Prostaglandin E_2 biosynthesis: changes in rabbit aorta and skin during experimental atherogenesis[1]

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Abstract The transformation of [1-^{14}C]arachidonic acid into [^{14}C]prostaglandin E_2(PGE_2) by rabbit aorta and skin was demonstrated by cell-free preparations, and the PGE_2 synthetase activity was located mainly in the microsomal fraction (105,000 *g* pellet) of both tissues. Rabbits fed an atherogenic diet (Purina rabbit chow plus 1% cholesterol and 2% cottonseed oil) developed atheroma in the aortas and skin lesions resembling xanthoma in 6 to 7 months. At the end of this period, increases in the conversion of [1-^{14}C]-arachidonic acid into [^{14}C]PGE_2 were observed in microsomal preparations of the intima–media of the aortas (2.5-fold of control) and normal-appearing skin (3.0-fold of control) of the experimental animal. Microsomal preparations of skin lesions particularly had greater ability to form PGE_2 (7-fold of control).

The level of PGE_2 in skin biopsy specimens of the rabbit was studied by gas-liquid chromatography over the first two months of cholesterol feeding. A 2- to 3-fold increase in the level of PGE_2 occurred in the first 2 weeks, reaching a peak of 30 ng/mg protein by the 35th day; thereafter, the level gradually declined to the control range at the end of two months. Control groups had a PGE_2 level of 2 to 12 ng/mg protein and did not show significant change throughout this period.

The results suggest the involvement of PGE_2 in atherogenesis, and the possibility of using the skin as an experimental model for the study of atherosclerosis.

Supplementary key words arachidonic acid • cholesterol feeding • xanthoma • intima–media

The beneficial effect of dietary polyunsaturated fats in lowering serum cholesterol has been reported (1–3), and the role of essential fatty acids (EFA) in retarding atherogenesis has been the subject of many investigations (4–8). Since EFA are precursors of prostaglandins (PG) (9), the possible involvement of the PG in atherogenesis was postulated, and was discussed by Thomasson (10). Experimentally, injuries to the arterial wall causing inflammation leading to atherogenesis have been demonstrated (11). Since PG are involved in inflammatory processes, and inflammation of the arterial wall is an important aspect of atherogenesis, it is of merit to examine the role of PG in the process. Additional consideration must be given to reports that PG inhibit the formation of cholesteryl esters in several tissues (12–14), and cholesteryl ester formation is an important event in lipid deposition in atherosclerotic plaques (15, 16).

It has been observed in rabbits fed an atherogenic diet that, besides the formation of vascular lesions, the animals lose their fur in certain areas of the skin and develop skin lesions resembling xanthoma (17, 18). Intrigued by the possibility that the lesions in the skin may develop through mechanisms similar to those in the aorta (17), and that the skin may be useful as a model for experimental purposes (19), we investigated the biosynthesis of PGE_2 from [1-^{14}C]arachidonic acid in both the skin and aorta of rabbits during experimental atherogenesis, and measured the level of PGE_2 in the skin. Our results indicate increases in the biosynthesis of PGE_2 in both tissues and elevated PGE_2 level in skin during an early stage of atherogenesis.

MATERIALS AND METHODS

Materials

[1-^{14}C]Arachidonic acid (sp act, 56.6 mCi/mmole) was obtained from Dhom Products (North Hollywood, Cal.). The methyl ester was prepared and analyzed by gas–liquid chromatography (GLC); 95% of the ^{14}C had the retention time of methyl arachidonate. [2-^{14}C]Prostaglandin E_1 (50 mCi/mmole) was obtained from New England Nuclear (Boston, Mass.). PGE_2 was a gift from Dr. John Pike of the Upjohn Co. (Kalamazoo, Mich.). ω-nor-PGE_2 (19:4, n-5)

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; EFA, essential fatty acids; PG, prostaglandins. Trivial names are: prostaglandin E_1 (PGE_1), 11,15-dihydroxy-9-oxoprosta-13-enoic acid; prostaglandin E_2 (PGE_2), 11,15-dihydroxy-9-oxoprosta-5-13-dienoic acid; prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 9,11,15-trihydroxy-prosta-5,13-dienoic acid; prostaglandin B_2 (PGB_2), 15-hydroxy-9-oxoprosta-5,8(12)13-dienoic acid; ω-norPGE_2, *cis*-5,8,11,14-noreicosatetraenoic acid; ω-homo-PGE_1, *cis*-5,8,11,14-homoeicosatetraenoic acid; arachidonic acid, *cis*-5,8,11,14-eicosatetraenoic acid; homo-γ-linolenic acid, all-*cis*-8,11,14-eicosatrienoic acid.

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and ω-homo-PGE_1 (21:4, n-7) were gifts from Professor van Dorp of the Unilever Research Laboratory (Netherlands). Silica gel G was a product of E. Merck Co. (Darmstadt, Germany). Diazald (*N*-methyl-*N*-nitroso-*p*-toluene-sulfonamide) for the generation of diazomethane, was purchased from Aldrich Chemical Co. (Milwaukee, Wis.), and gentamicin sulfate from the Schering Corp. (Port Reading, N.J.). BSA (bis-trimethylsilylacetamide) and reference fatty acid esters (FAME 19112) were purchased from Applied Science Laboratories, Inc. (State College, Penn.). Reagents were of analytical grade and solvents were redistilled before use.

Experimental animal

Adult male New Zealand white rabbits weighing 3–4 kg were maintained on three types of diet: Control 1, Purina rabbit chow; control 2, Purina rabbit chow plus 2% cottonseed oil (w/w); and atherogenic, Purina rabbit chow plus 1% cholesterol and 2% cottonseed oil (w/w). The number of animals used and length of feeding are specified for each experiment. Animals fed the atherogenic diet developed alopecia in about 3 months, and skin lesions resembling xanthoma, most prominently in the nape, in about 6 to 7 months. At autopsy, animals fed the atherogenic diet had fatty streaks in the arterial wall in about 1 month, and well-developed intimal atherosclerotic plaques in 6–7 months.

Skin biopsy

Biopsy specimens of the skin weighing 30–40 mg were obtained from the back of the rabbit with a device which we named "snipper", after the hair was carefully clipped and shaved. The snipper consisted of a metal tube (ID 0.6 cm) which was fitted in the inside with a perforated plate. The latter could be set at 0.3 mm from one end of the tube. The other end was attached to a vacuum pump. The end with the perforated plate was placed on the surface of the skin and a small piece of skin was lifted by suction into the tube in the space under the plate. This was quickly excised with a razor blade attached to the shaft of an electric oscillator. This procedure causes little pain and can be performed repeatedly without anesthesia. The excised skin was immediately placed in chloroform–methanol 2:1 (v/v) to arrest the biosynthesis of PGE_2. In control experiments, skin specimens left for 30 min on a piece of gauze moistened with 0.1 M phosphate buffer and chilled in an ice bath, contained 5 times the PGE_2 as in specimens immediately treated with chloroform–methanol as judged by GLC analysis as described below.

Subcellular preparations

For the preparation of subcellular fractions of skin and aorta, the animals were killed by cervical dislocation. Skin was taken along the ventral midline incision and from the nape, and whole aorta was removed within 15 min. The intima–media layer of the aorta was dissected, weighed, and homogenized in 10 volumes of phosphate buffer, pH 7.4, with a Polytron homogenizer (Model PT-20, Kinematica, Lucerne, Switzerland) while chilled in an ice bath. The skin was freed of hair and subcutaneous fat, and homogenized similarly. The homogenates were centrifuged at 4° C sequentially at 900 *g* for 15 min, 10,000 *g* for 30 min, and 105,000 *g* for 60 min. The final pellet was rinsed and resuspended in the same buffer and stored in ice. Aliquots of each of the subcellular fractions were analyzed for protein using the method of Lowry et al. (20). The protein contents of aorta subcellular fractions were: crude homogenate, 10.0 mg/ml; 10,000 *g* supernatant, 9.13 mg/ml; 10,000 *g* pellet, 1.31 mg/ml; 105,000 *g* supernatant, 3.78 mg/ml; 105,000 *g* pellet, 0.862 mg/ml.

The protein contents of skin subcellular fractions were: crude homogenate, 15.2 mg/ml; 10,000 *g* supernatant, 10.8 mg/ml; 10,000 *g* pellet, 2.1 mg/ml; 105,000 *g* supernatant, 5.6 mg/ml; 105,000 *g* pellet, 3.1 mg/ml.

Incubation

The procedure for the study of PGE_2 biosynthesis in human skin (21) was used. [1-^{14}C]Arachidonic acid (2.0 μCi) dissolved in benzene was evaporated in an incubation flask with a stream of N_2. The residue was mixed with 0.65 mM glutathione, 0.55 mM hydroquinone, and the subcellular fraction from 4.0–6.0 g of skin or 1.5–3.0 g of intima–media, in a final volume of 5 ml of phosphate buffer, pH 7.4. A boiled sample of the tissue preparation was used as control. The mixture was incubated aerobically with shaking at 37°C for 30 min, and the incubation was terminated by the addition of 9 volumes of 95% aqueous ethanol and heating for 15 min in a boiling water bath. The mixture was filtered through glass wool and the filtrate was reduced in volume under vacuum, acidified to pH 3 and extracted with dichloromethane. The residue was partitioned between equal volumes of 60% aqueous ethanol and of petroleum ether. Prostaglandins were partitioned mainly in the lower phase, and arachidonic acid was in the upper phase. The residues from both phases were subjected to thin-layer chromatography (TLC).

Separation of lipids

For the separation of PG from other lipids, the lipid mixture was subjected to TLC on silica gel G according to Struijk, Beerthuis and van Dorp (22) in the solvent system consisting of chloroform–methanol–acetic acid–water 90:6:1:0.75 (v/v). For further separation of PG, system AI of Green and Samuelsson (23) consisting of benzene–dioxane–acetic acid 20:20:1 (v/v) was used. Reference PGE_2 and arachidonic acid were chromatographed concurrently on separate plates and visualized by exposure to I_2 vapor. The radioactivity was located by scanning with a Radio-Chromatogram Scanner (Packard Model 7210, Packard Instruments Co., Downers Grove, Ill.). For quantitative determination of ^{14}C, the plates were scraped in successive 0.5 cm sections for counting in a Packard Tri-Carb Model 2002 liquid scintillation counter.

Identification of [^{14}C]PGE_2

The area in the chromatogram corresponding to PGE_2 from system AI was extracted with chloroform–methanol 1:1, and

diluted with carrier PGE_2. The diluted sample was chromatographed on a reversed phase partition column according to the procedure of Norman and Sjövall (24), using chloroform–isooctanol 1:1 (v/v) (4.0 ml) as the stationary phase supported on hydrophobic supercel (4.5 g). The column was developed with methanol–water 135:165 (v/v) as the moving phase, and the effluent was collected in 5 ml fractions. PGE_2 was located by conversion to PGB_2 after an aliquot of each fraction was treated with 0.5 N NaOH at 37°C for 30 min, and measuring absorbance at 278 nm. The ^{14}C in the column effluent was assayed by scintillation counting. For further identification of ^{14}C-labeled PGE_2, the remaining portions of PGE_2-containing fractions were combined. The PGE_2 was converted into PGB_2 and subjected to reverse phase partition chromatography using chloroform–heptane 45:15 (v/v) as the stationary phase and methanol–water 165:135 (v/v) as the movable phase.

Arachidonic acid content in the microsomal pellet

Samples of the 105,000 *g* pellets of normal and diseased skin and aorta were homogenized manually in a mixture of chloroform–methanol 2:1 (v/v). The lipid extract was evaporated in a rotary evaporator. After transesterification by refluxing for 60 min under N_2 in methanol containing 5% HCl, the methyl esters of fatty acids were separated by GLC on a 15% diethylene glycol succinate polyester (DEGS) column. Quantitation of arachidonic acid and eicosatrienoic acid was by triangulation of the chromatographic peaks, using Applied Science Laboratories FAME 19112 as reference.

Determination of PGE_2 in skin

Immediately after excision, the skin biopsy specimen was placed in chloroform–methanol 2:1 (v/v) and homogenized with a motor-driven glass homogenizer. An aliquot of the homogenate was taken for protein analysis by the method of Lowry et al. (20) and the lipid extract was evaporated to dryness in a rotary evaporator and further dried with N_2. The residue was dissolved in chloroform and subjected to column chromatography on silica gel H according to the method described by Jouvenaz et al. (25). The elution was accelerated by centrifugation, and the development of the column was marked by malachite green and phenol red. The PGE were eluted by 35% methanol in chloroform and marked by phenol red. ^{14}C-Labeled PGE_1 added to the tissue and carried through the procedure showed 90% recovery.

The PGE fraction was treated with methanolic KOH to transform the E-prostaglandins into B-prostaglandin (26). The mixture was acidified, extracted three times with ether, and evaporated to dryness under N_2. The residue was dissolved in methanol and methylated with diazomethane. After the esterification was completed in 1 hr, the methyl ester (PGB-ME) was silylated with bis-trimethylsilylacetamide (BSA). Reference ω-nor-PGE_2 and ω-homo-PGE_1 were treated similarly and the silylated methyl ester derivatives were mixed with the preparation from the skin in a hexane solution and injected into a gas chromatograph (Hewlett-Packard, Palo Alto, Cal.) with a ^{63}Ni electron capture detector. The two reference compounds were used because the retention time of the PGE_2 derivative was between those of ω-homo-PGE_1 and ω-nor-PGE_2 (25). Reference PGE_2 was similarly treated for quantitative comparison, and quantitation was by triangulation of the chromatographic peaks.

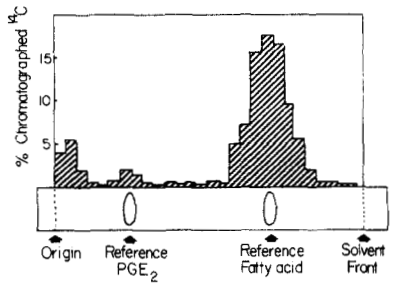

Fig. 1. Thin-layer chromatography of products obtained after incubating [1-^{14}C]arachidonic acid with a homogenate of rabbit aorta. [1-^{14}C]Arachidonic acid was incubated with the homogenate in the presence of 0.65 mM glutathione and 0.55 mM hydroquinone. Details of incubation conditions are described under Methods. The solvent system used for developing TLC was chloroform–methanol–acetic acid–water 90:6:1:0.75 (v/v). Reference PGE_2 and arachidonic acid were chromatographed concurrently and visualized by exposure to I_2 vapor. The height of the bars represents the percentage of the total chromatographed ^{14}C.

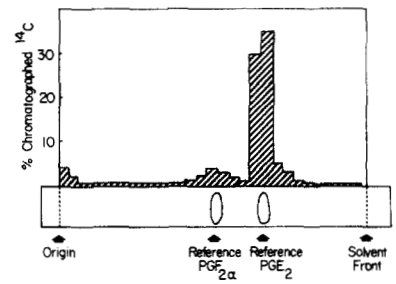

Fig. 2. Separation of [^{14}C]PGE_2. The fraction containing the PG from Fig. 1 was further separated in solvent system AI of Green and Samuelsson (22), consisting of benzene–dioxane–acetic acid 20:20:1 (v/v). The height of the bars represents the percentage of the total chromatographed ^{14}C.

RESULTS

PGE synthetase activity in aorta and skin homogenates

Incubation of [1-^{14}C]arachidonic acid with the homogenate of an intima–media preparation of rabbit aorta yielded radioactive products with chromatographic mobilities similar to PG **(Fig. 1).** The total fraction was subjected to TLC in solvent system A1 (23). Two peaks of radioactivity corresponding to $PGF_{2\alpha}$ and PGE_2 were observed **(Fig. 2).** The radioactive material in the peak corresponding to PGE_2 was extracted with chloroform–methanol 2:1 (v/v), mixed with

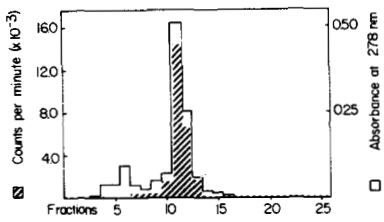

Fig. 3. Identification of [^{14}C]PGE_2. The radioactive material corresponding to PGE_2 in Fig. 2 was subjected to reverse phase partition chromatography on silicic acid, using chloroform–isooctanol 15:15 (v/v) as the stationary phase and methanol–water 135:165 (v/v) as the moving phase. The dashed bars indicate the amount of ^{14}C; the open bars indicate the absorbance at 278 nm after the PGE_2 was converted into PGB_2.

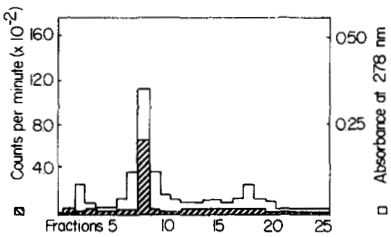

Fig. 4. Reverse phase partition chromatography of PGB_2 obtained after treating the radioactive material eluted in Fig. 3 with 0.5 N NaOH. Stationary phase was chloroform–heptane 45:5 (v/v) and moving phase, methanol–water 165:135 (v/v). The dashed bars indicate the amount of ^{14}C, and the open bars indicate the absorbance at 278 nm.

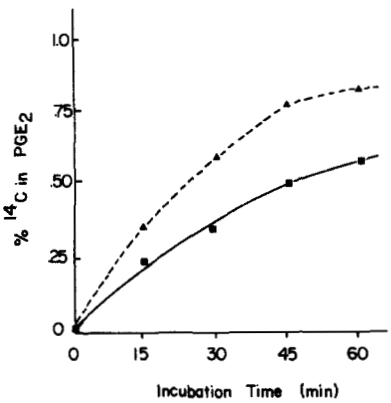

Fig. 5. Time course of conversion of [1-^{14}C]arachidonic acid into PGE_2 by microsomal preparations of aortic intima–media of control (■ — ■) and atherosclerotic rabbits (▲– –▲). [1-^{14}C]-Arachidonic acid was incubated with microsomal preparations of control and atherosclerotic aorta having 2.6 and 2.1 mg of protein per incubation, respectively. Conditions of incubation and identification of radioactive products are described under Methods. The results are expressed as percentages of ^{14}C recovered in the PGE_2 fraction. The experimental animal had been fed the atherogenic diet for 9 months.

TABLE 1. Biosynthesis of PGE_2 from [1-^{14}C]arachidonic acid by subcellular fractions of rabbit aorta and skin

Fraction	^{14}C in PGE_2	
	Aorta	Skin
	dpm/mg protein	
Homogenate	200	163
10,000 *g* supernatant	308	367
10,000 *g* pellet	306	465
105,000 *g* supernatant	106	178
105,000 *g* pellet	4,440	2,054

The incubation mixtures consisted of 5 ml of subcellular fraction obtained from normal rabbit aortas or skin, homogenized in 0.1 M potassium phosphate buffer (pH 7.4), 0.65 mM glutathione, 0.55 mM hydroquinone and [1-^{14}C]arachidonic acid (2.0 μCi). The incubation was at 37°C for 30 min. The protein concentrations of each subcellular fraction are described under Methods.

0.5 mg of PGE_2 and subjected to reverse phase partition chromatography (23) **(Fig. 3).** The elution of PGE_2 was traced by conversion of PGE_2 in an aliquot from each fraction into PGB_2, which was subjected to reverse phase partition chromatography in chloroform–heptane and methanol–water. The results are shown in **Fig. 4.** Again the ^{14}C was eluted with the material which exhibited absorbance at 278 nm. These results gave evidence that the radioactive product formed from [1-^{14}C]arachidonic acid by aorta homogenates was PGE_2. Additional identification was furnished by TLC of the radioactive material recovered from the column.

Incubation of [1-^{14}C]arachidonic acid with homogenates of skin from rabbits fed a control diet gave products with identical chromatographic mobilities as those shown in Figs. 1 and 2. The radioactive material corresponding to PGE_2 was treated with 0.5 N NaOH, and the product had the chromatographic mobilities of PGB_2.

Subcellular localization of synthetase activity

The conversion of [^{14}C]arachidonic acid into PGE_2 by the various subcellular fractions of rabbit aorta and skin is shown in **Table 1.** The results indicate that the microsomal fraction (105,000 *g* pellet) of both tissues had most of the biosynthetic activity of PGE_2. These findings are consistent with published reports that the endoplasmic reticulum is the site of prostaglandin biosynthesis (27, 28).

Time course of ^{14}C incorporation from [1-^{14}C]arachidonic acid into PGE_2

The time course of the incorporation of ^{14}C from [1-^{14}C]-arachidonic acid into PGE_2 by microsomal preparations of intima–media of control and experimental animals is shown in **Fig. 5.** The activity was linear for approximately 45 min. The preparation from atherosclerotic aorta exhibited greater activity. A similar comparison between preparations from skin lesions of an experimental animal and skin of a control rabbit is presented in **Fig. 6.** The activity was also linear for 45 min. and the preparation from skin lesions had activity approximately 3 times that of the control.

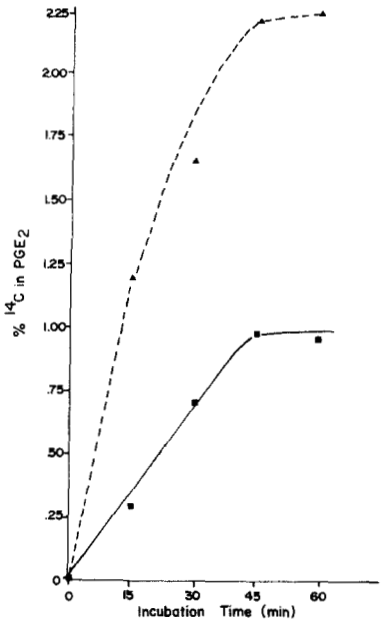

Fig. 6. Time courses of conversion of [1-^{14}C]arachidonic acid into PGE_2 by microsomal fractions of skin from a control rabbit (■—■), and skin lesions (▲– –▲) from an experimental rabbit that had been fed the atherogenic diet for 9 months. Arachidonic acid was incubated with microsomal fractions obtained from skin homogenized in 0.1 M potassium phosphate buffer. Control and lesion skin had 13.1 and 10.5 mg protein per incubation, respectively. Conditions of incubation and identification of radioactive products are described under Methods. The results are expressed as percentages of ^{14}C recovered in the PGE_2 fraction. Data are the averages of results from duplicate incubations.

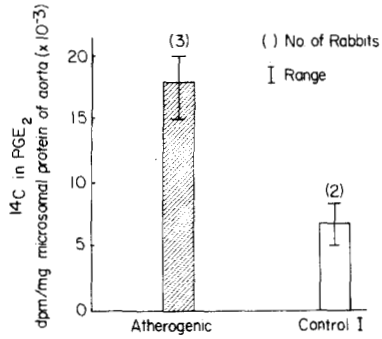

Fig. 7. PGE_2 biosynthesis by microsomal fraction of rabbit aorta. [1-^{14}C]Arachidonic acid was incubated with the microsomal fraction obtained from rabbits fed the atherogenic diet and rabbits fed the control 1 diet for 7 months. Control and atherosclerotic aortas had an average of 2.3 and 2.0 mg of protein per incubation, respectively. The open bar represents synthesis of controls. Duplicate determinations were performed for each animal. The [^{14}C]PGE_2 formed by preparations from experimental animals was 2.5 times that of controls.

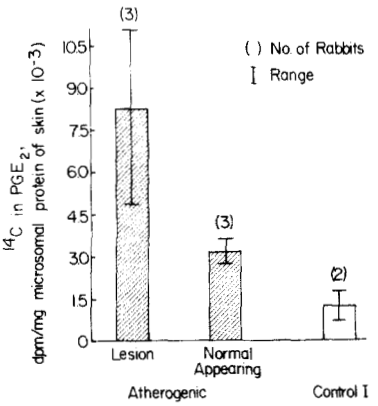

Fig. 8. PGE_2 biosynthesis by microsomal preparations of rabbit skin. [1-^{14}C]Arachidonic acid was incubated with microsomal preparations of skin lesions and normal-appearing skin of animals fed the atherogenic diet and animals fed control 1 diet for 7 months. Control, normal-appearing and lesion skin had an average of 15.5, 15.0, and 12.5 mg of protein per incubation, respectively. The preparations from skin lesions of the experimental animals had greater activity than that of the normal-appearing skin, which in turn had greater activity than that from skin of control animals.

TABLE 2. Arachidonic acid content of microsomal pellets of skin and aorta

Tissue	μg/mg Protein
Aorta	
Control	0.032
Experimental	0.071
Skin	
Control	0.101
Experimental	
Normal appearing	0.090
Lesion	0.414

Total lipids were extracted from the 105,000 *g* pellets and transesterified by refluxing for 60 min under N_2 in methanol containing 5% HCl. The methyl esters of fatty acids were subjected to GLC on a 15% DEGS polyester column. Quantitation was by triangulation. Protein was determined by the method of Lowry et al. (20).

Comparative study of aorta and skin

The above observation was repeated with microsomal fractions of aorta and skin from additional control and experimental rabbits. After 7 months of feeding, aortas of the experimental rabbits had extensive atherosclerosis and their skin manifested visible lesions. The conversion of [1-^{14}C]-arachidonic acid into PGE_2 per mg microsomal protein of aorta from three experimental animals during 30 min of incubation was approximately 2.5 times that of controls **(Fig. 7).** The comparison of the activities in skin from control animals versus lesioned and normal-appearing skin from experimental animals is shown in **Fig. 8.** The activity in the normal-appearing skin of the experimental animals was approximately 3 times that of the control animal, and the activity in skin lesions was approximately 7 times that of control.

To test whether the observed increased incorporation of ^{14}C into PGE_2 by microsomes of diseased tissues was due to a deficiency of endogenous precursor, arachidonic acid of the microsomal preparations was determined. **Table 2** shows

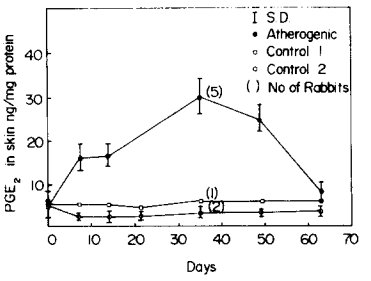

Fig. 9. Early changes in PGE_2 level in rabbit skin. Biopsy specimens of skin were obtained with the "snipper" from the backs of rabbits on three different diets: Control 1, Purina rabbit chow only; control 2, Purina rabbit chow plus 2% cottonseed oil (w/w); and atherogenic, Purina rabbit chow plus 1% cholesterol and 2% cottonseed oil (w/w). PGE_2 levels were determined by GLC according to Jouvenaz, et al. (25), and protein by the method of Lowry, et al. (20).

that the highest content was in the microsomal pellet of lesioned skin which also synthesized the greatest amount of [^{14}C]-labeled PGE_2. The ratio of 5,8,11-eicosatrienoic acid (20:3, n-9) to arachidonic acid (20:4, n-6) in the skin lesion was 0.073. This ratio did not meet the criterion for essential fatty acid deficiency as proposed by Aaes-Jørgensen and Holman (29).

Early changes in PGE_2 level in rabbit skin

The levels of PGE_2 in the skin of rabbits fed the control 1 and control 2 diets and the atherogenic diet during 63 days are shown in **Fig. 9.** The PGE_2 level in the skin of animals fed the control diets was 2–12 ng/mg skin protein and did not vary significantly throughout the experimental period, while the level in animals fed the atherogenic diet increased 2 to 3-fold in the first two weeks and continued to rise, reaching a peak of 30 ng/mg skin protein in 35 days. Thereafter, the level decreased gradually towards that in the controls. At the 63rd day, the level was within the range of the controls.

DISCUSSION

The present study demonstrated that rabbit aorta and skin can transform arachidonic acid into PGE_2. This transformation was established by column and thin-layer chromatography in several solvent systems (Fig. 1–4). A metabolite having chromatographic mobility similar to $PGF_{2\alpha}$ (Fig. 2) was also detected, and its identification needs further confirmation. Formation of PGE_2 from [1-^{14}C]arachidonic acid by rabbit aorta has been reported by Hollander, et al. (30), and conversion of homo-γ-linolenic acid into PGE_1 by rabbit aorta has been reported by Christ and van Dorp (31). Table 1 shows that this biosynthetic activity was mainly in the microsomal fraction of both the aorta and the skin. This subcellular localization is consistent with previous finding on PGE_2 synthetase activity in human epidermis (32).

The time courses of the conversion of arachidonic acid into PGE_2 by microsomal preparations were linear for approximately 45 minutes for both aorta (Fig. 5) and skin (Fig. 6). The diseased tissues converted [1-^{14}C]arachidonic acid into [^{14}C]-PGE_2 at greater rates.

The data in Figs. 7 and 8 indicate greater amounts of [^{14}C]-PGE_2 synthesized in 30 min by microsomal preparations of both the aorta and the skin from the experimental animal. Since these preparations had higher contents of arachidonic acid (Table 2), these results indicate greater PGE synthetase activity rather than a result of greater dilution of the isotope by endogenous arachidonic acid of the control preparations.

Fig. 9 shows the changes in PGE_2 level in skin of rabbits on the three different diets over 63 days. Only the atherogenic group had a marked increase in the level of PGE_2, which reached a maximum of 30 ng/mg skin protein in 35 days. This value was significantly higher than control values of 2–12 ng/mg skin protein ($P < 0.01$), which remained essentially unchanged throughout the experimental period. After the maximum was reached, the PGE_2 level decreased gradually to that of controls by the end of 2 months. However, prostaglandin synthetase activity remained high even after 7 months (Fig. 6). The decline in PGE_2 level in the face of increased synthetase activity may reflect increased degradation of PGE_2 during prolonged cholesterol feeding.

These results suggest that the skin may be useful as a model to study the biological and biochemical responses to cholesterol feeding. The easy accessibility to the tissue and the feasibility of obtaining serial biopsy specimens for longitudinal studies using the same animals offer special advantages in such experiments. In harmony with this line of thought, Kritchevsky (19) proposed using lipid metabolism in xanthomatoses as an approach to the study of atherosclerosis. It is also of interest to note that a report by Ho and Taylor (33) indicated that the skin and the aorta have greater affinity for cholesterol than many other tissues studied, and electron microscopic studies of Parker and Odland (17) showed comparable morphological changes, particularly increase of foam cells in xanthomas as in atheromas during experimental atherogenesis.

It has been reported that PGE_1 and PGE_2 inhibit cholesteryl ester synthetase (12, 13) and $PGF_{2\alpha}$ stimulates cholesteryl ester hydrolase (14). It is conceivable that PGE_2 level in skin during the early phase of diet-induced atherosclerosis may lead to decreased cholesteryl esters in this tissue and thus influence the course of xanthomatosis.

Although our results indicate enhanced prostaglandin synthetase activity and an elevated level of PGE_2 after cholesterol feeding, they provide no evidence that PGE_2 is causally related to atherogenesis. Further studies are necessary to clarify the possible role of PG in the atherogenic process, either primary or secondary to an inflammatory reaction.

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