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# DESENSITIZATION TO LOCALLY INJECTED $PGF_{2\alpha}$ AS REFLECTED IN THE VASCULAR PERMEABILITY AND COLLAGEN AND NONCOLLAGENOUS PROTEIN SYNTHESIS OF CARRAGEENIN-INDUCED GRANULATION TISSUE IN RATS*

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**Abstract**—Administration of $PGF_{2\alpha}$ at doses of 50 and 250 μg into pouch fluid (calculated concentration of $PGF_{2\alpha}$ was about 4 and 14 μg/ml of pouch fluid, respectively) of 7-day-old carrageenin granuloma in rats depressed vascular permeability, as measured by leakage of radioiodinated human serum albumin into the pouch fluid. However, 3 hr after $PGF_{2\alpha}$ treatment at a dose of 50 μg, the vascular permeability had recovered to control level; 3 hr after a dose of 250 μg $PGF_{2\alpha}$, the vascular permeability had not recovered completely. The uptake of [$^3$H]proline into collagen hydroxyproline and noncollagenous protein of carrageenin granuloma tissue was also examined. It was found that the first injection of $PGF_{2\alpha}$ at a dose of 50 or 250 μg depressed the uptake of [$^3$H]proline into both protein fractions, but 3 hr after the first injection of $PGF_{2\alpha}$, there was no significant difference in uptake between the control and the $PGF_{2\alpha}$-treated group. A study of the metabolism of $PGF_{2\alpha}$ in a homogenate of granulation tissue and in pouch fluid showed little activity in the former and very little activity in the latter. In the homogenate, about 70 per cent of the originally added [$^3$H]$PGF_{2\alpha}$ remained unmetabolized after 3 hr of incubation. In the inflammatory fluid, less than 20 per cent of the radioactivity present at 35 min after the injection had dissappeared from the pouch fluid at 3 hr after the [$^3$H]$PGF_{2\alpha}$ injection; all the remaining radioactivity was found to be unmetabolized [$^3$H]$PGF_{2\alpha}$. Consequently, it was suggested that the tissue became desensitized to the injected $PGF_{2\alpha}$. To confirm this suggestion, another 50 or 250 μg of $PGF_{2\alpha}$ was injected 3 hr after the first $PGF_{2\alpha}$ injection. However, a decrease in vascular permeability and in uptake of [$^3$H]proline into both protein fractions after the second injection of $PGF_{2\alpha}$ were not observed. It was concluded that, 3 hr after the first $PGF_{2\alpha}$ treatment, the sensitivity of the carrageenin-induced granulation tissue to $PGF_{2\alpha}$ had decreased.

During a series of investigations of drug action on chronic granulomatous inflammation [1–5], we found that a single local administration of prostaglandin $F_{2\alpha}$† ($PGF_{2\alpha}$) significantly diminished vascular permeability, as well as collagen and noncollagenous protein synthesis of preformed chronic granulomatous tissue provoked by carrageenin in rats [6]. These inhibitory effects caused by $PGF_{2\alpha}$ are similar to those caused by the anti-inflammatory steroid, hydrocortisone [5]. It is generally accepted that $PGF_{2\alpha}$, as well as PGE is rapidly metabolized in tissue first by 15-hydroxyprostaglandin dehydrogenase (PGDH), and that the effects of these prostaglandins are not persistent [7, 8]. With these observations in mind, we have made an effort in the present investigation to determine if we could obtain a continuous decrease in vascular permeability, and a depression of collagen and noncollagenous protein synthesis in the granuloma tissue by the repeated administration of $PGF_{2\alpha}$.

These studies show that the inhibitory effects of a single local administration of $PGF_{2\alpha}$ to chronic granulomatous tissue are brief, although there is little metabolic activity of $PGF_{2\alpha}$. A second treatment with $PGF_{2\alpha}$, after alleviating the inhibitory effects of the first treatment with $PGF_{2\alpha}$, failed to reproduce the inhibitory effects. These findings led us to conclude that the sensitivity of chronic granulomatous tissue to $PGF_{2\alpha}$ was decreased by the first treatment with $PGF_{2\alpha}$. We suggest that the combined application of $PGF_{2\alpha}$ and a specific inhibitor to PGDH, or the application of $PGF_{2\alpha}$ analogs which are resistant to PGDH, would not be of use in suppressing granuloma tissue development.

## MATERIALS AND METHODS

*Carrageenin granuloma pouch.* A carrageenin granuloma pouch was made according to a procedure described previously [5, 6]. Male rats of the Donryu strain (6-weeks-old), weighing 150–180 g, were injected with 8 ml of air on the dorsum, subcutaneously, to make an air-pouch, in which, 24 hr later, 4 ml of a 2 per cent (w/v) solution of carrageenin (Seakem No. 202, Marine Colloid Inc., Springfield, NJ, U.S.A.) in 0.9 per cent NaCl were

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† Abbreviations used are: PG, prostaglandin; HSA, human serum albumin; PGDH, 15-hydroxyprostaglandin dehydrogenase; DPO, 2,5-diphenyloxazole; and POPOP, 1,4-bis-[2-(methyl-5-phenyloxazolyl)]benzene.

injected. The carrageenin solution was sterilized, by autoclaving at 120° for 15 min, and injected after cooling to within the temperature range of 40–45°. Immediately before the injection, penicillin and streptomycin were added to the carrageenin solution. Seven days after the carrageenin injection, a granuloma pouch, producing an inflammatory exudate, with a volume of 15–20 ml had developed and was used in the following experiment.

*Vascular permeability measurement in the granuloma pouch.* Vascular permeability in the granuloma was measured according to the procedure of Tsurufuji *et al.* [5]. In brief, a 1 $\mu$Ci aliquot of purified [$^{125}$I]HSA (Iodinated [$^{125}$I]Human Albumin Injection, 173 $\mu$Ci/$\mu$M, Kaken Kagaku Co. Ltd., Tokyo, Japan) in 0.2 ml of 0.9 per cent NaCl was injected into the right femoral vein of the rat bearing the granuloma pouch. After 30 min, 1.0 ml of the inflammatory fluid in the granuloma pouch was withdrawn, through a syringe attached with a 1/3 mm needle, to measure the leakage of [$^{125}$I]HSA into the pouch fluid through the local vascular networks. Immediately after the sampling of the pouch fluid, $PGF_{2\alpha}$ (Prostaglandin $F_{2\alpha}$, Fuji Yakuhin Kogyo Co. Ltd., Tokyo, Japan) solution or its vehicle in a volume of 0.2 ml was injected locally into the pouch fluid. At 5 min or 2.5 hr after the $PGF_{2\alpha}$ injection, a 1 $\mu$Ci aliquot of the purified [$^{131}$I]HSA [Radioiodinated Serum Albumin (RISA), 345 $\mu$Ci/$\mu$M, Dainabot Co. Ltd., Tokyo, Japan] in 0.2 ml of 0.9 per cent NaCl was injected into the femoral vein, and after 30 min, 1.0 ml of the pouch fluid was again taken out and served as a sample to measure the leakage of [$^{131}$I]HSA. The animals were killed just after the second sampling of the pouch fluid, and all the fluid in the pouch was collected to measure its volume. The radioactivities of $^{125}$I and $^{131}$I were measured in an automatic well-type scintillation counter, Aloka JDC-751 (Aloka Co. Ltd., Mitaka, Japan). The radioactivity of $^{125}$I was counted in the operation mode adjusted for counting the pulses from the photoelectric effect of the 35 KeV gamma rays of $^{125}$I. The radioactivity of $^{131}$I was measured by selective counting of pulses which correspond to the 360 KeV gamma rays of $^{131}$I, separated from the radiation of coexisting $^{125}$I. The radioactivity of [$^{125}$I]HSA and [$^{131}$I]HSA in all the pouch fluid of each rat was calculated and expressed in terms of the percentage of the particular radioactivity injected into the rats. The percentages of [$^{125}$I]HSA radioactivity and of [$^{131}$I]HSA radioactivity which leaked into the granuloma pouch fluid were used as indices of vascular permeability in the granuloma pouch before and after $PGF_{2\alpha}$ treatment respectively. The ratio of the control groups was almost unity. Therefore, a ratio was determined in order to express the change in vascular permeability which occurred under the influence of $PGF_{2\alpha}$ treatment.

*Incorporation of* [$^{3}$H]*proline into collagen hydroxyproline.* Rats were injected subcutaneously with [$^{3}$H]proline (generally labeled L-[$^{3}$H] proline, 63.0 Ci/mmole, Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan), 10 $\mu$Ci/0.1 ml/100 g body weight, 5 min or 2.5 hr after treatment with $PGF_{2\alpha}$ or its vehicle, into the pouch fluid. Thirty min later, the rats were killed by cutting the carotid artery, and the granuloma tissue was carefully dissected free from the surrounding fat, muscle and nongranulomatous subcutaneous tissue. The granuloma tissue was washed with ice-cold 0.9 per cent NaCl and minced with scissors. A portion of it was autoclaved twice with distilled water at 120° for 1 hr to obtain the collagen fraction as gelatin, which was then hydrolyzed with 6 N HCl in a sealed glass tube at 105° for 16 hr. The hydrolysate was evaporated to dryness and [$^{3}$H]hydroxyproline was determined according to the procedure of Juva and Prockop [9]. The results were expressed as disintegrations per min per $\mu$g of collagen hydroxyproline.

*Incorporation of* [$^{3}$H]*proline into noncollagenous protein.* A portion of minced granuloma tissue was homogenized by a Vir-Tis 45 homogenizer in ice-cold water for 5 min, mixed with an equal volume of 10 per cent trichloracetic acid (TCA) and centrifuged at 1000 *g* for 5 min. The pellet was washed twice with ice-cold 5 per cent TCA containing 1 per cent L-proline and then boiled for 15 min at 90° to solubilize the collagen. The resultant insoluble fraction was washed twice with ice-cold 5 per cent TCA and dissolved in 1 N NaOH. A portion of it was used for the determination of protein by the method of Lowry *et al.* [10]. Another portion of it was mixed with 10 ml of a Triton–toluene scintillation mixture (7 g DPO, 0.1 g POPOP, in 667 ml toluene and 333 ml Triton X-100) and the radioactivity was measured in a Packard Tri-Carb model 3380 liquid scintillation spectrometer, correcting for quenching by external standardization. The results were expressed as disintegrations per min per $\mu$g protein.

*Metabolism of* $PGF_{2\alpha}$ *in the granuloma.* Carrageenin granulation tissue was homogenized by a Vir-Tis 45 homogenizer in 0.05 M phosphate buffer (pH 7.4) containing 4 mM $NAD^+$ in a volume of 1 ml/g wet tissue. Two ml of granuloma homogenate were incubated with 0.5 $\mu$Ci of [$^{3}$H]$PGF_{2\alpha}$ ([9-$^{3}$H (n)]prostaglandin $F_{2\alpha}$, 9.2 Ci/mmole, New England Nuclear, Boston, MA, U.S.A.) at 37° for 1 hr and 3 hr. Lung and liver homogenates were also incubated with [$^{3}$H]$PGF_{2\alpha}$ at 37° for 1 hr. Incubation was terminated by the addition of 9 vol. of 95 per cent EtOH. After filtration, the ethanol fraction was evaporated to a small volume, and the resultant solution was extracted with ethylacetate after acidification to pH 3 with 0.1 N HCl. The extracted radioactive substances were subjected to thin-layer chromatography (T.L.C.). Thin-layer chromatographic plates of silica gel 60 (layer thickness, 0.25 mm, E. Merck, Germany) were developed in the solvent system (chloroform–methanol–acetic acid–water, 90:8.5:1:0.65, by vol.) described by Pace-Asciak and Miller [11]. Zones of the radioactive substances on each thin-layer plate were detected by a thin-layer chromatoscanner (Aloka Co. Ltd., Mitaka, Japan) and scraped off the plate for measurement of radioactivity by the liquid scintillation counter. When 0.5 $\mu$Ci of [$^{3}$H]$PGF_{2\alpha}$ with 50 or 250 $\mu$g of cold $PGF_{2\alpha}$ were locally injected into the granuloma pouch fluid, total amounts of the pouch fluid were withdrawn at 35 min or 3 hr after $PGF_{2\alpha}$ injection. An aliquot of the exudate was counted for radioactivity by the liquid scintillation counter. The radioactive substance was extracted

Table 1. Effect of $PGF_{2\alpha}$ on vascular permeability of carrageenin granuloma*

| Time after $PGF_{2\alpha}$ treatment | Treatment | No. of rats | Exudate (ml) | Concentration of $PGF_{2\alpha}$ in the exudate ($\mu$g/ml) | Vascular permeability index ratio† | Per cent of control |
|---|---|---|---|---|---|---|
| First injection | | | | | | |
| 5–35 min | Control | 8 | 16.5 ± 3.1 | 0 | 0.96 ± 0.10 | 100 |
| | $PGF_{2\alpha}$ (50 $\mu$g) | 8 | 16.6 ± 2.1 | 3.7 ± 0.5 | 0.60 ± 0.05‡ | 62.5 |
| | Control | 6 | 20.0 ± 2.4 | 0 | 1.08 ± 0.06 | 100 |
| | $PGF_{2\alpha}$ (250 $\mu$g) | 8 | 19.3 ± 2.1 | 14.8 ± 2.1 | 0.43 ± 0.04‡ | 40.9 |
| 2.5–3.0 hr | Control | 8 | 12.5 ± 1.2 | 0 | 1.08 ± 0.06 | 100 |
| | $PGF_{2\alpha}$ (50 $\mu$g) | 8 | 14.4 ± 1.6 | 4.4 ± 0.9 | 0.98 ± 0.10 | 90.7 |
| | Control | 3 | 23.6 ± 4.7 | 0 | 1.00 ± 0.06 | 100 |
| | $PGF_{2\alpha}$ (250 $\mu$g) | 7 | 18.4 ± 0.7 | 14.1 ± 0.5 | 0.75 ± 0.08‡ | 75.0 |
| Second injection§ | | | | | | |
| 5–35 min | Control | 7 | 17.4 ± 1.9 | 0 | 1.06 ± 0.06 | 100 |
| | $PGF_{2\alpha}$ (50 $\mu$g) | 7 | 14.1 ± 2.1 | 4.3 ± 0.7 | 1.07 ± 0.11 | 100.9 |
| | Control | 6 | 20.0 ± 2.4 | 0 | 1.08 ± 0.06 | 100 |
| | $PGF_{2\alpha}$ (250 $\mu$g) | 6 | 19.5 ± 1.9 | 13.4 ± 1.2 | 0.78 ± 0.03‡ | 74.3 |

* Results are given as means ± S.E.M.

† Vascular permeability index ratio = $\frac{\%\text{ of }[^{131}\text{I}]\text{HSA radioactivity leaked into the pouch fluid (post-treatment)}}{\%\text{ of }[^{125}\text{I}]\text{HSA radioactivity leaked into the pouch fluid (pre-treatment).}}$

‡ Indicates a statistically significant difference ($P < 0.005$) between the $PGF_{2\alpha}$-treated group and the corresponding control group.

§ Rats were injected with 50 or 250 $\mu$g of $PGF_{2\alpha}$ into the pouch fluid. Three hr after the first injection, 50 or 250 $\mu$g of $PGF_{2\alpha}$ were again injected into the pouch fluid and the vascular permeability was examined during 5–35 min after the second injection of $PGF_{2\alpha}$.

Table 2. Effect of $PGF_{2\alpha}$ on [$^3$H]proline incorporation into collagen hydroxyproline*

| Time after $PGF_{2\alpha}$ treatment | Treatment | No. of rats | Specific activity (d.p.m./μg of Hyp) | Per cent of control |
|---|---|---|---|---|
| First injection | | | | |
| 5–35 | Control | 8 | 0.78 ± 0.09 | 100 |
| | $PGF_{2\alpha}$ (50 μg) | 7 | 0.36 ± 0.03† | 46.7 |
| 2.5–3.0 hr | Control | 8 | 0.62 ± 0.08 | 100 |
| | $PGF_{2\alpha}$ (50 μg) | 6 | 0.47 ± 0.09 | 75.1 |
| Second injection‡ | | | | |
| 5–35 min | Control | 8 | 0.84 ± 0.11 | 100 |
| | $PGF_{2\alpha}$ (50 μg) | 8 | 0.73 ± 0.13 | 86.9 |

* Results are given as means ± S.E.M.

† Indicates a statistically significant difference ($P < 0.01$) between the $PGF_{2\alpha}$-treated group and the corresponding control group.

‡ Rats were injected with 50 μg $PGF_{2\alpha}$ into the pouch fluid. Three hr after the first injection, 50 μg $PGF_{2\alpha}$ were again injected into the pouch fluid and the [$^3$H]proline incorporation into collagen hydroxyproline was examined during 5–35 min after the second injection $PGF_{2\alpha}$.

from another aliquot of the exudate and subjected to T.L.C. and scanned in a manner similar to that described above.

## RESULTS

*Changes in vascular permeability of carrageenin granuloma after the first injection of $PGF_{2\alpha}$.* The changes in vascular permeability of the granulation tissue by $PGF_{2\alpha}$ treatment are summarized in Table 1. The first injection of $PGF_{2\alpha}$ at doses of 50 and 250 μg decreased the vascular permeability during 5–35 min after the $PGF_{2\alpha}$ treatment to 62.5 per cent ($P < 0.005$) and 39.8 per cent ($P < 0.005$) of the control level respectively. However, 3 hr after the $PGF_{2\alpha}$ injection, the decreased vascular permeability caused by 50 μg $PGF_{2\alpha}$ had recovered almost to the control level and there was no statistically significant difference between the $PGF_{2\alpha}$-treated group and the control group. At a dose of 250 μg $PGF_{2\alpha}$, the decreased vascular permeability was still observed 3 hr after the treatment, but the level was only 75.0 per cent of the control ($P < 0.005$).

*Changes in [$^3$H]proline incorporation into collagen hydroxyproline and noncollagenous protein after the first injection of $PGF_{2\alpha}$.* As shown in Table 2, at a dose of 50 μg $PGF_{2\alpha}$ (first injection), [$^3$H]proline incorporation into collagen hydroxyproline was decreased to 46.7 per cent ($P < 0.01$) of the control during 5–35 min after $PGF_{2\alpha}$ treatment. However, at 3 hr after the $PGF_{2\alpha}$ injection, incorporation had recovered almost to the control level. There was no statistically significant difference between the control and the $PGF_{2\alpha}$-treated group. Table 3 summarizes the effect of $PGF_{2\alpha}$ on the incorporation of [$^3$H]proline into noncollagenous protein. At a dose of 50 μg $PGF_{2\alpha}$ (first injection), [$^3$H]proline incorporation into noncollagenous protein was decreased to 65.9 per cent ($P < 0.005$) of the control. However, at 3 hr after the $PGF_{2\alpha}$ treatment, incorporation had recovered almost to the control level (not significant). At a dose of 250 μg $PGF_{2\alpha}$ (not shown), the uptake of [$^3$H]proline into collagen hydroxyproline and noncollagenous protein was also inhibited significantly ($P < 0.005$), 37.4 and 33.8 per cent of the control, respectively, during 5–35 min after the first injection of $PGF_{2\alpha}$. However, 3 hr after the

Table 3. Effect of $PGF_{2\alpha}$ on [$^3$H]proline incorporation into noncollagenous protein*

| Time after $PGF_{2\alpha}$ treatment | Treatment | No. of rats | Specific activity (d.p.m./μg of protein) | per cent of Control |
|---|---|---|---|---|
| First injection | | | | |
| 5–35 min | Control | 8 | 0.65 ± 0.05 | 100 |
| | $PGF_{2\alpha}$ (50 μg) | 7 | 0.43 ± 0.03† | 65.9 |
| 2.5–3.0 hr | Control | 8 | 0.62 ± 0.03 | 100 |
| | $PGF_{2\alpha}$ (50 μg) | 6 | 0.50 ± 0.07 | 80.2 |
| Second injection‡ | | | | |
| 5–35 min | Control | 8 | 0.81 ± 0.04 | 100 |
| | $PGF_{2\alpha}$ (50 μg) | 8 | 0.68 ± 0.07 | 84.2 |

* Results are given as means ± S.E.M.

† Indicates a statistically significant difference ($P < 0.005$) between the $PGF_{2\alpha}$-treated group and the corresponding control group.

‡ Rats were injected with 50 μg $PGF_{2\alpha}$ into the pouch fluid. Three hr after the first injection, 50 μg $PGF_{2\alpha}$ were again injected into the pouch fluid and the [$^3$H]proline incorporation into noncollagenous protein was examined during 5–35 min after the second injection of $PGF_{2\alpha}$.

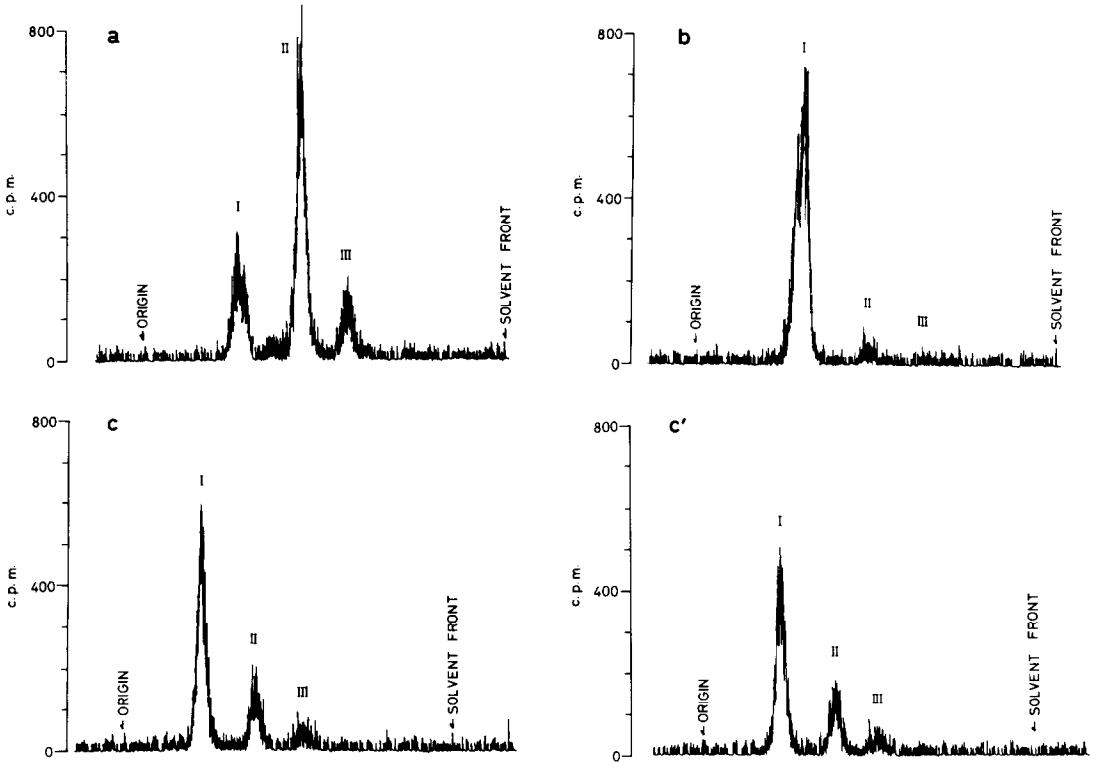


Fig. 1. Thin-layer radiochromatogram of [$^3$H]$PGF_{2\alpha}$ and its metabolites after incubation with lung (a) liver (b) and granuloma (c) homogenate. Each tissue was homogenized by a Vir-Tis 45 homogenizer with 0.05 M phosphate buffer (pH 7.4) containing 4 mM $NAD^+$ in a volume of 1 ml/g wet tissue. Two ml of homogenate were incubated with 0.5 $\mu$Ci of [$^3$H]$PGF_{2\alpha}$ (9.2 Ci/mmole) at 37° for 1 hr (a, b and c) and for 3 hr (c'). Incubation was terminated by adding 9 vol. of 95 per cent EtOH. After filtration, the ethanol fraction was evaporated to a small volume, and the resultant solution was extracted with AcOEt after acidification to pH 3 with 0.1 N HCl. The extracted radioactive substances were subjected to thin-layer chromatography. Thin-layer chromatographic plates of silica gel 60 (0.25 mm in thickness) were developed in the solvent system (chloroform–methanol–acetic acid–water, 90:8.5:1:0.65, by vol.) described by Pace-Asciak and Miller [11]. Peaks I, II and III represent $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$ and 13,14-dihydro-15-keto-$PGF_{2\alpha}$ respectively.

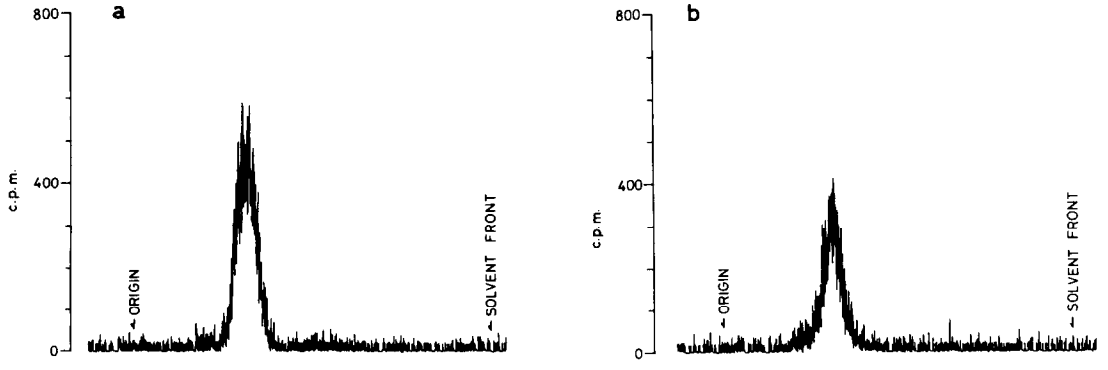


Fig. 2. Thin-layer radiochromatogram of [$^3$H]$PGF_{2\alpha}$ at 35 min (a) and at 3 hr (b) after injection into the pouch fluid. [$^3$H]$PGF_{2\alpha}$ (0.5 $\mu$Ci, 9.2 Ci/mmole) was injected into the pouch fluid with carrier $PGF_{2\alpha}$ (50 $\mu$g). After 35 min (a) or 3 hr (b), total pouch fluid was withdrawn and added to 9 vol. of 95 per cent EtOH solution. After filtration, the ethanol fraction was evaporated to a small volume and the resultant solution was acidified to pH 3 with 0.1 N HCl. The acid solution was extracted with AcOEt. The extracted radioactive substances were subjected to T.L.C. and developed in the same solvent system shown in Fig. 1. The recovery of [$^3$H]$PGF_{2\alpha}$ during the extraction procedure was reproducible and more than 95 per cent.

injection, the uptake was 87.8 per cent (not significant) and 76.1 per cent ($P < 0.005$) of the control, respectively, the inhibition of uptake having decreased.

*Metabolism of $PGF_{2\alpha}$ in carrageenin granuloma.* Figure 1 shows the radiochromatograms of [$^3$H]$PGF_{2\alpha}$ (I) and its metabolites, 15-keto-$PGF_{2\alpha}$ (II) and 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (III), after incubation with homogenates of lung, liver and granuloma. Lung homogenate showed the most active $PGF_{2\alpha}$ metabolism (Fig. 1a). The greater part of the original [$^3$H]$PGF_{2\alpha}$ (peak I) was metabolized to 15-keto-$PGF_{2\alpha}$. Slight metabolic activity was observed in the liver homogenate (Fig. 1b). In carrageenin granuloma, $PGF_{2\alpha}$ metabolism was greater than in liver, but 75 per cent of the original [$^3$H]$PGF_{2\alpha}$ was not metabolized after 1-hr incubation (Fig. 1c), and even after 3 hr of incubation, about 70 per cent of [$^3$H]$PGF_{2\alpha}$ remained unmetabolized (Fig. 1c').

Figure 2 (panels a and b) shows the radiochromatogram of the granuloma exudate 35 min and 3 hr after [$^3$H]$PGF_{2\alpha}$ injection with 50 $\mu$g of cold $PGF_{2\alpha}$ respectively. One single peak with an $R_f$ value that coincided with authentic $PGF_{2\alpha}$ was observed; no peak of the metabolite of [$^3$H]$PGF_{2\alpha}$ was seen. The radioactive peak was scraped off the plate and the radioactivity was counted. After correcting for recovery, it was calculated that only 18 per cent of the radioactivity at 35 min after the [$^3$H]$PGF_{2\alpha}$ injection had disappeared during the following 2 hr and 25 min. When [$^3$H]$PGF_{2\alpha}$ was injected into the granuloma pouch with 250 $\mu$g of cold $PGF_{2\alpha}$, only 15 per cent of the radioactivity present at 35 min had disappeared during the 3 hr (Table 4). The decrease in the total radioactivity during 35 min to 3 hr was only 6 per cent of the injected [$^3$H]$PGF_{2\alpha}$.

*Changes in the vascular permeability of carrageenin granuloma and the incorporation of* [$^3$H]*proline into collagen hydroxyproline and noncollagenous protein after a second injection of $PGF_{2\alpha}$.* Three hr after the first injection of $PGF_{2\alpha}$, when the decrease in vascular permeability and in the uptake of [$^3$H]proline into both protein fractions had disappeared or diminished, 50 or 250 $\mu$g of $PGF_{2\alpha}$ were again injected locally into the pouch fluid (second injection). However, as shown in Table 1, no decrease in vascular permeability was observed after 50 $\mu$g $PGF_{2\alpha}$. At a dose of 250 $\mu$g, the decrease was 25.7 per cent ($P < 0.005$), which was almost the same as at 3 hr after the first injection (25.0 percent). These results show that the second injection of $PGF_{2\alpha}$ caused no decrease in vascular permeability. As to the uptake of [$^3$H]proline into collagen hydroxyproline and noncollagenous protein, the second injection of $PGF_{2\alpha}$ at a dose of 50 $\mu$g did not cause a decrease (Tables 2 and 3). Also, at a dose of 250 $\mu$g $PGF_{2\alpha}$ (not shown), no further decrease was observed after a second injection.

Table 4. Radioactivity of [$^3$H]$PGF_{2\alpha}$ in granuloma exudate*

| Time after [$^3$H]$PGF_{2\alpha}$ injection | Exudate (ml) | Total radioactivity in exudate ($\times 10^{-3}$ c.p.m./ml) | per cent |
|---|---|---|---|
| 35 min | 15.5 ± 2.1 | 27 ± 7 (3) | 100 |
| 3 hr | 15.0 ± 1.1 | 23 ± 3 (3) | 85.0 |

* Results are given as mean ± S.E.M. [$^3$H]$PGF_{2\alpha}$ (0.5 $\mu$Ci) with 250 $\mu$g of cold $PGF_{2\alpha}$ were locally injected into the granuloma pouch fluid, and total amounts of the pouch fluid were withdrawn at 35 min or 3 hr after the $PGF_{2\alpha}$ injection. An aliquot of the pouch fluid was counted for radioactivity by the liquid scintillation counter.

## DISCUSSION

The carrageenin-induced granulomatous tissue employed in this investigation displays the two pathological hallmarks of inflammation, the development of a wall of proliferative tissue and the exudation of inflammatory fluid inside the wall. Around day 7 after carrageenin injection (chronic phase), the inflammatory exudate and the surrounding tissue are still in a stage of active development. We have shown in a previous report [6] that $PGF_{2\alpha}$, when injected locally into the pouch fluid, suppressed vascular permeability and collagen and noncollagenous protein synthesis of the chronic carrageenin-induced granulomatous tissue in rats. These effects of $PGF_{2\alpha}$ are similar to those of hydrocortisone, a potent anti-inflammatory drug.

The anti-inflammatory activity caused by local administration of $PGF_{2\alpha}$ may have relevance for possible clinical application if rapid metabolism *in vivo* and the large number of possible side effects can be overcome by the development of $PGF_{2\alpha}$ analogs. It is widely accepted that $PGF_{2\alpha}$ as well as PGE is rapidly metabolized in the tissue first by PGDH [7,8]. If this is the case, the effect by exogenously injected $PGF_{2\alpha}$ cannot be persistent. As shown in Tables 1–3, at 3 hr after the first injection of $PGF_{2\alpha}$, the depressive effects on vascular permeability and collagen and noncollagenous protein synthesis, observed during 5–35 min after the $PGF_{2\alpha}$ treatment, were diminished. By the application of reversed phase partition chromatography, we have shown in a previous report [12] that there is very little activity of PGDH in the exudate of carrageenin-induced granulation tissue. This finding was reconfirmed in the present experiment. As shown in Fig. 2, no detectable peak of [$^3$H]$PGF_{2\alpha}$ metabolites in the exudate was observed at 35 min or at 3 hr after the injection of [$^3$H]$PGF_{2\alpha}$ into the pouch fluid. Accordingly, it was expected that the proliferative wall of the granuloma tissue, but not the exudate, had strong PGDH activity since, as mentioned above, at 3 hr after the injection of $PGF_{2\alpha}$, the effects of $PGF_{2\alpha}$ were diminished. When [$^3$H]$PGF_{2\alpha}$ was incubated for 1 hr with the homogenate of the granuloma tissue in the presence of $NAD^+$, only 30 per cent of the originally added [$^3$H]$PGF_{2\alpha}$ was metabolized into 15-keto-$PGF_{2\alpha}$ and 13, 14-dihydro-15-keto-$PGF_{2\alpha}$ (Fig. 1c), and no further prominent degradation was observed during the next 2 hr (Fig. 1c'). About 70 per cent of the added [$^3$H]$PGF_{2\alpha}$ remained unmetabolized. Consequently, the metabolic activity of $PGF_{2\alpha}$ in the granuloma was not as great as we had expected.

Another possible explanation for the decrease in the effect of $PGF_{2\alpha}$ during the 3 hr is the rapid

disappearance of injected $PGF_{2\alpha}$ from the tissue through the local circulation system. Three hr after the local injection of [$^3$H]$PGF_{2\alpha}$ with 50 or 250 $\mu$g of cold $PGF_{2\alpha}$, only 18 or 15 per cent of the total radioactivity at 35 min had disappeared, respectively, during 2 hr 25 min. These results show that the disappearance rate of injected $PGF_{2\alpha}$ was very low between 35 min and 3 hr after the $PGF_{2\alpha}$ injection. Consequently, the rapid disappearance of $PGF_{2\alpha}$ from the tissue could not be the cause of the decrease in the effect of $PGF_{2\alpha}$ during 3 hr. The most plausible explanation is that the sensitivity of the granulation tissue to exogenously added $PGF_{2\alpha}$ is decreased for 3 hr after the first injection of $PGF_{2\alpha}$. To confirm this possibility, 50 and 250 $\mu$g of $PGF_{2\alpha}$ were again injected locally into the pouch fluid at 3 hr after the first injection of $PGF_{2\alpha}$. As shown in Tables 1–3, no changes in vascular permeability or in uptake of [$^3$H]proline into protein fractions were observed. From these results, we have reached the conclusion that continuous anti-inflammatory activity of $PGF_{2\alpha}$ on preformed rat carrageenin-granulation tissue is not to be expected, since the sensitivity of this tissue to exogenously added $PGF_{2\alpha}$ appeared to diminish within 3 hr after the local injection and no further response could be observed by the second injection of $PGF_{2\alpha}$.

Anti-inflammatory activity of $PGF_{2\alpha}$ has already been reported by several investigators who focused mainly on acute inflammation [13–16], but no efforts have been made to examine whether the anti-inflammatory activity of $PGF_{2\alpha}$ was long-lasting. In chronic granuloma tissue, no anti-inflammatory activity of $PGF_{2\alpha}$ was reported, except in our previous report [6]. Chang and Tsurufuji [3] reported that local injection of $PGF_{2\alpha}$ caused no statistically significant decrease of vascular permeability, although there was a tendency to suppress 7-day-old carrageenin-induced granulation tissue in rats employing the single [$^{131}$I]HSA tracer technique. By the application of the double isotope tracer method, which is more accurate than the single radioisotope method, as described in previous papers [5, 6], it was found that $PGF_{2\alpha}$ did depress vascular permeability [6]. According to Glenn *et al.* [17], local injection of $PGF_{2\alpha}$ twice each day for 7.5 days, on a chronic basis into croton oil or *d*-$\alpha$-tocopherol-induced granuloma pouches of rats (6.5 to 52 $\mu$g/pouch) showed no effect. DiPasquale *et al.* [18] also reported no effects of $PGF_{2\alpha}$ on croton oil pouch exudation. Repeated injection of $PGF_{2\alpha}$ into chronic inflammatory tissue is of no use in showing cumulative anti-inflammatory activity of $PGF_{2\alpha}$, since, as clarified in the present investigation, continuous anti-inflammatory activity cannot be expected. The tissue quickly reaches the desensitized stage to exogenously injected $PGF_{2\alpha}$. We are now investigating how long it takes tissue to be restored to $PGF_{2\alpha}$ sensitivity after the first treatment by $PGF_{2\alpha}$.

The existence of a receptor for $PGF_{2\alpha}$ has been reported in stomach [19], corpus luteum [20, 21], adrenal [22], thyroid [23], uterus [24], liver [25], thymocytes [26] and fat cells [27]. Provided that granulation tissue has receptors to $PGF_{2\alpha}$, all the receptors might be occupied by the first injection of $PGF_{2\alpha}$, followed by a brief decrease in vascular permeability and in uptake of [$^3$H]proline into both of the protein fractions. A second injection of $PGF_{2\alpha}$ could cause no further binding of $PGF_{2\alpha}$ to receptors as all of the receptors would have been occupied by the first injection of $PGF_{2\alpha}$. The mechanism of the effect of $PGF_{2\alpha}$ on vascular permeability and protein synthesis remains to be shown. When the granulation tissue was minced into small pieces (less than 1 mm$^3$) and incubated in a medium containing [$^3$H]proline with or without $PGF_{2\alpha}$, the uptake of [$^3$H]proline into both protein fractions was inhibited significantly by $PGF_{2\alpha}$ (H. Sato, K. Ohuchi and S. Tsurufuji, manuscript in preparation).

Contradictory to our results obtained in the present experiments, there is one report which shows anti-inflammatory activity of $PGF_{2\alpha}$ on chronic inflammation. According to Aspinall *et al.* [28], $PGF_{2\alpha}$ actively suppressed the development of adjuvant arthritis of *Mycoplasma*-induced arthritis. We assume that the anti-inflammatory activity of $PGF_{2\alpha}$ on chronic arthritis inflammation reported by them may be due to the stimulation of the adrenal cortex by $PGF_{2\alpha}$ injection, as pointed out by Louis *et al.* [29].

Although there was little $PGF_{2\alpha}$ metabolism in granulation tissue, the anti-inflammatory activity of $PGF_{2\alpha}$ was not long-lasting. The application to granulation tissue of $PGF_{2\alpha}$ together with an inhibitor of PGDH or $PGF_{2\alpha}$ analogs which are resistant to PGDH, would not be of use in maintaining the anti-inflammatory activity for long periods of time, judging from the results obtained in this investigation.

## REFERENCES


1. M. Fukuhara and S. Tsurufuji, *Biochem. Pharmac.* **18**, 475 (1969).
2. K. H. Shin, H. Nakagawa and S. Tsurufuji, *Chem. pharm. Bull. Tokyo*, **23**, 1137 (1975).
3. W. C. Chang and S. Tsurufuji, *Eur. J. Pharmac.* **36**, 7 (1976).
4. W. C. Chang, S. Murota and S. Tsurufuji, *Biochem. Pharmac.* **25**, 2045 (1976).
5. S. Tsurufuji, K. Sugio and Y. Endo, *Biochem. Pharmac.* **26**, 1131 (1977).
6. K. Ohuchi, H. Sato and S. Tsurufuji, *Biochem. Pharmac.* **26**, 2049 (1977).
7. M. Hamberg and B. Samuelsson, *J. biol. Chem.* **246**, 6713 (1971).
8. E. Granström, *Eur. J. Biochem.* **27**, 462 (1972).
9. K. Juva and D. J. Prockop, *Analyt. Biochem.* **15**, 77 (1966).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
11. C. Pace-Asciak and D. Miller, *Prostaglandins* **4**, 351 (1973).
12. K. Ohuchi, H. Sato and S. Tsurufuji, *Biochem. biophys. Acta* **424**, (1976).
13. D. A. Willoughby, *J. Path. Bact.* **96**, 381 (1968).
14. S. Arora, P. K. Lahiri and K. Sanyal, *Int. Archs Allergy appl. Immun.* **39**, 186 (1970).
15. P. Crunkhorn and A. L. Willis, *Br. J. Pharmac.* **41**, 49 (1971).
16. A. J. Lewis, P. W. Wells and P. Eyre, *J. Pharm. Pharmac.* **24**, 326 (1972).
17. E. M. Glenn, B. J. Bowman and N. A. Rohlhoff, in *Prostaglandins in Cellular Biology* (Eds. P. W. Ramwell and B. B. Phariss), p. 329. Plenum Press, New York (1972).

18. G. DiPasquale, C. Rasaert, R. Richter, P. Welaj and L. Tripp, *Prostaglandins* **3**, 741 (1973).
19. O. V. Miller and W. E. Magee, *Adv. Biosci.* **9**, 83 (1973).
20. C. V. Rao, *J. biol. Chem* **249**, 7023 (1974).
21. W. S. Powell, S. Hammarström and B. Samuelsson, *Eur. J. Biochem.* **41**, 103 (1974).
22. A. Dazard, A. M. Morera, J. Bertrand and J. M. Saez, *Endocrinology* **95**, 352 (1974).
23. W. V. Moore and J. Wolff, *J. biol. Chem.* **248**, 5705 (1973).
24. A. E. Wakeling and L. J. Wyngarden, *Endocrinology* **95**, 55 (1974).
25. M. Smigel and S. Fleischer, *Biochim. biophys. Acta* **332**, 358 (1974).
26. B. P. Schaumburg, *Biochim. biophys. Acta* **326**, 127 (1973).
27. R. R. Gorman and O. V. Miller, *Biochim. biophys. Acta* **323**, 560 (1973).
28. R. L. Aspinall, P. S. Cammarata, A. Nakao, J. Jiu, M. Miyano, D. E. Baker and W. F. Patsch, *Adv. Biosci.* **9**, 419 (1973).
29. T. M. Louis, J. N. Stellflug, H. A. Tucker and H. D. Hafs, *Proc. Soc. exp. Biol. Med.* **147**, 128 (1974).