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ROLE OF PROSTAGLANDIN E IN CARRAGEENIN-INDUCED INFLAMMATION IN RATS

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Abstract—The effect of prostaglandin E on the vascular permeability and the prostaglandin synthesizing and metabolizing activities in rat carrageenin granuloma were studied. Radioiodinated human serum albumin was used as an indicator for the measurement of vascular permeability. The dose necessary to induce significant increase of vascular permeability in the inflammatory tissue was found at least in the order of 0.5 μg for PGE_1 and 5 μg for PGE_2. The prostaglandin synthesizing system was characterised by a radiometric assay. Two main products (PI and PII) were formed from [1-^{14}C]arachidonic acid, while PGE_2 and $PGF_{2\alpha}$ were hardly formed. The prostaglandin metabolizing activity was measured by a reversed-phase partition chromatography technique. Both of the granuloma exudate and granuloma pouch wall were found to have little enzymatic activity for metabolizing PGE_2. The results strongly suggest that contribution of endogenous PGE as a mediator of vascular permeability response in the granulomatous inflammation is minor at best.

Prostaglandins (PG) are hormone-like lipids that have been proposed to be implicated in various physiological and pathological processes involving inflammation. The role of PGE in the development of inflammatory reactions is considered to be that it increases local vascular permeability and it is chemotactic for polymorphonuclear leukocytes and it causes pain [1]. Among these inflammatory reactions evoked by PGE, reaction of increasing local vascular permeability has been extensively investigated and regarded as the main manifestation of the pro-inflammatory property of PGE. PGE induces an increase of local cutaneous vascular permeability in the skin of guinea pig [2], bovine [3], rat [4, 5] and human [6]. When infused intra-arterially, PGE also causes oedema and vasodilation in the human forearm [7].

The existence of PGE in the inflammatory locus is also evident—the perfusate of human contact eczema contained 0.4–1.6 ng/ml of PGE_1 [8]; the exudate of carrageenin-induced hind foot oedema in rats contained 3–6 ng/ml of PGE_2 [9]; the fluid of human blister contained about 1.9 ng/ml of PGE_2 [10]. However, it still remains unknown whether the concentration of endogenous PGE as determined in the inflammatory locus is enough to evoke significant increase of vascular permeability or not, because the amount of PGE recoverable from the locus of inflammatory lesions is generally very low, though it is broadly suspected to be a chemical mediator of inflammation.

The present experiments were undertaken in order to investigate whether PGE level as reported in a previous paper for the inflammation locus of carrageenin granuloma pouch [11] is sufficiently high or not, to evoke vascular permeability response of the granuloma pouch tissue itself. Biosynthetic activity for PGE of the granuloma tissue and PGE metabolizing activity of the granuloma tissue were also examined. Overall results strongly suggest that contribution of endogenous PGE as a mediator of vascular permeability response in the granulomatous inflammation is minor at best.

MATERIALS AND METHODS

Carrageenin (Seakem No. 202) was supplied from Marine Colloid Inc., Springfield, N.J., U.S.A. PGE_1 was a generous gift of Ono Pharmaceutical Co., Ltd., Osaka, Japan. PGE_2 and $PGF_{2\alpha}$ were generous gifts of Japan Upjohn Limited, Tokyo, Japan. Thin-layer chromatography (t.l.c.) plates of Silica gel 60 F_{254}, 0.25-mm in thickness, were supplied by E. Merck, Darmstadt, Germany. ^{131}I-Human serum albumin (^{131}I-HSA) was purchased from Dainabot Radioisotope Institute, Tokyo, Japan. ^{125}I-Human serum albumin (^{125}I-HSA) (0.0025 mCi/mg), [1-^{14}C]arachidonic acid (58 mCi/m-mole), [5, 6, 8, 11, 12, 14, 15-(*n*)-^{3}H]PGE_2 (140 Ci/m-mole) and [5, 6, 8, 11, 12, 14, 15(*n*)-^{3}H]$PGF_{2\alpha}$ (140 Ci/m-mole) were purchased from The Radiochemical Centre, Amersham, England.

Granulomatous inflammation. Granuloma pouch was induced by the modification of Selye's method using carageenin as a phlogistic agent as described in a previous paper [12]. Rats bearing 8-day-old granuloma pouch were used throughout this series of experiments.

Effect of PG on the enhancement of vascular permeability. ^{125}I-HSA and ^{131}I-HSA were used as the tracer for vascular permeability measurement, since the radioactive tracer technique have been established by Movat and DiLorenzo [13] as a rapid, sensitive and reliable method for measuring the vascular permeability response in inflammation. Both of the radionated human serum albumins were purified by subjecting to Sephadex G-100 column chromatography to

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remove radioactive low mol. wt. impurities before use. The method of the exudative reaction induced by PGE was according to methods described in a previous paper [14] with slight modification.

Preparation of enzyme source and assay for PG biosynthetic activity. All procedures were performed at 4°. Rat granulomas or bovine seminal vesicles (from a slaughter house) were homogenized in Ca^{2+}- and Mg^{2+}-free phosphate buffered saline [15] (tissue to buffer ratio, 1:2.5, w/v). The homogenate was centrifuged at 600 *g* for 5 min, and the resulting supernatant was used for the enzyme source. The microsomal fraction of bovine seminal vesicle was prepared according to the method of Parkes and Eling [16].

Each incubation tube contained 4 ml of the 600 *g* supernatant, 0.2 μCi of [^{14}C]arachidonic acid and 5 μg of unlabeled arachidonic acid. In case of microsomal fraction of bovine seminal vesicle, it contained 0.2 μCi of [^{14}C]arachidonic acid, 5 μg of unlabeled arachidonic acid, 2 ml of boiled 105,000 *g* supernatant, 1 ml of 0.25 M phosphate buffer (pH 8.0) and 4 mg of microsomal protein resuspended in 1 ml of 0.25 M sucrose [16]. Incubation was carried out in air at 37° for 30 min with shaking. Reaction was terminated by chilling them followed by quickly adding an appropriate amount of 1 N HCl to bring the pH of the reaction to 3.0. Ten μg of PGE_2 and $PGF_{2\alpha}$ were added to the mixture and the mixture was then extracted twice with 8 vol of ethyl acetate. The resulting organic phase was evaporated to dryness under reduced pressure. Residues were dissolved in a small amount of ethanol and applied to t.l.c. plates. Ten μg each of PGE_2 and $PGF_{2\alpha}$ were also applied to the plates as the references. The plates were developed in the AI solvent system (benzene-dioxane-acetic acid 20:20:1, v/v) [17], and the radioactive products were detected by a Dünnchicht scanner. Zones corresponding the radioactive products were extracted with chloroform–methanol (1:1, v/v) for further analysis. In some cases, the developed plates were autoradiographed using Fuji X-ray film No. Kx medical. Reference standards of PGE_2 and $PGF_{2\alpha}$ were visualized in a tank with iodine vapour, if necessary.

Identification of the radioactive products. An aliquot of the extracted [^{14}C]radioactive products separated by t.l.c. in the AI solvent was mixed with an appropriate quantity of [^{3}H]PGE_2 or [^{3}H]$PGF_{2\alpha}$, respectively, and the mixture was successively chromatographed on thin layers with three kinds of solvent systems. The sequence was (A) the AI solvent system, (B) chloroform–ethyl acetate–methanol (1:1:1, by vol) and (C) the AII solvent system (ethyl acetate–acetic acid–methanol–2,2,4-trimethylpentane–water 110:30:35:10:100, by vol) [17]. Plates impregnated with $AgNO_3$ [18] were used in the AII solvent system. Zones of the radioactive substances on each thin-layer chromatogram were detected by a radiochromato scanner and eluted with a mixture of methanol–chloroform (1:1, v/v), except in case of silver impregnated plate, in which the method of Kunze and Bohn [19] was used for the extraction. The radioactivities due to ^{3}H and ^{14}C in each zone were separately counted in the operation mode of double isotope setting by a liquid scintillation counter.

PGE metabolizing activity in granuloma. Rat granuloma and lung of male guinea pig were homogenized in Bücher medium [20] (tissue to medium, 1:4, w/v). The homogenate was centrifuged for 15 min at 900 *g*, and the supernatant was recentrifuged for 60 min at 105,000 *g*. Four ml each of the 105,000 *g* supernatant fractions as well as of exudate of granuloma were mixed with 0.5 μCi of [^{3}H]PGE_2 and incubated at 37° for 30 min. The incubation was terminated by adding 9 vol of 96% ethanol. After centrifugation, the ethanol fraction was evaporated to a small volume, and the resultant solution was acidified to pH 3 with 1 N HCl and extracted with ether. To the ether extract 1 mg of PGE_2 was added as the reference, and the mixture was evaporated to dryness. Residue was analyzed by reversed-phase partition chromatography as described by Änggard and Samuelsson [21]. The system used was C-47. In the case of investigating PGE metabolizing activity in granuloma *in vivo*, 0.5 μCi of [^{3}H]PGE_2 was injected into the granulomatous pouch, and the exudate was collected 35 min later and analyzed.

RESULTS

Effect of PGE on the vascular permeability in rat carrageenin granuloma. The data are shown in Table

Table 1. Dose–response relationship for the effect of PGE on vascular permeability of carrageenin-induced granuloma in rats

Treatment	Dose (μg)	Number of rat	Exudate (ml)	Initial concentration of exogenous PGE in pouch fluid (ng/ml)	^{125}I* (%)	^{131}I* (%)	^{125}I/^{131}I	Increase (%)
Control		6	8.3 ± 1.9		0.344 ± 0.033	0.349 ± 0.037	1.030 ± 0.092	
PGE_1	0.05	6	11.6 ± 1.9	4.3	0.426 ± 0.030	0.497 ± 0.085	1.134 ± 0.136	10
PGE_1	0.5	6	13.3 ± 3.0	37.6	0.412 ± 0.061	0.654 ± 0.122	1.670 ± 0.269	62‡
PGE_1	5	6	11.8 ± 1.8	424.0	0.426 ± 0.059	1.091 ± 0.081	2.711 ± 0.314	163§
PGE_2	0.05	6	10.7 ± 2.5	4.7	0.399 ± 0.089	0.435 ± 0.089	1.165 ± 0.140	13
PGE_2	0.5	6	10.8 ± 1.8	46.3	0.499 ± 0.086	0.538 ± 0.085	1.496 ± 0.188	16
PGE_2	5	5	9.8 ± 2.0	510.0	0.430 ± 0.075	0.646 ± 0.103	1.542 ± 0.131	50§

* Total radioactivity in pouch fluid/Total injected radioactivity × 100.

† $P < 0.05$, ‡ $P < 0.001$, § $P < 0.02$. P value was given by *t*-test.

Rats bearing the granuloma pouch were given i.v. 1 μCi of ^{125}I-HSA in a vol of 0.3 ml of 0.9% NaCl. Thirty min later, 1 ml of exudate in the granuloma pouch was sampled; 1 min later, PG dissolved in 0.5 ml of Krebs saline serum substitute solution was injected into the pouch. Control group received the vehicle only. Five min after PG treatment, rats were given again i.v. 1 μCi of ^{131}I-HSA in a vol of 0.3 ml of 0.9% NaCl and after 30 min, 1 ml of exudate in the pouch was sampled again. The radioactivities of ^{125}I-HSA in the exudate of the first sampling and of ^{131}I-HSA in the exudate of the second sampling were counted in an Aloka JDC-715 auto well gamma system.

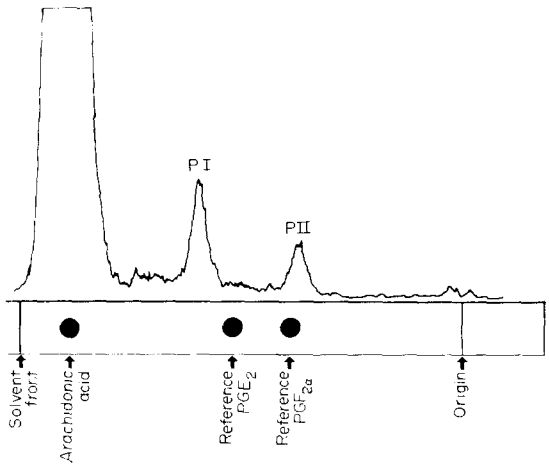

Fig. 1. Radiochromatogram showing the prostaglandin synthesizing activity by the 600 *g* supernatant fraction of rat granuloma. The extracted sample was spotted on Silica gel thin-layer plates on which standards were also spotted and developed in the AI solvent system. See Materials and Methods for details.

1. A marked enhancement of the permeability was provoked by injecting 0.5 and 5 μg of PGE_1 and 5 μg of PGE_2 into the pouch, resulting in 62, 163 and 50% increase in the exudation of ^{131}I-HSA respectively. While 0.05 μg of PGE_1 and 0.05 and 0.5 μg of PGE_2 showed no significant effect. From these results it was concluded that the dose necessary for inducing significant increase of vascular permeability in the inflammatory tissue of carageenin-induced granuloma was at least in the order of 0.5 μg for PGE_1 and 5 μg for PGE_2. Recovery of PGE in the pouch fluid throughout the experimental period (35 min) was about 80–85 per cent.

Transformation of arachidonic acid in inflammatory tissue. Incubation of [^{14}C]arachidonic acid in the presence of 600 *g* supernatant fractions of rat granuloma resulted in forming two main products (PI and PII) as shown in Fig. 1. PI was slightly less polar than PGE_2 on thin-layer chromatography. PII was more polar than PI, and its chromatographic mobility was very close to $PGF_{2\alpha}$, but it was not identified with $PGF_{2\alpha}$ (Table 2). This transformation was also confirmed by autoradiography. The yields of PI and PII were found to be about 2–3 per cent of the substrate respectively. In order to ensure PI and PII were not the further metabolites of PGE_2 and $PGF_{2\alpha}$ which might have been formed from arachidonic acid during the incubation, each tritiated standard of PGE_2 and $PGF_{2\alpha}$ was incubated with 600 *g* supernatant fractions of rat granuloma for 30 min at 37° and examined the metabolizing activity in the same manner as described above in the case of arachidonic acid. No conversion of the tritiated standards to PI and PII or any other products was observed. Moreover, it was also known that PI and PII were not the PG endoperoxides (PGG and PGH) [22] because of their stability, i.e. when PI or PII was incubated further with 600 *g* supernatant fractions of granuloma no detectable conversion of PI and PII to PGE_2, $PGF_{2\alpha}$ or any other products was observed. From these findings it is concluded that the main metabolites from arachidonic acid in the granuloma were PI and PII, while PGE_2 and $PGF_{2\alpha}$ were hardly formed.

To ensure that the formation of PI and PII from arachidonic acid in rat granuloma was not due to the arbitrary incubation and isolation procedures used, 600 *g* supernatant fractions of bovine seminal vesicle, which was obtained by the same procedures as in the case of granuloma, was substituted for 600 *g* supernatant fractions of granuloma. Moreover, further to ensure the biosynthetic activity of prostaglandins in bovine seminal vesicle, transformation arachidonic acid in microsomes of bovine seminal vesicle was also investigated. Both of the 600 *g* supernatant fraction and microsomal fraction converted arachidonic acid into two main products: the less polar one (MI) had chromatographic mobility similar to PGE_2 and the other more polar one (MII) was chromatographically very close to $PGF_{2\alpha}$ as shown in Fig. 2 and Fig. 3. This transformation was also confirmed by autoradiography. MI was identified with PGE_2 as indicated in Table 2, but MII was not identified with $PGF_{2\alpha}$ as indicated in Table 2. Judging from these results, it was confirmed that the formation of PI and PII from arachidonic acid in the granuloma shown in Fig. 1 were not due to the arbitrary incubation and isolation procedures used.

PGE metabolizing activity in rat carrageenin granuloma. After incubating granuloma exudate or

Table 2. Identification of the radioactive products from arachidonic acid

Enzyme source	^{3}H-authentic standard	^{14}C-^{3}H			
		Original mixture	(A)	(B)	(C)
PII from 600 *g* supernatant of granuloma	$PGF_{2\alpha}$	0.180 (0.38)	0.210 (0.38)	0.120 (0.42)	0.017*(0.26) 2.200 (0.59)
MII from 600 *g* supernatant of bovine seminal vesicle	$PGF_{2\alpha}$	0.643 (0.42)	0.386 (0.42)	0.223*(0.55) 14.319 (0.61)	
MII from microsome of bovine seminal vesicle	$PGF_{2\alpha}$	0.638 (0.42)	0.424 (0.42)	0.274*(0.58) 7.039 (0.63)	
m 600 *g* supernatant minal vesicle	PGE_2	0.691 (0.59)	0.654 (0.59)	0.654 (0.69)	0.620 (0.53)
me sicle	PGF_2	0.617 (0.58)	0.565 (0.59)	0.538 (0.68)	0.560 (0.52)

vI in the t.l.c. plate.
were described in the text. R_f values were indicated in the parentheses.

anol–ethyl acetate, 1:1:1.
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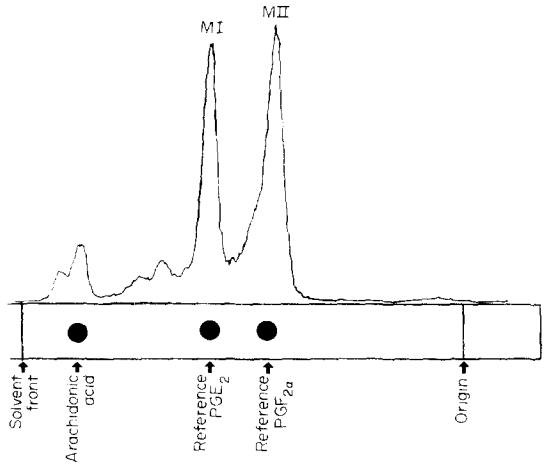

Fig. 2. Radiochromatogram showing the prostaglandin synthesizing activity by the 600 g supernatant fraction of bovine seminal vesicle. Spotting and development system were the same as those described in Fig. 1. See Materials and Methods for details.

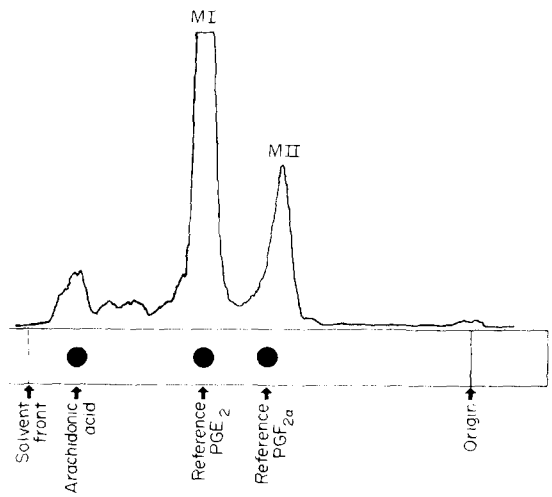

Fig. 3. Radiochromatogram showing the prostaglandin synthesizing activity of the microsomal fraction of bovine seminal vesicle. Spotting and development system were the same as those described in Fig. 1. See Materials and Methods for details.

105,000 g supernatant fraction of granuloma pouch wall with [^{3}H]PGE_2, the extracted radioactive substances were subjected to reversed phase partition chromatography. The radioactivity appeared in one peak upon the reversed phase partition chromatography as shown in Fig. 4 and Fig. 5. This peak could be identified as the original [^{3}H]PGE_2 itself because the spot derived from the unlabeled reference PGE_2 was consistent with this radioactive peak. The preliminary experiments revealed that when [^{3}H]PGE_2 was incubated with 105,000 g supernatant fractions of guinea pig lung in the same incubation conditions, two less polar metabolites designated Metabolites I and II were found as shown in Fig. 6. Metabolite I was described as 11α,15-dihydroxy-9,15-ketoprostanoic acid and Metabolite II was described as 11α-hydroxy-9,15-diketoprostanoic acid by Änggård and Samuelsson [21]. In the case of PGE metabolizing activity *in vivo*, also no enzymatic activity was found as shown in Fig. 7. Therefore it was indicated that both of the granuloma exudate and granuloma pouch wall had little enzymatic activity capable of metabolizing PGE_2.

DISCUSSION

In the literature E type of prostaglandins were reported to have a pharmacologic activity of increasing vascular permeability locally at the site of injection [2–7]. The authors also previously reported that 0.5, 5 and 50 μg of PGE_1 and 5 and 50 μg of PGE_2, when injected into the granuloma pouch of the carrageenin-induced inflammation, exerted significant elevation of the local vascular permeability in 7-day-old granuloma pouch [14]. In the present investigation, dose–response relationships for vascular permeability increasing action of PGE_1 and PGE_2 were examined more precisely using 8-day-old granuloma pouch (Table 1). Consequently, minimum effective concentration was confirmed to be around 37.6 ng/ml (0.5 μg

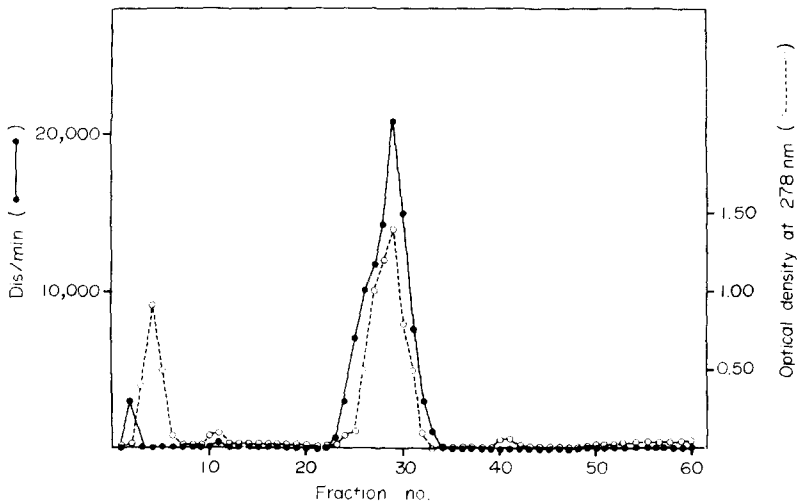

Fig. 4. Reversed phase partition chromatography of an extract from an incubation mixture of [^{3}H]PGE_2 with 4 ml of exudate of 8-day-old granuloma. Conditions: 4.5 g of hydrophobic Hyflo Super-Cel; solvent, System C-47. Reference of PGE_2 was determined by measuring the absorbance at 278 nm after treating aliquots of the fractions with 0.5 N methanolic NaOH.

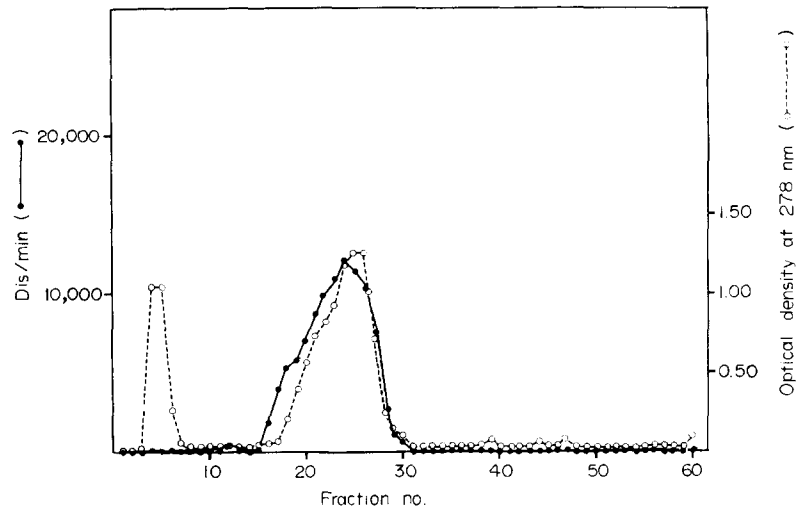

Fig. 5. Reversed phase partition chromatography of an extract from an incubation mixture of [^{3}H]PGE_2 with 4 ml of 105,000 g supernatant fraction of granuloma. Conditions were the same as those described in Fig. 4.

per 13.3 ml of the pouch fluid) for PGE_1 and around 510 ng/ml (5 μg per 9.8 ml of the pouch fluid) for PGE_2, respectively.

Besides this demonstration of the minimum effective concentration of exogenously given PGE, we also reported some data on the levels of PGE in the exudates of the carrageenin granuloma pouches at 1, 2, 3, 4, 5, 8 and 11 days after injection of carrageenin solution with the aid of radioimmunoassay technique [11]. The highest concentration demonstrated was 8.2 ng/ml, which was obtained at the third day after carrageenin injection. At the 8th day after carrageenin injection, only 2.3 ng/ml of PGE were found in the exudate. Thus, the concentration of endogenous PGE in the exudate was shown to be the order of 10^{-1}–10^{-2} of pharmacologically active local concentration of PGE. Therefore, significance of endogenous PGE as a condidate for the natural chemical mediator appears to be doubtful. Of course, further evidence for eliminating the possibility of rapid degradation of PGE in the exudate should be given to confirm this concept, because rapid degradation, if it occurs in the exudate, would make it unpractical to estimate tissue PGE level by its concentration in the exudate.

PGE has been known to be metabolized rapidly by prostaglandin 15-hydroxydehydrogenase [23]. If the exudate and the granuloma of the carrageenin-induced inflammation had prostaglandin 15-hydroxy dehydrogenase, PGE would be metabolized and consequently the contents of PGE in the exudate would be kept at a low level. However, both of the granuloma exudate and the granuloma pouch tissue of the 8-day-old granuloma were found to have little enzymatic activity capable of metabolizing PGE_2 (Fig. 4 and Fig. 5). These results were consistent with those reported by Ohuchi *et al.* [24] who showed that the exudates of the granuloma pouch at 24 hr and 72 hr after carrageenin injection in the Donryu strain rats had little activity of prostaglandin 15-hydroxy dehydrogenase. It was also found that the inflammatory tissue has little PG metabolizing activity *in vivo* (Fig.

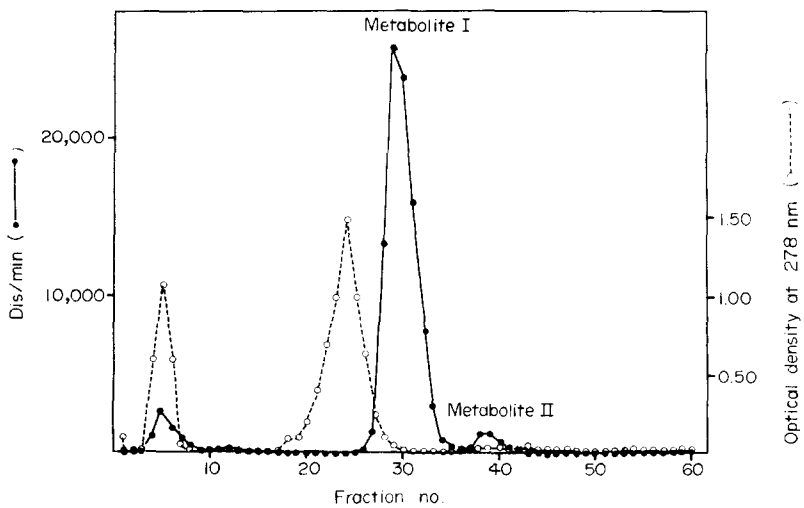

Fig. 6. Reversed phase partition chromatography of an extract from an incubation mixture of [^{3}H]PGE_2 with 4 ml of 105,000 g supernatant fraction of guinea-pig lung. Conditions were the same as those described in Fig. 4.

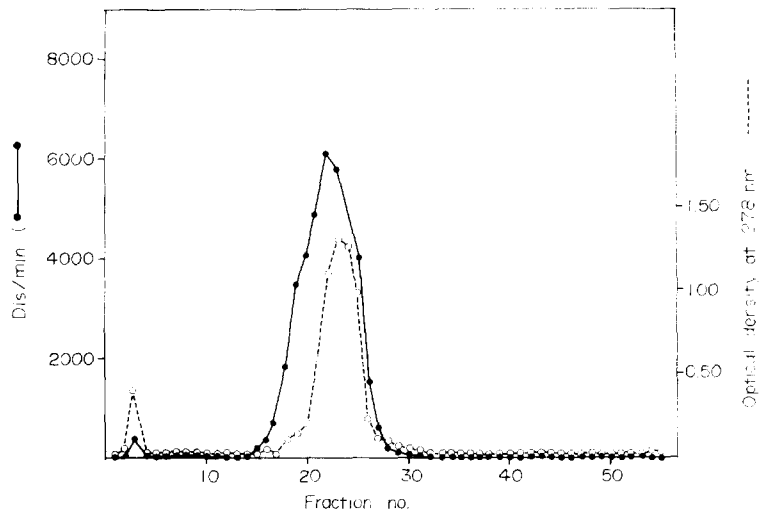

Fig. 7. Reversed phase partition chromatography of an extract from a granulomatous exudate which [^{3}H]PGE_2 was injected *in vivo*. Conditions were the same as those described in Fig. 4.

7). Therefore, the concentration of 2.3 ng/ml of PGE in the exudate at the 8th day after carrageenin injection seems reliable as a mirror of its tissue concentration and this is far from its effective concentration for increasing vascular permeability.

The prostaglandin synthesizing activity in the granuloma was also investigated in the present experiments. Metabolizing activity for arachidonic acid of the granuloma was found to belong to the group of moderate yields (about 5–10% conversion from the precursor) as classified by Christ and Van Dorp. [25]. In the granuloma PI and PII were shown to be the main metabolites from arachidonic acid, and PGE_2 and $PGF_{2\alpha}$ were not formed in a detectable amount (Fig. 1 and Table 2). It was also disclosed that PI and PII are neither the metabolites of PGE_2 and $PGF_{2\alpha}$ nor the intermediates from arachidonic acid prior to the production of PGE_2 and $PGF_{2\alpha}$. Identification of PI and PII, and investigation of their biological activities is under way in our laboratory.

Overall results obtained in the present experiments strongly suggest that contribution of endogenous PGE as a mediator of vascular permeability response in the carrageenin-induced inflammation in rats is minor at best. However, it still remains unsettled whether PGE has nothing to do with inflammation at all or not, since Williams and Morley [28] have reported that PGE plays as a potentiator of increased vascular permeability for other chemical mediators in inflammation.

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