

The Inhibition of Prostaglandin Biosynthesis by Human Plasma and its Relationship to Albumin and Haptoglobin

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Abstract: Blood plasma was found to contain endogenous inhibitor of prostaglandin (PG) synthase (EIPS) and this activity was associated with at least two plasma components, albumin and haptoglobin. Hemoglobin, a stimulant of PG biosynthesis which is bound by haptoglobin, reversed the inhibitory effect of haptoglobin and reduced the inhibition by albumin, acetylsalicylate or indomethacin. It is proposed that in physiological situations the albumin-associated EIPS mainly helps to prevent excessive PG production, whereas in tissue injury the acute phase protein haptoglobin plays a more important role. Possible clinical applications of EIPS are discussed.

Key words: Blood plasma, EIPS, albumin, haptoglobin, hemoglobin

Introduction

The blood plasma or serum of adult, but not fetal, mammals inhibits the biosynthesis of prostaglandins (PGs) by bovine seminal vesicle PG synthase *in vitro* (Collier *et al.*, 1980; Saeed *et al.*, 1977). Human serum inhibits the production of PGE₂, PGF_{2α} and PGI₂ (Denning-Kendall *et al.*, 1981) and since it inhibits cyclooxygenase (Collier *et al.*, 1980), we may assume that serum also inhibits production of thromboxane A₂ (TXA₂). This assumption is supported by the finding that human plasma inhibits arachidonate-induced aggregation of washed human platelets, suspended in buffer (Collier and McDonald-Gibson, 1980). Rat serum inhibits the production of prostaglandins by leukocytes that are phagocytosing killed bacteria (Di Rosa *et al.*, 1979). Such observations indicate that adult mammalian plasma or serum inhibits the biosynthesis of the main products of prostaglandins synthase, including TXA₂. These observations have led to the conclusion that adult mammalian plasma contains one or more circulating endogenous inhibitors of PG synthesis (EIPS).

Since the existence in plasma of such inhibitor(s) was discovered, attempts have been made to determine the identity, function and mode of action of EIPS. This paper outline these attempts, dealing in more detail with recent studies and discusses their implications and the conclusions that may be drawn.

Materials and Methods

The materials and methods used have been described elsewhere (Collier *et al.*, 1980; Saeed *et al.*, 1980; Saeed *et al.*, 1977). They are briefly outlined below.

Materials

Serum samples and Cohn fractions of human plasma were obtained from commercial suppliers. Table 1 gives details of the origin of the vein and human plasmas used. For collection of fetal lamb plasma, at the beginning of the last month of pregnancy, the ewe was premeditated with 1 g pentobarbital intravenously and then anesthetized with a mixture of halothane and oxygen. Catheters were implanted in the fetal and maternal circulations. Samples were taken at daily or longer intervals from fetus and mother. Except in three samples from adult men, disodium ethylenediaminetetraacetic acid (EDTA), 0.7 mg ml⁻¹ final concentration in whole blood, was used as anticoagulant. In the three exceptions, heparin, 10 units ml⁻¹ whole blood, was used. Human donors were required not to have taken any nonsteroidal anti-inflammatory drug during the week before the collection of sample. Human donors were not under treatment with sex hormones. Hemolyzed samples were rejected.

Haptoglobin was purified from dialyzed Cohn fraction IV-4 of human plasma by ammonium sulfate precipitation followed by ionexchange chromatography on DE-52 cellulose (Saeed *et al.*, 1980). As albumin, Cohn fraction V was mainly used, but a highly purified albumin (>99%) was kindly supplied by Dr. D.D. Schroeder (Cutter Laboratories), Inc. USA).

Methods

The ability of test samples to inhibit the synthesis of prostaglandins ("EIPS activity") was measured in three ways: (a) bioassay of total prostaglandins produced by incubation with bovine seminal vesicle PG synthase in the presence of added arachidonic acid, (b) measuring cyclooxygenation during incubation with bovine vesicular synthase by monitoring oxygen uptake and © measuring arachidonate induced aggregation of washed human platelets suspended in buffer.

To measure the inhibition of PG production, a lyophilized preparation of bovine seminal vesicle PG synthase (Miles Laboratories) was used, in a 1 ml volume of a standard assay mixture containing 1.3 mM reduced glutathione, 5.0 mg PG synthase and a dilution of the test plasma in 50 mM phosphate buffer, pH 7.4. The positive control was similar incubate without test plasma and the negative control was an incubate containing heat-inactivated enzyme. The reaction was started by addition of 61 µM sodium arachidonate and the mixture was incubated aerobically with gentle shaking for 15 min at 37°C, as described previously (Collier *et al.*, 1976). Prostaglandins were extracted in acidified ethylacetate and assayed on hamster stomach fundus (Ubatuba *et al.*, 1973). Total prostaglandin activity was expressed in terms of a reference standard PGE₂, which is the main production of the reaction in these conditions, as a ratio of the test value to that of the positive control, after subtracting from both the negative control value.

To measure cyclooxygenation, a comparable standard assay mixture was used, as previously described (Collier *et al.*, 1980). By means of a Yellow Springs biological oxygen monitor

Table 1: Sources of plasma^a

Subject		
Type	Number	Collection
Ram	6	Jugular vein, percutaneous needle
Pregnant ewe, last month gestation	11	Jugular vein or carotid artery, indwelling catheter
Fetal lamb	6	Femoral vein or artery, indwelling catheter
Adult man ^b	12	Antecubital vein percutaneous needle
Nulipara	13	Antecubital vein, percutaneous needle
Pregnant woman	11	Antecubital vein, percutaneous needle
Human cord	6	Umbilical vein, postdelivery, needle

^aEDTA, 0.7 mg ml⁻¹ whole blood, used as anticoagulant

^bIn three men, heparin, 10 units ml⁻¹ whole blood, was used as anticoagulant

(Model 53), oxygen uptake during 15 min incubation at 37°C of test and control mixtures was recorded. The proportion of oxygen uptake attributable to cyclooxygenation was determined by comparison of incubates in the presence and absence of 5.6 mM acetylsalicylate.

To measure arachidonate-induced aggregation of washed platelets, platelet suspensions were prepared by centrifuging whole blood at 200 g for 15 min. The resulting platelet-rich plasma was centrifuged at 600 g for 15 min and the resulting platelet pellet washed with saline, containing 10 ng ml⁻¹ PGI₂ to inhibit platelet stickiness during processing. The pellet of washed platelets was then suspended in half the original plasma volume of 0.1 M Tris-HCl buffer, pH 7.4, containing 10 ng ml⁻¹ PGI₂ and left at 22°C for 1 hr to allow decay of the PGI₂. The total volume of platelet suspension was adjusted to approximately 2×10^8 platelets ml⁻¹. Test plasma was added to 0.5 ml of platelet suspension at 37°C, the volume was made up to 0.7 ml with saline and the mixture was preincubated at 37°C for 5 min, before challenge with sodium arachidonate. Aggregation was recorded by Born or coulter aggregometer and expressed as percentage inhibition, compared with control, at 4 min after challenge.

Haptoglobin was determined by the peroxidase method of Connell and Smithies (Connell and Smithies, 1959). The content of human haptoglobin was determined with reference to a purified sample and expressed in mg ml⁻¹. As no reference sample of sheep haptoglobin was available, its content in sheep plasma was expressed as Δ optical density at 470 nm (OD) $\times 10^3$ sec⁻¹. Albumin was determined according to Sigma Technical Bulletin No. 630, using sheep or human plasma albumin for reference.

Results and Discussion

Early observations

It was initially observed that EIPS is nondialyzable and labile to heating for 10 min at 98°C or to incubation with pronase at 37°C, indicating that EIPS is either proteinaceous or closely associated with plasma protein. Study of various Cohn fractions of human plasma showed that several had little or no EIPS activity. Activity was high, however, in Cohn fractions IV, especially IV-4 (Saeed *et al.*, 1977), which contains haptoglobin, other α - and β -globulins and albumin and in Cohn V, which is largely albumin (Collier *et al.*, 1980; Collier *et al.*, 1980 and Saeed *et al.*, 1980).

Table 2: Inhibition of arachidonate-induced aggregation of washed human platelets in tris-HCl buffer, pH 7.4^a

Test material	Conc.	(n)	Mean % inhibition $\pm$ SEM
Plasma	0.07% v/v	9	19.4 $\pm$ 3.1
	0.3% v/v	19	36.1 $\pm$ 3.7
	0.7% v/v	23	63.4 $\pm$ 3.8
	1.4% v/v	10	68.8 $\pm$ 3.5
Cohn I	14.3 mg ml ⁻¹	7	11.9 $\pm$ 11.5
Cohn IV	3.6 mg ml ⁻¹	3	47.2 $\pm$ 4.5
	7.2 mg ml ⁻¹	3	75.7 $\pm$ 2.4
Cohn IV-4	1.13 mg ml ⁻¹	5	53.2 $\pm$ 2.5
	2.81 mg ml ⁻¹	5	84.9 $\pm$ 2.1
Cohn V (albumin)	0.29 mg ml ⁻¹	6	23.5 $\pm$ 6.8
	0.71 mg ml ⁻¹	11	63.4 $\pm$ 8.5
	1.42 mg ml ⁻¹	6	91.7 $\pm$ 3.6
Haptoglobin	3.6 mg ml ⁻¹	4	30.8 $\pm$ 10.5
(76% pure)	7.2 mg ml ⁻¹	5	55.9 $\pm$ 6.7
Dextrant T40, T70	14.3 mg ml ⁻¹	5	8.5 $\pm$ 4.9
	14.3 mg ml ⁻¹	4	14.6 $\pm$ 2.8

^aAggregation in test suspension was compared with that in control, 4 min after challenge with 95 μ M arachidonate. Number of experiments

Table 3: Relationship of haptoglobin and albumin contents of plasmas to EIPS activity^a

Plasma	Haptoglobin (* Δ OD $\times$ 10 ³ sec ⁻¹ ; **, mg ml ⁻¹)	Albumin (mg/ml)	EIPS activity; IC ₅₀
Pregnant ewe	ND - 19.3' (5)	36.2 $\pm$ 3.0 (6)	0.99 $\pm$ 0.11 (11)
Fetal lamb	ND - 0.6' (5)	26.4 $\pm$ 2.2 (5)	3.91 - >20 (6)
Adult man	NT	53.2 $\pm$ 0.5 (8)	0.31 $\pm$ 0.03 (12)
Nullipara	1.50 $\pm$ 0.10" (13)	51.0 $\pm$ 0.9 (13)	0.20 $\pm$ 0.02 (13)
Pregnant woman	1.67 $\pm$ 0.16" (11)	42.4 $\pm$ 0.8 (11)	0.28 $\pm$ 0.02 (11)
Human cord	ND (6)	36.5 $\pm$ 3.1 (4)	0.44 $\pm$ 0.07 (6)

^aNumber of subjects in brackets. IC₅₀ is the concentration to inhibit by 50% production of PGs by bovine seminal vesicle PG synthase; the IC₅₀ is therefore the reciprocal of potency

OD, optical density at 470 nm; ND, none detectable; NT, not tested

Table 4: Comparative effects of human serum, haptoglobin (76% pure), albumin (Cohn fraction V) and indomethacin on the inhibition of biosynthesis of prostaglandins D, E, F_{2 α} and 6-keto-F_{1 α}

Test	IC ₅₀ (μ g protein/ml) $\pm$ SEM in absence of Cofactor ^a				IC ₅₀ (μ g protein/ml) $\pm$ SEM in presence of Cofactor ^a		
	D ₂	E ₂	F _{2α}	6-keto-F _{1α}	E ₂	F _{2α}	6-keto-F _{1α}
Serum	NT	NT	NT	NT	292 $\pm$ 30	411 $\pm$ 113	406 $\pm$ 125
Haptoglobin	236 $\pm$ 19	285 $\pm$ 29	341 $\pm$ 16	> 600	162 $\pm$ 23	186 $\pm$ 38	271 $\pm$ 17
Albumin	480 $\pm$ 30	650 $\pm$ 91	800 $\pm$ 122	1,250 $\pm$ 109	250 $\pm$ 27	298 $\pm$ 51	377 $\pm$ 82
Indomethacin (μ M)	0.72 $\pm$ 0.05	0.65 $\pm$ 0.05	0.82 $\pm$ 0.04	1.11 $\pm$ 0.34	0.31 $\pm$ 0.06	0.49 $\pm$ 0.11	0.29 $\pm$ 0.13

^aReduced glutathione, 1.3 mM

Table 2 gives the ability of various Cohn fractions to inhibit platelet aggregation and indicates that potency is greatest in Cohn V. Experiments in which human serum was depleted by affinity chromatography of haptoglobin and albumin, separately or together, showed that depletion of

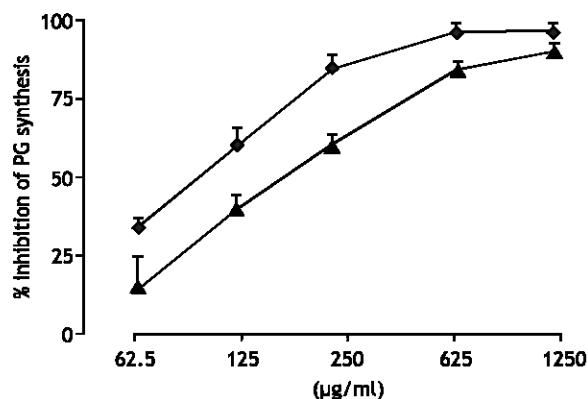


Fig. 1: Inhibition of prostaglandin synthesis by highly purified plasma albumin (♦) and by Cohn V (▲). Concentration is expressed as µg albumin per ml of incubate. Vertical bars represent standard error of the mean

haptoglobin removed about 33% of the activity of whole serum in inhibiting PG synthesis; depletion of albumin removed about 85% of this inhibitory activity; and depletion of both removed about 96% of the activity (Kendall *et al.*, 1970). These experiments suggested that haptoglobin and albumin or a substance that is attached to these proteins in the depletion process are largely responsible for activity in inhibiting PG synthesis.

Purified haptoglobin and albumin

Partly purified haptoglobin was compared with human plasma or serum, with Cohn IV-4 and with the relatively pure albumin that constitutes Cohn V, in various tests of ability to inhibit prostaglandin synthesis. Haptoglobin was about eight times more potent than whole serum inhibiting prostaglandin production by bovine vesicular synthase; but it was only partly effective in inhibiting cyclooxygenation, in contrast to plasma and serum, which were fully effective and to albumin, which had low potency and effectiveness (Collier *et al.*, 1980 and Saeed *et al.*, 1980). Haptoglobin was less potent and albumin was more potent than Cohn IV-4 in inhibiting arachidonate-induced aggregation of washed human platelets (Table 2).

Recently, we have tested a highly purified sample of human plasma albumin against PG synthesis and platelet aggregation. In both tests, the purified albumin was 1.5 to 2.0 times more potent than Cohn V (Fig. 1). That the increase in potency (50-100%) was disproportionately greater than the extent of purification (<10%). This suggest that some kind of inhibitory effect, due to occupation of a binding site, was removed by purification.

Vein and human plasmas

Very recently we have obtained further evidence on the identity of plasma EIPS by studies on the inhibitory activities and the haptoglobin and albumin contents of various vein and human plasma (Brennecke *et al.*, 1981; Brennecke *et al.*, 1981; Collier *et al.*, 1980). Table 3 compares the

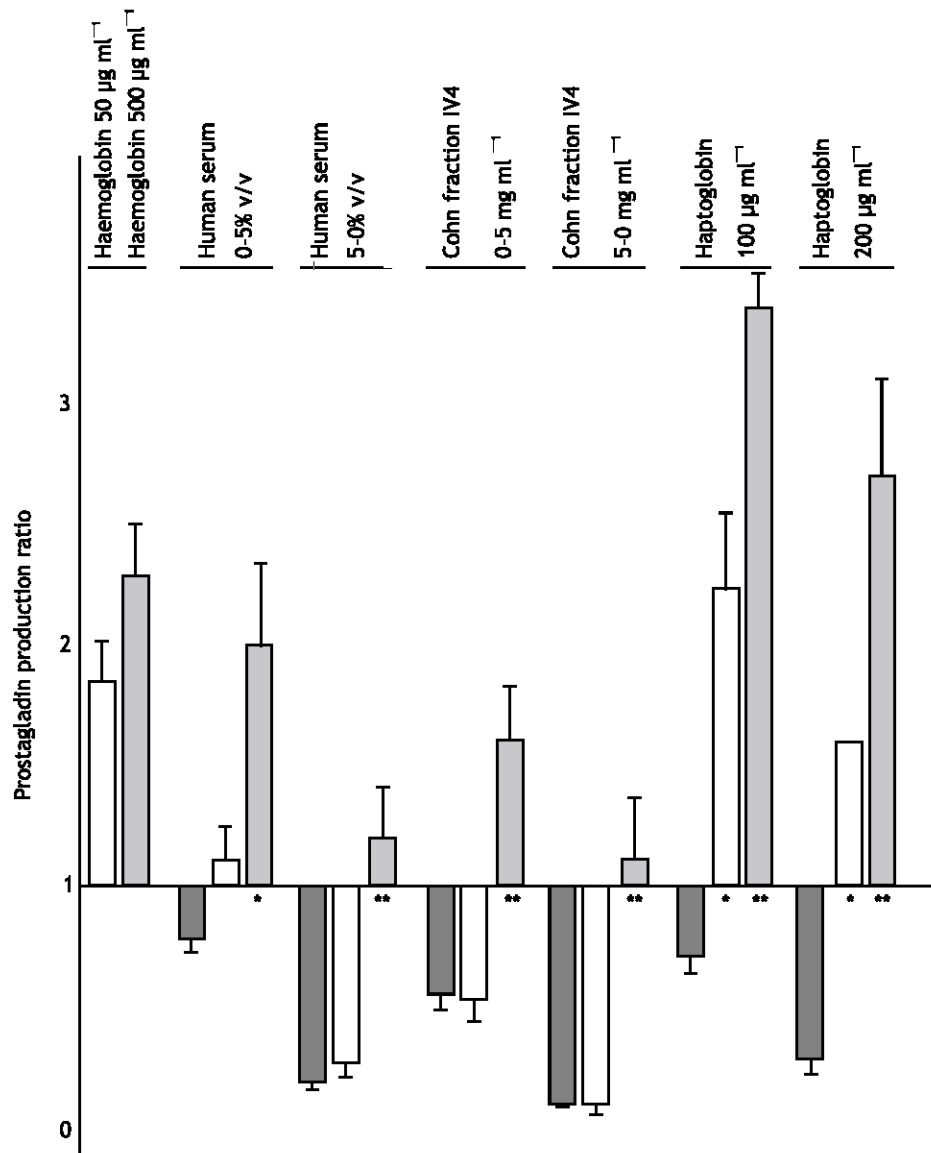


Fig. 2: Human hemoglobin stimulates PG production by bovine seminal vesicle synthase and antagonizes the inhibitory effects of whole serum and Cohn IV-4. Haptoglobin synergies with hemoglobin in stimulating PG production. *, $P < 0.005$ and **, $p < 0.0005$, for difference from corresponding control in the absence of hemoglobin (8)

haptoglobin and albumin contents of plasmas from different vein and human sources with their activities as inhibitors of PG synthesis.

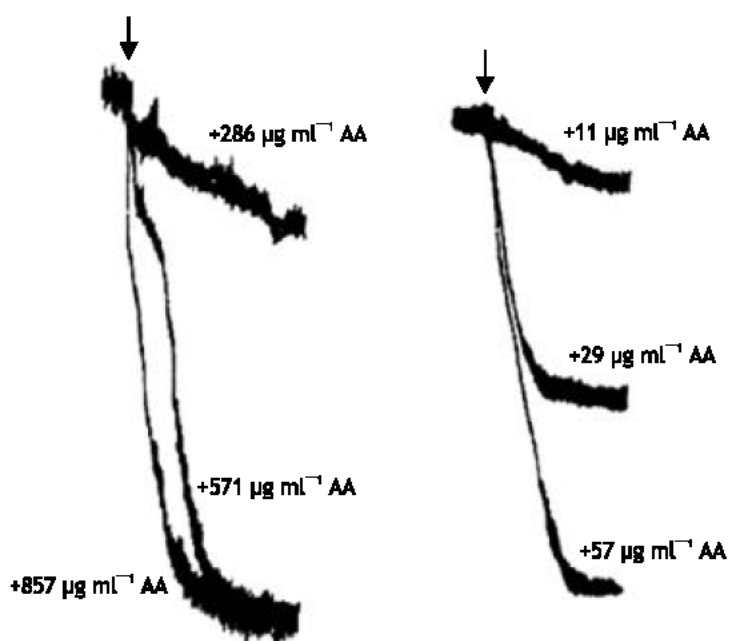


Fig. 3: Induction of platelet aggregation by various concentrations of arachidonic acid (AA) in platelet-rich plasma (left-hand tracing) and in washed platelet suspension in Tris-HCl buffer, pH 7.4 (right-hand tracing). Aggregation, recorded by Born aggregometer, is indicated by the downward movement of the tracing. Arrows indicate point of addition of drug

Table 3 shows that plasma containing no detectable haptoglobin (pregnant ewe and human cord) nonetheless has considerable EIPS activity. It also shows that plasma without measurable EIPS activity (fetal lamb) has a considerable concentration of albumin. Since it seemed possible that fetal plasma contained an antagonist of EIPS, we tested mixtures of this with pregnant ewe plasma; but no evidence of an antagonist in fetal plasma emerged.

That, in some instances, plasma or serum is more effective than albumin or haptoglobin alone might perhaps be due to mutual potentiation of the two factors. Table 6 (below), shows, however, that plasma from pregnant women has distinctly greater activity in inhibiting PG synthesis than it has against platelet aggregation ($P < 0.05$), whereas the activities of the plasma of men and of nulliparae were equal against both. This does not seem to be explicable in terms of a synergism between albumin and haptoglobin, but would be consistent with the existence of another factor in pregnancy plasma with high activity against biosynthesis of prostaglandins.

The evidence so far obtained on the identity of EIPS suggest that haptoglobin itself is unlikely to be responsible for more than a small part of EIPS activity in plasma. Albumin, on the contrary, appears to be responsible for a large part of this activity. There remains some evidence suggesting that a third EIPS also exists in plasma.

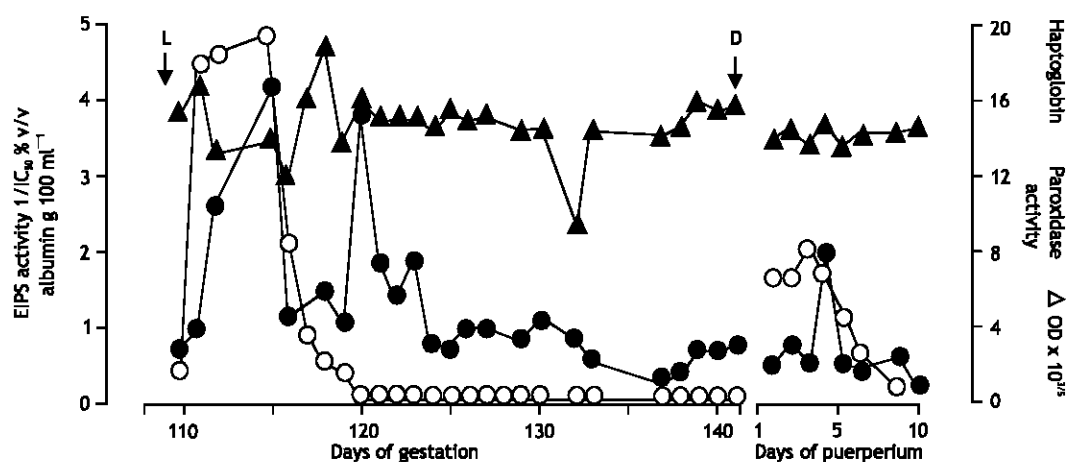


Fig. 4: Levels of EIPS activity (●), haptoglobin (○) and albumin (▲) in the plasma of a ewe during late pregnancy, parturition and the puerperium (2). EIPS activity is expressed as the reciprocal of the IC₅₀ for inhibition of PG biosynthesis by bovine vesicular PG synthase. L, day of laparotomy; D, day of delivery

Functions of EIPS

Early experiments showed that Cohn subfraction IV-4 of human plasma, which has high EIPS activity, lessens adjuvant arthritis in the rat and arachidonate-induced bronchospasm in the guinea pig (Saeed *et al.*, 1977). It has shown at the same time that injection of dexamethasone or hydrocortisone in the rat raised the EIPS activity of plasma, so that this was approximately doubled between 24 and 48 hr after the injection (Saeed *et al.*, 1978 and Saeed *et al.*, 1977). Although these experiments suggested that EIPS might help to mediate corticosteroid action, they did not enable function of plasma EIPS to be determined.

Control of excessive PG production

It has been argued that prostaglandins, as local hormones, are largely concerned with defensive, including exclusive, processes (Collier, 1971; Collier, 1974 and Collier, 1980). Mainly, prostaglandins activate such processes, which include fever, pain and inflammation, vomiting and diarrhea, cough and mucus production, hemostasis and expulsion of the fetus. Some prostaglandins, however, have the secondary function of regulating these defensive exclusive processes; for example, prostacyclin may regulate platelet aggregation induced by thromboxane A₂. Since the effects of prostaglandins can be damaging, the body has safety mechanisms to control their excessive or misdirected production. Six ways in which the body probably controls excessive PG production have been noted (Collier, 1980). These are control mechanisms, of which EIPS may be regarded as one. Regarding EIPS as one or more endogenous regulators of PG synthesis, we have considered its possible function in several conditions in which prostaglandin levels may become excessive: hemolysis, a rise in free arachidonic acid in plasma, premature birth and premature labour.

Hemolysis

The well-known ability of haptoglobin to bind hemoglobin suggested that haptoglobin might have the function of inhibiting the stimulation of PG synthase exerted by free hemoglobin in the plasma. Experiments on the interaction of haptoglobin and hemoglobin, however, gave an unexpected result. Although haptoglobin can inhibit the stimulatory activity of a relatively small amount of hemoglobin, mixtures ranging in proportion from 1 part hemoglobin: 2 parts haptoglobin to 5 parts hemoglobin: 1 part of haptoglobin stimulate PG synthase more than does hemoglobin alone (Fig. 2). This figure also shows that whole serum or Cohn fraction IV-4 appear, on the contrary, only to antagonize the stimulatory action of hemoglobin. For example, a 1:1 mixture of hemoglobin and Cohn IV-4 has less stimulatory activity on PG synthesis than does hemoglobin alone. These findings point to a special role of haptoglobin, not as an antagonist, but as a synergist of the stimulation of PG synthase by hemoglobin, which is likely to occur after hemolysis. Further evidence on such a possibility is, however, required.

Plasma arachidonic acid

Addition of arachidonic acid to platelet-rich plasma rapidly leads to aggregation of the platelets. As Fig. 3 shows, considerably less arachidonic acid is needed to give the same result after the platelets have been washed free of plasma and suspended in buffer (Collier *et al.*, 1980). In these conditions, aggregation is presumably due to formation by the platelet of the powerful aggregating agent. That more arachidonic acid is required to induce this process in plasma than in buffer may be attributable to the EIPS activity of plasma.

As Table 2 shows, Cohn V, consisting largely of albumin, was the most powerful of the plasma fractions tested for inhibition of platelet aggregation. Highly purified albumin has proved still more potent. Hence it seems that the ability of plasma and its fractions to inhibit arachidonate-induced aggregation of washed platelets can largely be attributed to their albumin content (Collier *et al.*, 1980).

These observations suggest a hitherto unrecognized function for plasma albumin: to inhibit platelet aggregation in the bloodstream that might otherwise be elicited by small amounts of free arachidonic acid, arising from its ingestion, its untoward liberation, or some other cause (Collier *et al.*, 1980). Since aggregation of platelets tends to be self-recruiting and since the amounts of prostacyclin in the circulating plasma are too small to inhibit aggregation therein, this proposed function of albumin would be expected to have survival value. Since this effect on aggregation has a ceiling (Fig. 3), aggregation due to massive liberation of arachidonic acid, as might occur with damage to the vessel wall, would be unimpaired.

Closure of the ductus arteriosus

Table 4 shows that the plasma of pregnant ewe has high EIPS activity, whereas that of fetal lamb has little. This pattern is consistent with reproductive requirements, because primary prostaglandins in the mother can induce uterine contraction at any stage of pregnancy and so may cause abortion; but in the fetus, they relax the circular muscle of the ductus arteriosus and thus maintain its patency (Coceani *et al.*, 1975; Coceani *et al.*, 1976; Sharpe *et al.*, 1974; Starling and Elliot, 1974 and Turnbull *et al.*, 1981). *In vitro*, it is essential for the fetus that the ductus

arteriosus should be sufficiently open to short-circuit the pulmonary circulation. After birth, it is equally essential for the neonate that the ductus should be closed and so allow gas exchange in the lungs. It has been suggested that this closure may be another function of EIPS (Lucas *et al.*, 1980). Such a function might be served by a rise of EIPS activity of the plasma after birth. Although EIPS activity has not yet been measured in the neonate, the presence of some activity in the plasma of the human umbilical cord is consistent with the possibility of such an increase.

Premature labor

Since the primary prostaglandins are potent abortifacients, it seems important that the maternal uterus should not be presented with high levels of these PGs until term. One means of achieving this might be a high level of EIPS activity. Evidence obtained by repeated sampling of plasma from one ewe during the last month of pregnancy was consistent with this possibility, since the EIPS activity fell to its lowest level (IC_{50} , 2.74% v/v) 4 days before delivery (Fig. 4).

These findings support the suggestion that a function of maternal plasma EIPS may be to suppress synthesis of prostaglandins during pregnancy sufficiently to prevent abortion or premature labor (Robinson *et al.*, 1978). The data obtained from human plasma are consistent with this hypothesis and furthermore suggest the existence of a pregnancy-associated EIPS, in that pregnancy plasma, in contrast to that of men or nulliparae, has greater ability to suppress prostaglandin synthesis and platelet aggregation.

Inhibitors of lipoxygenase

The findings described or cited above indicate the existence of a widespread system of endogenous inhibitors of arachidonic acid metabolism via the cyclooxygenase path. That this system may also extend to the lipoxygenase path is indicated by the recent finding that blood plasma or serum and some of its fractions inhibit soybean lipoxygenase and probably also a lipoxygenase of guinea-pig lungs (Collier *et al.*, 1981 and Saeed *et al.*, 1980). Albumin is active in this way, but haptoglobin is of low activity and Cohn fraction IV is more active than IV-4. Unlike EIPS, the inhibitor(s) of lipoxygenase also occur in fetal and in chicken plasma or serum. These lipoxygenase inhibitor(s) also appear to be proteins. Thus a system of lipoxygenase inhibitors, partly involving different proteins, appears to parallel that of cyclooxygenase inhibitors.

Mode of action

Access to site of action

In addition to the EIPS of plasma, a number of proteinaceous inhibitors of prostaglandin biosynthesis have been described in tissue extracts. Such inhibitors have been found in extracts of kidney (Limas *et al.*, 1979 and Terragno *et al.*, 1978), of inflammatory granulomas (Splawinski *et al.*, 1978) and of rat stomach (Saeed *et al.*, 1980). An inhibitor of PG synthesis has also been found in placenta (Harrowing and Williams, 1979) and in red blood corpuscles (Harrowing and Williams, 1980); but Harrowing and Williams, (1980) have recently concluded that the placental inhibitor may be derived from trapped blood. It is uncertain whether and to what extent the inhibitors of PG synthesis obtained from tissue extracts differ from those of blood. It is known that albumin, for example, is widely distributed in body fluids (Katz *et al.*, 1970; Rosendoer *et al.*,

1977 and Rothschild *et al.*, 1955). Hence at least the exterior surfaces of cells in soft tissues are accessible to albumin.

Albumin

In considering the mode of action of EIPS, we will confine ourselves to albumin, which is the most clearly identified protein having EIPS activity. Since it seems unlikely that albumin would readily penetrate the cell surface to reach the PG synthase within, it is postulated that albumin may act at the surface of the cell in which PG synthesis is in progress, by sequestering substrates, intermediates, or cofactors used in the biosynthetic process.

Albumin is well-known to bind long-chain fatty acid and so it would be tempting to assume that it acts by sequestering arachidonic acid. Our preliminary experiments suggest, however, that sequestration of arachidonic acid only accounts for a part of the activity of albumin in inhibiting PG synthesis (Denning-Kendall *et al.*, 1981). Furthermore, that albumin can inhibit PG synthesis without affecting cyclooxygenation (Collier *et al.*, 1980 and Denning-Kendall *et al.*, 1980) implies that it also works at a step beyond the initial formation of endoperoxide (Samuelsson *et al.*, 1979). Since albumin blocks the formation from arachidonic acid of all the main 2-series prostaglandins (Table 5), it presumably acts at a step before the separate formation of each. These considerations suggest that albumin may inhibit the conversion of PGG_2 to PGH_2 .

According to Samuelsson *et al.* (Samuelsson *et al.*, 1979), the conversion of arachidonic acid to PGG_2 requires heme and oxygen and the conversion of PGG_2 to PGH_2 uses heme and L-tryptophan. Insofar as albumin sequesters heme, which it is known to bind (Morgan *et al.*, 1976), it may therefore be expected to block the stimulation by heme of both the conversion of arachidonic acid to PGG_2 and of PGG_2 to PGH_2 . Albumin also binds L-tryptophan, for which it has a stereospecific binding site (Cunningham *et al.*, 1975 and McMenamy and Oncley, 1958). Insofar as albumin sequesters L-tryptophan, it may therefore be expected to inhibit the conversion of PGG_2 to PGH_2 . Hence it seems possible that the EIPS activity of albumin may reside to some extent in its ability to bind L-tryptophan.

A problem about considering albumin as a major EIPS arise from the finding that some samples of fetal lamb plasma, although without measurable EIPS activity, contain considerable amounts of albumin (Table 3). The resolution of this problem may lie in the fact that mammalian embryos produce albumin with an amino terminal extension consisting of several amino acids (Hannah *et al.*, 1980), which might be without EIPS activity. This possibility, however, remains to be explored experimentally.

COX exists in more than one form, constitutively expressed as COX-1, which is present in most cells and produces prostanoids which display physiological effects. Whereas COX-2, an isoform induced by pro-inflammatory cytokines and mitogens is present in inflamed tissues.

Regulation of COX-2

The two genes including COX-1 and COX-2 are clearly descended from a common ancestor. Each contains near identical intron replacement, suggesting that they are those from a common gene through gene duplication (Xie *et al.*, 1993).

In all organisms induction of COX-2 is largely at mRNA level and mostly transcriptionally driven. Like other immediate early genes its transcription increases abruptly within 15 minutes of treatment with a mitogen or other inducers (Evelt *et al.*, 1993). In addition COX-2 is down regulated at this level by synthetic glucocorticoids (O'Banion *et al.*, 1994), inhibitors of tyrosine phosphorylation (Chanmugam *et al.*, 1995), activated oxygen scavengers (Feng *et al.*, 1995) and prostanoids (Akarasereenont *et al.*, 1995).

The instability of the COX-2 gene and protein make it ideal for transient expression. COX-2 mRNA contains more than 10 copies of Shaw/ Kamen sequence, AUUUA, that is responsible for stabilizing RNA.

Following mitogen stimulation of established cell lines, there is a large increase in PGE2 and its release. Antisense oligonucleotides to COX-2 but not those directed against COX-1, prevent this release (Reddy and Herschman, 1994) which demonstrates that PGE2 is released solely from COX-2. Arachidonic acid delivery proteins that selectively feed substrate to COX-2 or proteins that block the channel by which COX-1 reaches arachidonate delivered from phospholipase A2 (PLA2) could be involved.

Possible clinical applications

The therapeutic potential of EIPS may be broad and one of its attractive characteristics is that its toxicity is likely to be limited because it is a natural constituent of human plasma. The possible uses that may present be envisaged for EIPS include: (a) control of platelet aggregation in conditions liable to give rise to thrombotic episodes, for example, in the surgery of hip joints; (b) control of fever and malaise in hemolytic disease; © medical management of neonates with patent ductus arteriosus; and (d) treatment of premature labor. To exploit such clinical possibilities requires not only identification and purification of the active substance but also its pasteurization before administration to man. So far, human plasma albumin is the only EIPS in which this process has progressed sufficiently for clinical trial to be envisaged.

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