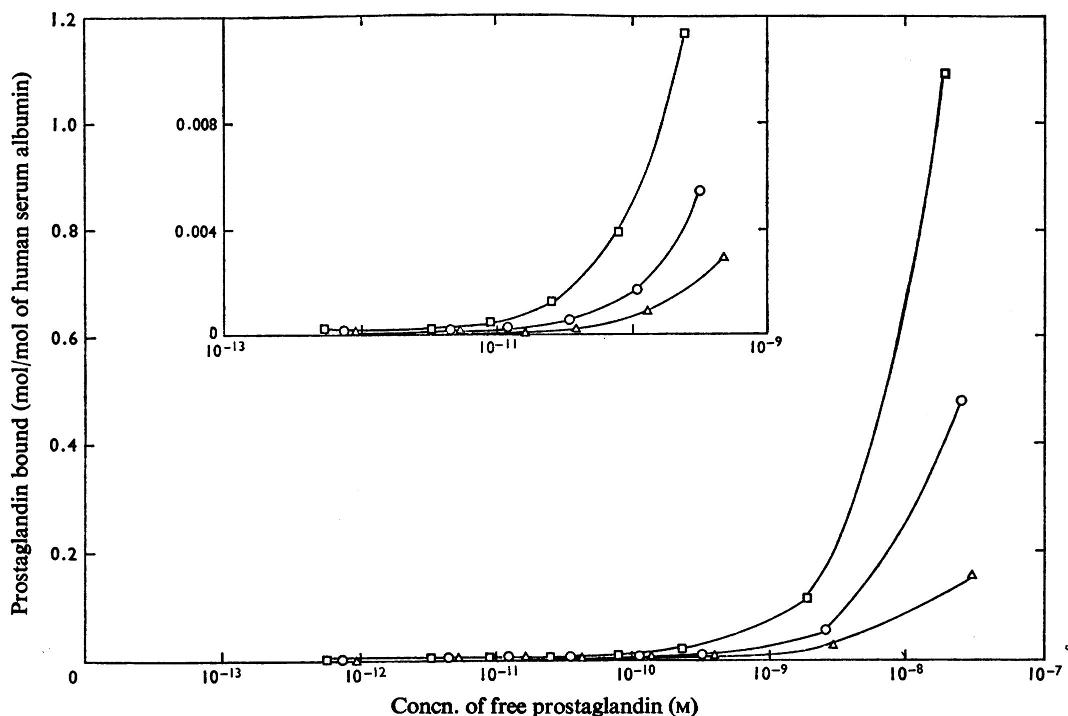

Fig. 3. *Effect of concentration of prostaglandins A_2, E_2 and $F_{2\alpha}$ on their binding to human serum albumin*

Various amounts of radioactive prostaglandins were added to a solution of human serum albumin (5mg/ml) and each mixture was subjected to equilibrium dialysis. The concentration of free and albumin-bound prostaglandin is plotted as mol of prostaglandin bound/mol of albumin versus concentration of free (unbound) prostaglandin (log scale) for prostaglandins A_2 (□), E_2 (○) and $F_{2\alpha}$ (△). Differences in the molar binding at low prostaglandin concentration (10^{-12}–10^{-9}M) can be seen in the inset figure, with enlarged ordinate scale. The association constant between each prostaglandin and albumin was obtained by assuming a 1:1 interaction system (human serum albumin+prostaglandin → human serum albumin–prostaglandin) in which

$$K_{\text{ass.}} = \frac{[\text{Human serum albumin–prostaglandin}]}{[\text{Human serum albumin}] \times [\text{prostaglandin}]} = \frac{[\text{Bound prostaglandin}]}{[\text{Free prostaglandin}]} \times \frac{1}{[\text{Human serum albumin}]}$$

In the low concentration range (10^{-12}–10^{-11}M) of prostaglandin the value of [human serum albumin] is very close to the total [human serum albumin] in the solution. By using this approximation, the association constants for prostaglandins A_2, E_2 and $F_{2\alpha}$ were calculated to be 4.8×10^4, 2.4×10^4 and 0.9×10^4 litre/mol respectively.

Effects of variations in the concentration of human serum albumin on the binding of prostaglandins E_2, A_2 and $F_{2\alpha}$

Additional information on the prostaglandin–human serum albumin interaction was obtained from studies in which the prostaglandin concentration was held constant while the concentration of human serum albumin was varied between 0 and 50mg/ml. The results (Fig. 4) show the binding of all three prostaglandins to be markedly dependent on the concentration of human serum albumin in the medium. A minimum concentration of human serum albumin was necessary for significant binding to be detected: 0.13mg/ml for prostaglandin A_2, 0.40mg/ml for prostaglandin E_2 and 1.33mg/ml for prostaglandin $F_{2\alpha}$. The data in Figs. 3 and 4 indicate that, within the range of prostaglandin concentrations employed, the absolute concentration of human serum albumin in the medium and not the molar ratio human serum albumin/prostaglandin E_2 is the key factor determining the resulting distribution of free and albumin-bound prostaglandin in equilibrium. The experiments described here were designed to provide information necessary to perform a

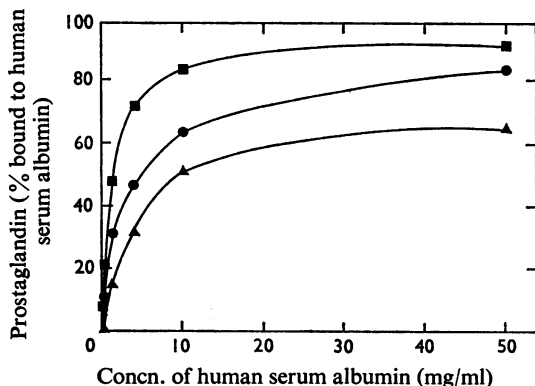

Fig. 4. *Effect of concentration of human serum albumin in the medium on the binding of prostaglandins A_2 (■), E_2 (●) and $F_{2\alpha}$ (▲)*

Curves represent the changes in the percentage of prostaglandins bound to human serum albumin after equilibrium dialysis of mixtures containing various amounts of human serum albumin.

Scatchard analysis (Scatchard *et al.*, 1950*a,b*), from which it would have been possible to obtain the number of classes of binding sites, the number of binding sites in each class and the association constant for each class of binding sites. However, owing to the apparent limited solubility of the prostaglandins in the aqueous medium, a molar ratio in excess of 1 for prostaglandin bound/albumin could not be attained, and therefore insufficient data were available for a meaningful Scatchard analysis. However, the data presented in Fig. 3 were utilized to obtain a rough approximation of the association constants for the binding of prostaglandins E_2, A_2 and $F_{2\alpha}$ to human serum albumin, by using a simple 1:1 interaction system (see the legend to Fig. 3). The apparent association constants for the interaction of human serum albumin with each of the three prostaglandins were thus estimated to be 4.8×10^4, 2.4×10^4 and 0.9×10^4 litre/mol for A_2, E_2 and $F_{2\alpha}$ respectively. These values are considerably smaller than the corresponding values for the binding of several long-chain fatty acids to human and bovine albumins. Spector *et al.* (1969) reported values of approx. 10^7 for the binding of palmitic acid to bovine and human albumins, and approx. 10^6 for the binding of palmitoleic acid, oleic acid and linoleic acid to bovine serum albumin. The differences in the binding affinities of prostaglandins and long-chain fatty acids may simply be due to a weaker association of prostaglandins with human or bovine serum albumins. However, these differences may also be due, at least in part, to differences in the techniques employed to assess the extent of binding.

Radioactive prostaglandin E_1 injected intravenously has been shown (Samuelsson, 1964) to disappear rapidly from the blood circulation of the rat and man. Prostaglandins injected into the blood appear to be rapidly metabolized in the lungs (Ferreira & Vane, 1967), yielding the physiologically inactive 15-oxo or 13,14-dihydro derivatives (Anggard & Samuelsson, 1966). On the other hand, prostaglandin A_2 appears to be inactivated very slowly or not at all during passage through the lungs (McGiff *et al.*, 1969) and was therefore suggested as a possible circulating hormone (Horton & Jones, 1969; McGiff *et al.*, 1969). The studies reported here suggest that, to the extent that prostaglandins are transported in the blood circulation, they are probably transported mainly in the albumin-bound form. Further work is needed to determine the rate of binding of prostaglandins to plasma albumin, both *in vitro* and *in vivo*, to enable the effects of the albumin–prostaglandins interaction on the uptake and metabolism of prostaglandins in various tissues to be evaluated.

This work was initiated at the Research Laboratories of the Upjohn Co., Kalamazoo, Mich., U.S.A.

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