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Prostaglandin uptake and metabolism by the perfused rat liver

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Summary

1. The prostaglandins are C20 unsaturated fatty acids which exhibit diverse physiological effects of short duration. We have investigated the speed of removal of PGE_1 and $PGF_{1\alpha}$ from the circulating blood and their subsequent metabolism by the isolated perfused rat liver.

2. Following either a single injection of radiolabelled PGE_1 or $PGF_{1\alpha}$ into the hepatic artery or portal vein, or recirculation of prostaglandins through the liver for 2·5 h, the distribution of radioactivity within extracts of bile, blood and liver was determined. The nature of the radioactive products of metabolism was inferred by comparison of the distribution of radioactivity after injecting carbon and tritium labelled standards, and by thin-layer chromatography, gas-liquid chromatography, ultraviolet and bioassay analysis.

3. A single injection of 1-^{14}C PGE_1 indicated that the liver could efficiently remove 89–95% of circulating PGE_1 on a single passage. Biliary excretion was excluded as a major route for elimination of unchanged PGE_1, because only 0·3–0·8% of the injected radioactivity was detected in the bile. During recirculation of 1-^{14}C PGE_1, 11–19% of the injected radioactivity was detected as exchanged $^{14}CO_2$. The radioactivity detected within liver was identified with further fragments resulting from decarboxylation of PGE_1, which were incorporated into fatty acids and then phospholipids.

4. Studies with 5,6-^{3}H PGE_1, and comparison with the results obtained using 1-^{14}C PGE_1, revealed a 30-fold increase in the percentage of radioactivity excreted into the bile, suggesting that biliary excretion may be a major route for elimination of compounds smaller than C20 prostaglandin. Evidence that the cyclopentane ring was intact was inferred by formation of a PGB compound on treatment with alkali; similar biliary excretion of 9-^{3}H $PGF_{1\alpha}$ also occurred. In addition, the increased radioactivity detected within the liver (37%) and blood (43%) after a single injection of 5,6-^{3}H PGE_1 had the solvent partition and thin-layer chromatography properties of PGE_1, but were associated with a less polar compound smaller than the C20 parent structure.

5. These results indicate rapid uptake of circulating prostaglandins by the rat liver. Decarboxylation of prostaglandins results in pharmacological inactivation. The products are excreted into the bile and venous effluent. These processes would curtail the duration of effects following prostaglandin injection.

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In addition, we infer from these results that any physiological action of these ubiquitous endogenous substances is likely to be localized within their tissue of origin.

Introduction

Prostaglandins exhibit a wide range of potent pharmacological effects *in vivo* (Bergström, Carlson & Weeks, 1968). One hour after injection of radiolabelled PGE_1 into mice or rats, autoradiographic and other studies revealed that radioactivity was excluded from the central nervous system (Holmes & Horton, 1968) but otherwise generally distributed throughout the body, and localized mainly in the lungs, liver and kidney (Samuelsson, 1964 ; Hansson & Samuelsson, 1965).

A specific dehydrogenase enzyme which oxidizes the prostaglandin molecule at the C15 position has been isolated from lungs of various species (Änggård & Samuelsson, 1966 ; Änggård, Matschinsky & Samuelsson, 1968, 1969) and direct evidence for β-oxidation of prostaglandins has been obtained in rabbit intestine (Parkinson & Schneider, 1969), in rat liver mitochondria (Hamberg, 1968), and intact rats (Miller & Krake, 1968). Analyses of urinary metabolites of the prostaglandins have indicated that the prostaglandin molecule can undergo not only oxidation of the 15-hydroxyl group and β-oxidation, but also reduction of the Δ13,14 bond ; in the guinea-pig, PGE_2 also undergoes reduction of the keto group within the five membered ring to a β-hydroxyl moiety (Hamberg & Samuelsson, 1969a), while in man PGE_2 also undergoes ω-oxidation with formation of a dicarboxylic acid (Hamberg & Samuelsson, 1969b). From our previous studies (Ramwell & Shaw, 1967 ; Shaw & Ramwell, 1968) and those of other workers (Coceani & Wolfe, 1965 ; Vogt & Distelkotter, 1967 ; Davies, Horton & Withrington, 1968 ; Vane, 1968), it is clear that prostaglandins are released from most animal tissues spontaneously and following drug, nervous or hormonal stimulation, and thus their release into the circulation can be anticipated (Ramwell & Shaw, 1970). However, circulating pharmacologically active prostaglandins have only been detected during well identified physiological and pathological conditions, including anaphylactic shock (Piper & Vane, 1969), the initial stages of labour (Karim, 1969), hypertension (Edwards, Strong & Hunt, 1969) and certain types of carcinoma (Williams, Karim & Sandler, 1968). The knowledge that the pharmacological effects measurable after injection of prostaglandins into animals or humans are of short duration, and also that the parent prostaglandins are not detectable in circulating blood under normal conditions, suggests that possibly rapid modification of circulating prostaglandins can occur, with formation of less potent compounds. Indeed 15-keto PGE_1 and 15-ketodihydro PGE_1 were detected in circulating rat blood following injection of the parent compound (Samuelsson, 1964) and studies with these naturally occurring metabolites, and the recently available synthetic analogues, has made it apparent that any modification of the parent prostaglandin structure tends to reduce pharmacological potency of these compounds (Beerthuis, Nugteren, Pabon & van Dorp, 1968).

The lungs and liver of the cat can efficiently inactivate circulating PGE_1 during a single passage (Ferreira & Vane, 1967). However, the rate of uptake and the extent of modification of prostaglandins by a particular tissue has not been studied. We have thus examined these parameters using radiolabelled PGE_1 and $PGF_{1\alpha}$ in a blood-perfused isolated rat liver preparation (Abraham & Dawson, 1967 ; Abraham,

Dawson, Grasso & Goldberg, 1968). Our initial studies with $1^{-14}C$ PGE_1 indicated that the rat liver efficiently removed 95% of the radioactivity associated with PGE_1 on a single passage, and that of the small percentage of radioactivity detected in the hepatic venous effluent only a minor fraction had the partition or pharmacological properties of unaltered PGE_1. Preliminary results were communicated to the British Pharmacological Society (Dawson, Ramwell & Shaw, 1968).

Methods

Abbreviations

The following abbreviations are used throughout: prostaglandin E_1, PGE_1 (11α, 15-dihydroxy-9-ketoprost-13-enoic acid); prostaglandin $F_{1\alpha}$, $PGF_{1\alpha}$ (9α,11α,15-trihydroxyprost-13-enoic acid); and prostaglandin A_1, PGA_1 (15-hydroxy-9-keto-prosta-10,13-dienoic acid).

Isolated liver preparation

Livers from fed and 16 h fasted male Sprague-Dawley rats (200–300 g) were perfused via both the hepatic artery (90–110 mmHg) and portal vein (7–10 mmHg) (1 mmHg≡1·333 mbar). The dissection was performed on artificially ventilated rats previously pithed under light ether anaesthesia; the bile duct was cannulated. The perfusion medium was defibrinated rat blood (150 ml), previously dialysed for 16–24 h at 4° C against Krebs bicarbonate solution containing amino-acids (Mayes & Felts, 1966). This procedure achieved a uniform glucose concentration and removed vasoconstrictor substances which otherwise interfere with liver perfusion. Blood was equilibrated with gas mixtures to a pO_2 and pCO_2 of 100–120 and 40 mmHg respectively. The portal blood flow (approximately 5 ml/min) and arterial pressure were monitored; the pO_2, pCO_2 and pH of aliquots of hepatic vein blood were measured with a Radiometer (model 27 pH meter) with a gas monitor attachment. Blood samples were collected anaerobically into glass syringes and immediately analysed. The liver was perfused for 30 min before prostaglandin injection.

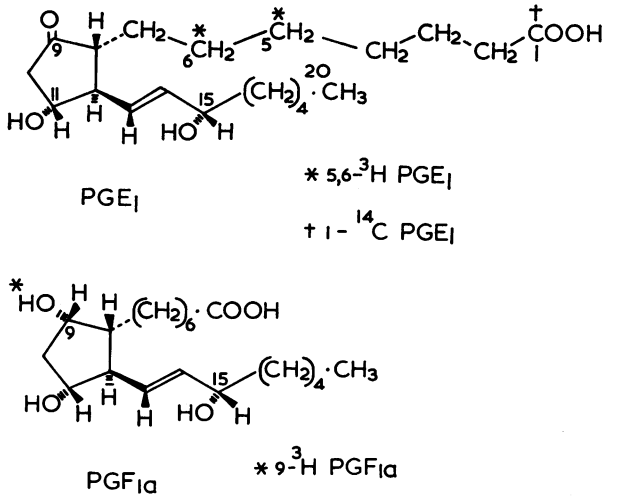

FIG. 1. Prostaglandin E_1 and prostaglandin $F_{1\alpha}$. Position of tritium and carbon labelling is indicated.

Prostaglandin injection

Single transit experiments, lasting 15 min, involved injection of 1–42 μg 1-^{14}C PGE_1 (3·4 μCi/mg) or 5,6-^{3}H PGE_1 (1·36 mCi/mg) (Fig. 1) dissolved in 20 μl saline, via the portal vein or the hepatic artery. The blood from the hepatic vein was not permitted to re-enter the blood reservoir. Samples of hepatic vein blood (15 × 1 min, approximately 5 ml by volume) were collected into 95 ml of ethanol (95% v/v). The radioactive content of bile, blood and liver extracts was determined in a Packard 3310 liquid scintillation spectrometer.

In recirculation experiments, 1-^{14}C PGE_1 (44 μg), 5,6-^{3}H PGE_1 (23·5 and 35·0 μg) or 9-^{3}H $PGF_{1\alpha}$ (7·0 μCi/mg, 20·7 and 53·0 μg) (Fig. 1) was injected via the portal vein and allowed to recirculate for 2·5 h through the liver via a gas exchanger. Hepatic vein blood samples (2·5 ml) were collected at various intervals throughout the experiment into 47·5 ml aqueous ethanol (95% v/v); bile samples were removed at similar times. In some experiments a single liver lobe was removed at 15 min and frozen.

Preparation of samples

From each liver sample, two 50 mg samples were removed and each digested with 1 ml NaOH (5 N); an aliquot of each sample (0·1 ml) was neutralized with BioSolv (BBS2, Beckman), dissolved in 10 ml of toluene scintillator fluid, for determination of the total liver radioactivity. The remaining liver was homogenized in 0·9% saline (5 ml/g tissue at 4° C), and ethanol was added to 95% (v/v). After standing for at least 3 h at 4° C, and centrifuging (3,000 *g* for 10 min) the precipitate was washed with 95% ethanol; the supernatants were combined and evaporated to near dryness at 40° C under reduced pressure; the residue from each sample was dissolved in ethanol-water (2 : 1 v/v) and extracted three times with equal volumes of petroleum ether (40°–60° C). Ethanol was then removed from the aqueous phase in a stream of nitrogen before acidifying to pH 3 with HCl (1 N). The acid aqueous phase was extracted three times with an equal volume of ethyl acetate. The radioactive content of the ethyl acetate and petroleum ether extracts and the acid aqueous residue was determined; counting efficiency was estimated by the channels ratio method. The radioactivity associated with the ethanol denatured protein was determined as described for each of the 50 mg samples of the total liver. Ethanol extracts of blood and bile were similarly extracted, and the distribution of radioactivity was determined.

Thin-layer chromatography (TLC)

Prostaglandins and their methyl esters were separated on thin layers of silica gel H in the AI, AII, AIV and MI systems described by Gréen & Samuelsson (1964). Phospholipids were separated on thin layers of silica gel H incorporating 1 mM Na_2CO_3 (Skipski, Peterson & Barclay, 1964), and fatty acids and their methyl derivatives as described by Freeman & West (1966) and Paulouse (1966). After development of chromatograms at 21° C, bands corresponding to the R_f values of simultaneously developed prostaglandins (PGE_1, $PGF_{1\alpha}$, PGA_1), free fatty acids (arachidonic, linoleic, linolenic), or phospholipid standards (lecithin, lysolecithin, phosphatidyl inositol and phosphatidyl serine), which were visualized by spraying with ethanolic phosphomolybdic acid (10%) and heating to 100° C for 4 min, were

eluted with 95% acetone (3 × 5 ml) for subsequent bioassay, biochemical analysis or further TLC. For location of radioactivity, small areas of silica were scraped directly into vials containing scintillation fluid and counted.

Methyl esters

Methyl esters of fatty acids were prepared by boiling with boron trifluoride (Supelco) in methanol in tightly capped tubes on a water bath at 100° C for 2 min. After cooling, the reaction mixture was diluted with water (2 ml), and extracted three times with petroleum ether (v/v). The combined petroleum ether supernatants were dried in a stream of nitrogen before being reconstituted in chloroform-methanol (2 : 1 v/v) for chromatography.

Phospholipase A

Phospholipase A (15 μg, Boehringer) was dissolved in 100 μl of an aqueous solution (composition (M): NaCl, $2{\cdot}2 \times 10^{-1}$; $CaCl_2$, 2×10^{-2}; EDTA, 10^{-3}); the pH was adjusted to 7·4 with 0·1 N KOH. Phospholipid standards and petroleum ether extracts of liver were dissolved in diethyl ether (4·0 ml) and 40 μl of the enzyme solution was added. The mixture was shaken intermittently for 2 h, then dried in a stream of nitrogen. The residue was reconstituted in chloroform-methanol (2 : 1 v/v) for TLC. Incubation with lecithin indicated that the reaction was 95% complete in 2 h.

Gas-liquid chromatography (GLC)

Analysis of the trimethyl silyl ether derivatives of prostaglandin standards (Ramwell & Daniels, 1968) and radioactive extracts of bile was performed as described elsewhere (Shaw & Ramwell, 1969). For separation of the methyl esters of fatty acids, a column consisting of 10% EGS (Supelco) on Chromosorb W (80–100 mesh) was prepared. The fatty acid constituents of liver extracts were identified by comparison with relative retention times of known fatty acid standards, and peaks containing radioactivity were identified following attachment of an effluent stream splitter.

Bioassay preparation

Female Sprague-Dawley rats were ovariectomized at least 12 days before use. A single uterine horn was suspended in 0·5 ml of de Jalon's solution (de Jalon, Bayo & de Jalon, 1945) in a Lucite bath, and contractions were recorded isotonically with a transducer (Phipps & Bird) on an ink recorder. This preparation is sensitive to 1–5 ng PGE_1 and 1–10 ng $PGF_{1\alpha}$. Eluates of silica gel were dried in a stream of nitrogen, and reconstituted in de Jalon's solution for bioassay.

Results

The rate of uptake of differently labelled prostaglandins from circulating blood, and the rate of appearance of radioactive products in bile, blood and liver was determined by injecting authentic prostaglandins into the portal vein or hepatic artery and either permitting the blood to pass once through the liver (single transit studies) or allowing recirculation via a gas exchanger.

Single transit studies

In two experiments, following injection of 1-^{14}C PGE_1 (1 or 42 μg) into the portal vein, only 11% and 5·4% of the injected radioactivity could be detected in the venous effluent collected over the subsequent 15 min: thus the liver removed 89–95% of circulating PGE_1 during a single passage. Extraction of the 15 × 1 min samples of hepatic venous blood indicated that the polarity of the radioactive material appearing within the sequential samples progressively increased (Fig. 2a). and thus could not be identified with unchanged 1-^{14}C PGE_1.

Radioactivity equivalent to only 0·8% and 0·3% of the injected dose was detected in the bile, and the rate of biliary excretion was found maximal at 4 min (Fig. 2a). In two additional experiments, following injection of 1-^{14}C PGE_1 (33 μg) into the hepatic artery, slightly more radioactive material appeared in the bile (1·3 and 1·6%); the peak output occurred at 6 min when using the liver from a fed rat, and 11 min with the liver from a fasted animal. Thus the time of maximum output of radioactive material into the bile seemed to depend on the route of administration of 1-^{14}C PGE_1 and the dietary condition of the rat. These results indicated that biliary excretion was unlikely to be a principal route for elimination of unchanged PGE_1 in the rat.

In these experiments, however, total recovery of radioactivity was not obtained, for only 28% and 33·5% of the injected dose was detected in the liver on termination of the experiment; 91–93% of this radioactive material was extracted by petroleum ether, and was thus less polar than PGE_1 (Table 1).

When compared with the 1-^{14}C PGE_1 experiments, administration of 5,6-^{3}H PGE_1 resulted in a very different distribution of radioactive material between blood, bile and liver. There was a marked increase in the percentage of the injected radioactivity which appeared in the venous effluent (43%) and >70% of the radioactivity in each of the hepatic venous samples had the solvent partition properties of the prostaglandins. Furthermore, while a similar proportion of the injected dose (37·3%) was detected in the liver in these tritium experiments, 56% of this material partitioned into ethyl acetate and only 7·5% was soluble in petroleum ether (Table 1). The contrast between these studies with 1-^{14}C PGE_1 and 5,6-^{3}H PGE_1 indicated that the major fraction of the tritiated material in blood and liver, though it had the solvent partition properties of PGE_1, was unlikely to be associated with unchanged PGE_1 but possibly with structures less than C20 whose presence in blood resulted from a rapid uptake of PGE_1 by the liver, modification, and release into the venous effluent.

Further comparisons between the two series of isotopic experiments indicated that there was a 30 fold increase in the percentage of tritium excreted into the

TABLE 1. *Distribution of radioactivity in solvent extracts of liver following single transit of PGE_1.*

	Total radioactivity (% injected dose)	Activity recovered (%)			
		Ethyl acetate	Petroleum ether	Aqueous residue	P.p.t.
1-^{14}C PGE_1 (1 μg)	28	3·5	90·9	5·6	—
1-^{14}C PGE_1 (42 μg)	33·5	7·1	92·9	—	—
5,6-^{3}H PGE_1 (25 μg)	37·3	55·6	7·6	25·7	11·1

Liver was removed 15 min after injection of PGE_1 through the portal vein.

bile ; the maximum excretion was evident 6–7 min after injection, suggesting that biliary excretion may be a major route for elimination of compounds smaller than the C20 prostaglandin parent structure.

Recirculation studies

1-^{14}C PGE$_1$

The low recovery of radioactivity in the 1-^{14}C PGE_1 studies prompted us to investigate whether decarboxylation of PGE_1 took place in the liver. 1-^{14}C PGE_1 was therefore allowed to recirculate through the liver, in a closed system with a CO_2 trap incorporated. Evidence for decarboxylation was obtained, for in two experiments 19% and 10·6% of the injected radioactivity was detected as exchanged $^{14}CO_2$, with peak output at 10–15 min (Fig. 2b). As in the single transit experiments rapid biliary secretion of radioactive material (5·96% and 0·93%, maximum at 5–10 min) was detected (Fig. 2b).

In the second of these experiments, a liver lobe (4·98 g) removed after 15 min recirculation, was found to contain 34·2% of the injected radioactivity (Table 2), indicating that within this time the liver (total weight 12·4 g) has sequestered some 85% of the injected prostaglandin, a figure which correlated well with the values obtained in the single transit studies. In addition, the major fraction (72–85%) of

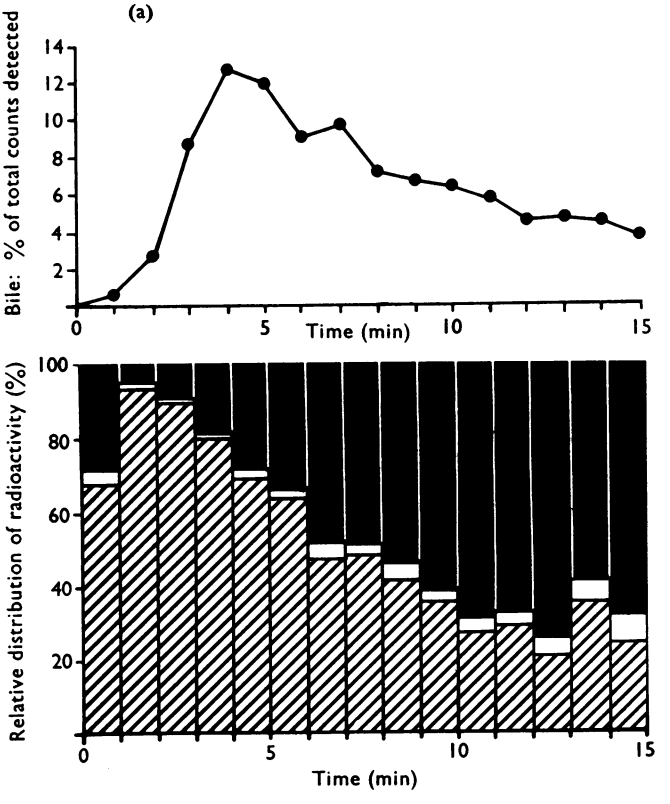

FIG. 2(a). *See legend on page 592.*

the radioactivity within the liver lobe, and that within the remaining liver on termination of the experiment, was again found to partition into petroleum ether (Table 2). An attempt was made to identify the nature of this nonpolar material derived from 1-^{14}C PGE_1.

Chromatography

On TLC in the AI solvent system, an aliquot of the radioactive material within petroleum ether extracts of the liver was found to separate into two main zones which were located at the origin (19–23%), and R_f (0·8–0·9) (51–71%); only 0·5–7% of the radioactivity co-chromatographed with authentic PGE_1 (R_f 0·68). This suggested that the ^{14}C label from PGE_1 was now associated with phospholipids and fatty acids, since all phospholipids simultaneously developed remained on or near the origin, while unsubstituted long chain fatty acids migrated near the solvent front.

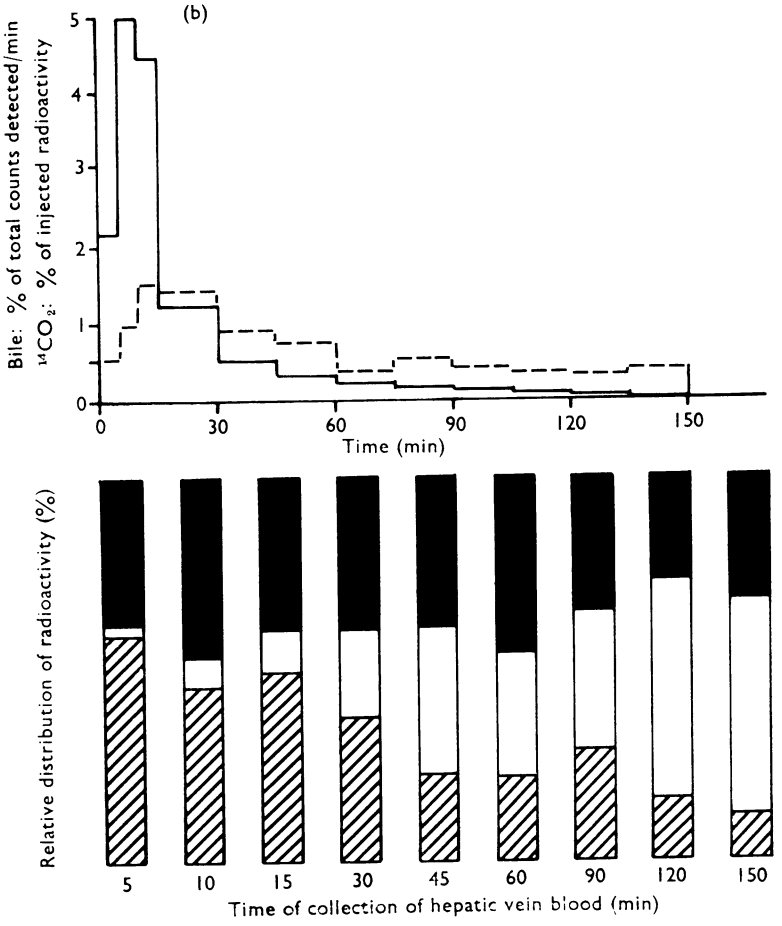

FIG. 2. Distribution of ^{14}C in bile and venous effluent of the isolated liver preparation. (a) 0–15 min following injection and a single transit; bile, total radioactivity 0·3% of injected dose; blood, total radioactivity 5·4% of injected dose. (b) 0–150 min during recirculation of 1-^{14}C PGE_1 (42 and 44 μg respectively); bile, total radioactivity 5·96% of injected dose; blood, total radioactivity 12·7% of injected dose. In the recirculation experiments evolution of $^{14}CO_2$ was also monitored (dashed line, total radioactivity 10·6% of injected dose). Filled columns, aqueous residue; open columns, petroleum ether; hatched columns, ethyl acetate.

Aliquots of the less polar material (R_f 0·83) were developed on TLC in the AII and AIV solvent systems. Of the total radioactive material recovered (75%), 90–92% co-chromatographed with simultaneously developed fatty acids. Identification of this radioactive material with free fatty acids was supported by further TLC in the Freeman & West (1966) system and also in the Paulouse (1966) system, where the radioactive material, after methylation, was found to co-chromatograph with the methyl esters of long chain unsaturated fatty acids. GLC of these methyl esters revealed nine fatty acid peaks, but 80% of the material was localized within two peaks with retention times equal to those of C18 : 1 and C22 : 1.

These results suggest that fragments resulting from decarboxylation of PGE_1 had been utilized during fatty acid biosynthesis within the liver. Detection of labelled fatty acids within but 15 min of 1-^{14}C PGE_1 injection provide a further index as to the speed of PGE_1 uptake and metabolism in the rat liver.

The more polar radioactive material, which remained on the origin of the chromatogram in the AI solvent system, did not migrate in either the AII or AIV systems. In the solvent system of Skipski *et al.* (1964) the R_f of this material (0·2) was found similar to that of lecithin and lysolecithin (0·33, 0·17) but more polar than phosphatidyl serine (0·75), phosphatidyl inositol (0·71) and PGE_1 (1·0). After refluxing an aliquot with borontrifluoride in methanol and redeveloping on TLC, 75% of the radioactivity was separated from the phospholipids and migrated with the methyl esters of fatty acids. After incubation of a further aliquot with phospholipase A (efficiency 50%, using lecithin as substrate) 49% of the radioactive material migrated with concomitantly developed fatty acid standards on TLC. These results all suggested that the radioactive material associated with phospholipids in the liver may derive from initial decarboxylation of 1-^{14}C PGE_1 and incorporation of the labelled fragments into fatty acids, and they in turn into phospholipids.

The speed of this process is evident when we consider that only 15 min after injection of 1-^{14}C PGE_1 the liver had retained some 85% of the injected material, and 80% of this material was soluble in petroleum ether.

5,6-^{3}H PGE_1 and 9-^{3}H $PGF_{1\alpha}$

As a corollary to the 1-^{14}C PGE_1 studies, the distribution of radioactivity within bile, blood and liver during recirculation of prostaglandins labelled with tritium on the side chain (5,6-^{3}H PGE_1 ; 23·5 and 35 μg) or cyclopentane ring (9-^{3}H $PGF_{1\alpha}$; 53 and 20·7 μg) was studied.

When compared with results obtained with carboxyl labelled PGE_1, the maximum secretion of tritiated material into the bile was delayed (peak at 10–15 min), and the total biliary excretion over 2·5 h was greatly increased (18·8–30% and 25·8–27·5% in the PGE and PGF studies respectively) (Fig. 3). This suggested that the tritiated material in bile was likely to differ from PGE_1 by at least one methylene group, but that the carboxyl side chain had not shortened sufficiently to liberate the tritium located originally in the 5,6 position. A further difference between the biliary secretion product and PGE_1 was revealed on extraction, when only 59% of the radioactivity partitioned into ethyl acetate and 41% remained in the acid aqueous residue. The radioactive material within such an ethyl acetate extract co-chromatographed with authentic PGE_1 on TLC in both the AI and AII solvent

systems ; the increment in R_f after treatment with alkali indicated formation of a PGB compound, which inferred that the cyclopentane ring of the prostaglandin molecule had remained intact in the biliary excretion product. GLC analysis of this PGB-like compound indicated a single peak, with a retention time corresponding to that of a monounsaturated PGB compound. Since the GLC columns used do not resolve prostaglandins of differing chain length, no evidence to support the suggestion of decarboxylation was obtained. Bioassay of the radioactive material eluted from the PGE zone of the AII chromatogram, on the isolated rat uterus, however, revealed that the material had only 7·5% of the expected potency when compared with authentic PGE_1 ; this suggested a modification of the parent structure associated with loss of pharmacological activity, not inconsistent with the possibility of decarboxylation.

The radioactive material within the bile which remained in the aqueous residue after extraction with petroleum ether and ethyl acetate remained on the origin following chromatography in the AI solvent system. Treatment with NaOH (1 N, 100° C, 20 min) did not modify the polarity, neither did treatment with phospho-

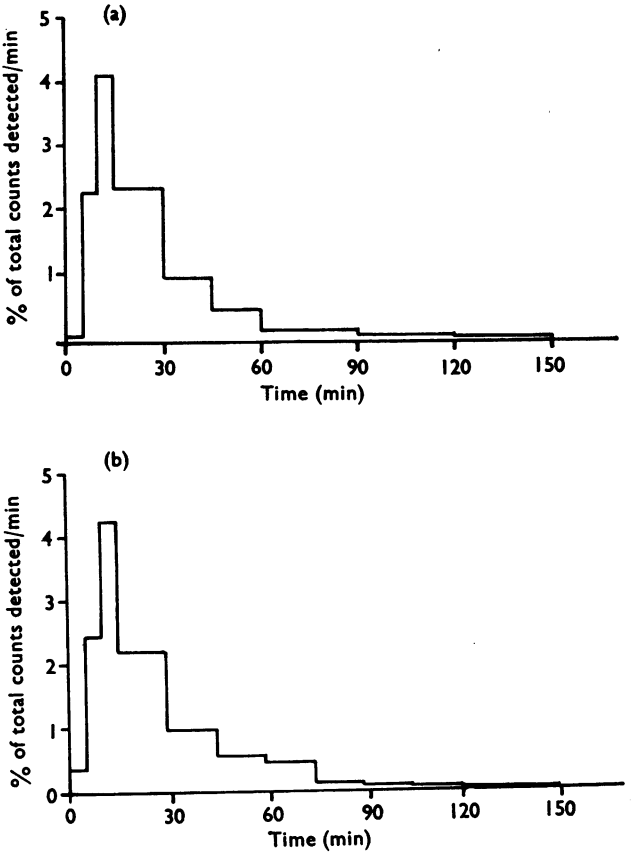

FIG. 3. Radioactivity detected within bile during recirculation of (a) 5,6-^{3}H PGE_1 (23·5 μg) (total radioactivity 18·8% injected dose) and (b) 9-^{3}H $PGF_{1\alpha}$ (53 μg) (total radioactivity 25·8% injected dose), 0–150 min following portal administration. In these experiments, 12·0 and 34·4% respectively of injected radioactivity was detected in the venous effluent and 85–98% of this radioactivity was soluble in ethyl acetate.

lipase A, which suggests that the radioactive product had neither the TLC nor hydrolysis properties of the phospholipids.

In further contrast to the 1-^{14}C experiments it was found that 29–60% of the radioactivity within the liver was soluble in ethyl acetate, and only 1–5% was soluble in petroleum ether (Table 2). In addition, it was found that 85–98% of the radioactive material detected in samples of blood removed during 2·5 h recirculation of 5,6-^{3}H PGE_1 or 9-^{3}H $PGF_{1\alpha}$ (12·0–16·7% and 34·4–49·6% of injected dose, respectively) was soluble in ethyl acetate. On TLC little radioactivity was identifiable with the parent compounds but was associated with less polar material (Table 3), which could not be identified with PGA or PGB material on ultraviolet analysis. The properties of this radioactive material compare well with those of the dihydro and 15 keto dihydro derivatives of the parent compounds. Such derivatives of PGE_1 have previously been isolated from rat plasma after administration of 5,6-^{3}H PGE_1 to the intact animal.

Discussion

PGE_1 is a potent vasodepressor substance, smooth muscle stimulant and nasal vasoconstrictor ; it is also an inhibitor of gastric acid secretion, platelet aggregation

TABLE 2. *Distribution of radioactivity in solvent extracts of liver following recirculation of labelled PGE_1 and $PGE_{1\alpha}$*

	Time (min)	Total radioactivity in liver (% injected dose)	Relative distribution (%)			
			Ethyl acetate	Petroleum ether	Aqueous residue	P.p.t.
1-^{14}C PGE_1 (44 μg)	15	34·2	11·0	74·0	10·5	4·5
	150	20·9	23·5	72·0	2·7	1·5
1-^{14}C PGE_1 (44 μg)	150	56·4	4·8	85·7	1·7	7·8
1-^{14}C PGE_1 (44 μg)*			88·7	7·3	2·3	1·7
5,6-^{3}H PGE_1 (35 μg)	150	4·7	37·5	5·4	4·7	52·4
5,6-^{3}H PGE_1 (23·5 μg)	150	2·7	54·6	5·2	17·4	22·7
9-^{3}H $PGF_{1\alpha}$ (20·7 μg)	15	8·5	59·7	—	29·5	10·8
	150	1·5	49·6	0·8	16·2	33·3
9-^{3}H $PGF_{1\alpha}$ (53 μg)	150	1·0	29·0	1·3	19·7	49·9

* Control experiment: 1-^{14}C PGE_1 was added to a liver homogenate at 4° C, and immediately extracted.
The liver was removed 150 min after injection of the prostaglandin through the portal vein. Where indicated a liver lobe was removed at 15 min and similarly extracted.

TABLE 3. *Percentage distribution of the total radioactivity eluted from a chromatogram after development of ethyl acetate extracts of blood in the AII solvent system*

	Rf values			
	Origin	$PGF_{1\alpha}$ (0·21)	PGE_1 (0·44)	Less polar (0·75)
Expt. 1	6·0	1	20	53
2	17	3	10·5	51·5
3	15	10	15	51
4	5	4·5	34·5	53·1
5,6-^{3}H PGE_1	1	3·6	60	10·3
9-^{3}H $PGF_{1\alpha}$	0	90	2	1 5

The blood samples were removed 2·5 h after recirculation of 5,6-^{3}H PGE_1 (experiments 1 and 2) and 9-^{3}H $PGF_{1\alpha}$ (experiments 3 and 4) and extracted as described in **Methods**. The results obtained following similar extraction and TLC of blood samples after incubation with 5,6-^{3}H PGE_1 or 9-^{3}H $PGF_{1\alpha}$ (50 ng/ml) are also indicated.

and lipolysis (Bergström *et al.*, 1968). Reduced adhesiveness of circulating platelets has been detected in both humans and dogs for hours following intravenous administration of PGE_1 (Elkeles, Hampton, Harrison & Mitchell, 1969; Hissen, Fleming, Bierwagen & Pindell, 1969), however, the general systemic effects following PGE_1 injection are usually of short duration. Although infusion of PGE_1 prolongs its action, these effects are quickly curtailed on cessation of the infusion. Thus the prostaglandins may bind to some blood constituent and thereby become inactive, or the prostaglandins may themselves undergo rapid conversion to less active compounds.

We have demonstrated that the isolated blood perfused rat liver, which stimulates many of the conditions which prevail *in vivo*, will effectively and rapidly remove circulating prostaglandins (85–90%) on a single passage. In the absence of sophisticated GLC-mass spectrometric analytical techniques, which provide the only definitive identification of prostaglandin metabolites (see Granström & Samuelsson, 1969), we have been able to make certain inferences regarding the nature of the biliary and venous metabolites by comparison of the distribution of tritium and carbon labelled prostaglandins.

The results obtained indicated that C20 prostaglandins are unlikely to be excreted as such into the bile, owing to very rapid and complete decarboxylation within the rat liver. The labelled fragments resulting from decarboxylation were liberated as $^{14}CO_2$ and also utilized during fatty acid and phospholipid biosynthesis. The increased biliary secretion of radioactive material obtained when using tritium labelled PGE_1 indicated that the biliary excretion products are likely to be of shorter chain length than the parent compound. Partition studies indicated that the biliary metabolites included far more polar material than the parent compounds; a similar unidentified polar metabolite of PGE_1 has previously been isolated from urine (Granström, 1967).

The percentage of the injected tritium which was excreted into the bile over 2·5 h (18·8–30%) agreed well with the figure of 16% over a period of 2 h, which one can derive from the results of Samuelsson (1964), who injected 5,6-^{3}H PGE_1 to intact rats and followed the biliary and urinary excretion of tritium. Thus combined decarboxylation and biliary excretion would seem an effective method for inactivation and removal of circulating prostaglandins.

Little of the radioactive material liberated from the liver into the venous blood over a period of 2·5 h (up to 40% of the injected tritium) was identifiable with unchanged PGE_1 or $PGF_{1\alpha}$. The similar decrease in polarity, in the PGE and PGF studies, of the radioactive material within blood, when compared with the parent compounds, indicated that the decrease in polarity could not be ascribed to formation of PGA or PGB compounds, for the PGF compounds are markedly resistant to dehydration of the five membered ring. In addition, ultraviolet analysis of the less polar material derived from PGE_1 did not reveal any chromophore at 217 nm (PGA) or at 278 nm (PGB) after base treatment. Furthermore, these less polar, blood borne metabolites had the correct partition and TLC properties to be associated with dihydro-PGE_1 and 15 keto-dihydro-PGE_1 derivatives of the parent compound, which have already been reported present in plasma of rats after injection of 5,6-^{3}H PGE_1; these metabolites are believed to be further modified by the kidney before urinary excretion (Samuelsson, 1964). Our current knowledge concerning structure activity relationships indicates that any modification of the parent prosta-

glandin structure will reduce biological activity in most assay systems. The only exception to this is the finding that homo-PGE_1 is more potent in inhibiting ADP-induced aggregation of platelets than PGE_1 (Kloeze, 1969) and also that dihydro-PGE_1 is more active on guinea-pig blood pressure than PGE_1 (Änggård, 1966).

The possibility of binding of circulating prostaglandins to a specific plasma protein, to afford protection from inactivation, has been indicated in a preliminary report (Holmes, Horton & Stewart, 1968). However, our studies concerning the ubiquitous biosynthesis and release of prostaglandins from tissues, on nervous and hormonal stimulation, and the evidence now accumulating which indicates that when reapplied directly to tissues *in vitro*, prostaglandins can effectively modify a wide range of hormonal responses (Ramwell & Shaw, 1970), has led us to consider the possibility that any physiological action of the prostaglandins may be localized within their tissue of origin. This conclusion is perhaps strengthened by the knowledge that many of the pharmacological effects of the prostaglandins manifest *in vitro* are not evident on systemic injection, possibly, as reported here, owing to rapid removal from the circulation and modification of structure.

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