standard (AES) method. The scintillators used were a) dioxan–toluene–ethylcellosolve (75:15:10) containing 4.0 g/liter of PPO, 0.4 g/liter of dimethyl–POPOP, and 100 g/liter of naphthalene, b) toluene containing 4.0 g/liter of PPO and 0.3 g/liter of dimethyl–POPOP.

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9) Reproducibility was not so good probably due to variation in the activity of alumina catalyst. Details are now being investigated in our laboratory.
10) S. Okada and O. Tamemasa, *Radioisotopes* (Tokyo), **14**, 42 (1965).

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Prostaglandin E_2 like Activity in Monkey Lung[1)]

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Recently, the occurrence of derivatives of prostaglandin (PG) has been demonstrated in the lungs of some species, such as sheep,[3)] pigs,[3a)] cattle,[4)] guinea pigs,[3a)] monkies,[3a)] and humans.[3a,5)] Prostaglandin F (PGF) series, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was found and identified in the lungs of all species, whereas prostaglandin E (PGE) series, prostaglandin E_2 (PGE_2) was proved to be present in only two species, namely sheep[3a)] and humans.[5)] The amount of the above compound in these species was found to be approximately one percent of the $PGF_{2\alpha}$ content. It seems of interest to investigate the occurrence and the contents of the PGE series compounds in the lungs of other species.

The present paper reports the occurrence of a compound which has a PGE_2 like activity in the lung of monkies (*Macaca irus*), in addition to $PGF_{2\alpha}$, the presence of which in monkey lungs has been previously reported by Äggård.[3a)]

An acidic lipid substance, which stimulates smooth muscle and reduces blood pressure, was separated from monkey lung by the use of organic solvent distribution, column chromatography and thin-layer chromatography (TLC). Treatment of this lipid compound is briefly outlined in Chart 1.

Fig. 1 shows the distribution of smooth muscle stimulating activity of the active component in fraction X on silver nitrate silicagel plate, which was found to be useful for the separation of the derivatives of PG with different degrees of unsaturation to each other.

1) Part of this work was presented at the Meeting of the Chugoku–Shikoku Branch, Pharmaceutical Society of Japan, Nov. 1968.
2) Location: *No. 78, Shomachi–1–chome, Tokushima.*
3) *a*) E. Äggård, *Biochem. Pharmacol.*, **14**, 1057 (1965); *b*) S. Bergström, F. Dressler, L. Krabisch, R. Ryhage and J. Sjövall, *Arkiv. Kem.*, **20**, 63 (1962); *c*) E. Äggård and B. Samuelsson, *Acta Physiol. Scand.*, **59**, suppl., **213**, 170 (1963).
4) B. Samuelsson, *Biochem. Biophys. Acta* (Amst.), **84**, 707 (1964).
5) S.M.M. Karim, M. Sandler, and E.D. Williams, *Brit. J. Pharmacol.*, **31**, 340 (1967).

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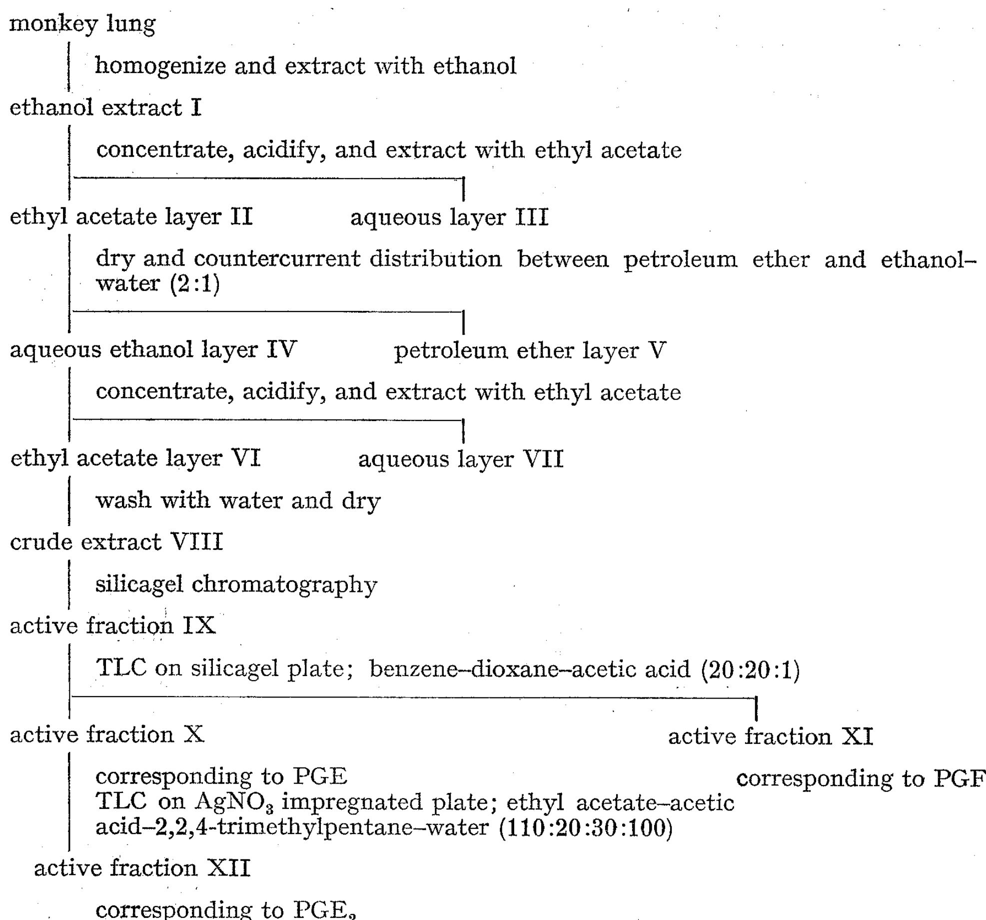

Chart 1. Separation Procedure

The *Rf* value of the active component in fraction X, which was obtained from the active zone corresponding to the PGE group on another silicagel plate, is close to that of authentic PGE_2.

In addition to strong smooth muscle stimulating activity, the active fraction XII shows a marked depressor activity on rats and rabbits. This strong depressor action of this fraction persists after treatment with atropine, diphenhydramine, or propranolol on rats.

Furthermore, we compared the derivatives of PGE and the active fraction XII with the effects on some isolated smooth musle organs and those on blood pressure in two species. The results are shown in Table 1. The relative activities of fraction XII are parallel to those of PGE_2 in each biological effect. Judging from this result, the active component of fraction XII may be regarded as PGE_2.

Considering these results, we assume that the isolated material from monkey lung may contain the compound which has the biological and chemical properties closely resembling PGE_2. Assuming that the active compound is PGE_2,

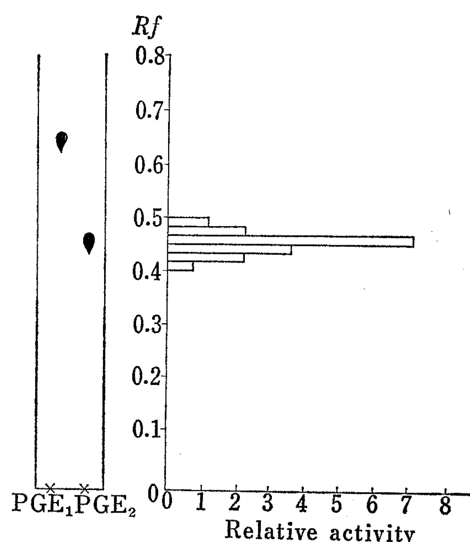

Fig. 1. Distribution of Smooth Muscle Stimulating Activity on TLC

Silver nitrate impregnated plate.
AcOEt-AcOH-2,2,4-trimethylpentane-water (110:20:30:100)

the content of which can be estimated as being approximately 0.02—0.03 μg per gram tissue: The result of which was obtained from the recovery of authentic PGE_2 through the same procedure. This content is approximately one per cent of that of $PGF_{2\alpha}$[3a] as in other species.

Table I.[a] Comparison of the Biological Activities between the Derivatives of PG and the Active Fraction XII on Some Isolated Smooth Muscle Organs and the Blood Pressures.

	PGE_1	PGE_2	PGE_3[b]	Fraction XII	PGE_2/Fraction XII
Rabbit duodenum	1	5.5	0.5	1.00	5.5
Rat jejenum	1	0.3		0.05	6.0
Rat stomach	1	1.1		0.21	5.2
Guinea pig ileum	1	1.6	0.23	0.27	5.9
Rat uterus	1	1.2	0.31	0.25	4.8
Rat blood pressure	1	0.5		0.10	5.0
Rabbit blood pressure	1	0.6	0.32	0.10	6.0

a) Each value is the average of three experiments and expresses the relative activity in relation to the activity of PGE_1. As to fraction XII, its aliquot portion is calculated in the same manner. In the last column are shown the ratios of the relative activity of fraction XII to those of PGE_2 in each biological effect.

b) B. Samuelsson, *J. Biol. Chem.*, **238**, 3229 (1963); E. W. Horton and I.H.M. Main, *Brit. J. Pharmacol. Chemotherap.*, **21**, 182 (1963)

Experimental

Material——Monkey Lung: Nine hundred grams of monkey (*Macaca irus*) lung obtained at bleeding were kept frozen at −20°. Silicagel plates for TLC: Commercially obtained plates "Spot film silicagel" (Tokyo Kasei Kogyo K.K., Tokyo) were used. Silver nitrate impregnated plate: The "Spot film silicagel" was dipped into a silver nitrate solution (40 g $AgNO_3$+100 ml Water+300 ml EtOH) for one minute, and activated in a vacuum oven at room temperature for one hour prior to use. Silicagel for column chromatography: "Kiesel gel" (Merck, Germany) was activated at 105° for one hour before use.

Extraction and Preliminary Separation[3a]——The lungs were homogenized and four times the volume of 96% ethanol was added. The suspention was left at room temperature over night with stirring. After centrifugation the clear supernatant was siphoned off. The insoluble residue was resuspended in the same volume of 70% ethanol, and after recentrifugation the second clear supernatant was added. The combined ethanol extract was evaporated under reduced pressure to about one twentieth of the original volume. This solution was acidified to pH 3 with 0.1N HCl and then extracted four times with the same volume of ethyl acetate. After washing the combined ethyl acetate layer was evaporated to dryness under reduced pressure. The residue was subjected to a three stage countercurrent distribution between petroleum ether and ethanol–water (2:1). The combined aqueous ethanol layer was evaporated under reduced pressure to about one twentieth of the original volume with nitrogen leak at 40°. This solution was acidified to pH 3 with 0.1N HCl and then extracted four times with the same volume of ethyl acetate. The combined ethyl acetate layer was washed with water to make the solution neutral and evaporated to dryness. These procedures give the crude extract VIII.

Silicagel Chromatography——Two grams of silicagel were suspended in ethyl acetate–benzene (20:80), and poured in the glass column. The obtained crude extract VIII was dissolved in the same solvent and applied to the column. The column was eluted successively with 10 ml of ethyl acetate-benzene (20:80), 40 ml of benzene–dioxane–acetic acid (20:20:1) and 40 ml of methanol. The smooth muscle stimulating activity on isolated rabbit duodenum was concentrated in the second fraction. This active fraction IX was dried under reduced pressure.

Separation of PGE like Substance——PGE like substance was isolated from fraction IX with TLC. The sample was applied on line on the plate with authentic samples on both sides. The plate was developed with benzene–dioxane–acetic acid (20:20:1).[6]

After cutting off of the region of authentic samples, the reference spots were detected by spraying with 10% alcoholic phosphomolybdic acid and heating at 105° for 15 minutes. The remaining region was scraped off with the range of *Rf* value 0.016, and the each region was transferred to the test tube. Each sample was extracted with methanol, and aliquot portion of extract was taken, dried and dissolved in saline solution.

6) K. Green and B. Samuelsson, *J. Lipid Res.*, **5**, 117 (1964).

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The solution were applied for the assay on isolated rabbit duodenum preparation. Two active zones were found, and their *Rf* values were found to correspond to those of the PGE group and PGF group respectively. The extracts, corresponding to the PGE group, were combined, dried and applied to further purification.

Separation of PGE_2 like Substance——The active fraction X which showed PGE like activity was further purified with TLC[6] on silver nitrate impregnated silicagel plate. Fraction X was applied on line with authentic samples of PGE_1 and PGE_2 on both sides. The plate was developed with ethyl acetate–acetic acid–2,2,4-trimethylpentane–water (110:20:30:100). Both reference sides were cut off and the reference spot were detected with 10% alcoholic phosphomolybdic acid. Distribution of smooth muscle stimulating activities on the remaining region of the plate was demonstrated as mentioned above. This procedure gives fraction XII.

Bioassay——The isolated organs were suspended in a bath of controlled temperature, oxigenated with air. The movements were recorded isotonically on a smoked drum with a linear frontal writing lever. Uterus Ringer solution was used for isolated uterus and Tyrode solution for other isolated organs. Compositions of these solutions are as follows: Uterus Ringer solution: NaCl 0.9%, KCl 0.02%, $CaCl_2$ 0.004%, $NaHCO_3$ 0.05% and glucose 0.05%. Tyrode solution: NaCl 0.8%, KCl 0.02%, $CaCl_2$ 0.02%, $MgCl_2$ 0.0093%, $NaHCO_3$ 0.1%, NaH_2PO_4 0.004%, and glucose 0.1%. Experiments *in vivo* were performed on rabbits and rats anesthetized with urethane (2 g/kg body weight *s.c.*). The trachea was canulated and arterial blood pressure was measured by means of mercury manometer.

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On the Structure of Morphine-6-glucuronide in a Solid State

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Recently we have reported the first synthesis of morphine-3- and -6-glucuronides together with the synthesis of codeine glucuronide.[2] It has been subsequently established that both the morphine glucuronides are excreted as the metabolites of morphine in several experimental animals and in human, although the amount of 6-glucuronide excreted is much less than that of 3-isomer.[3,4] For the pharmaceutical study of these glucuronides, a rather large amount of morphine-6-glucuronide became necessary to be synthesized, and therefore several runs of the synthesis of this glucuronide were carried out by the same method as reported previously.[2] However, subsequently obtained crystals of morphine-6-glucuronide (I_b), mp 251—256° (decomp.), exhibited a completely different IR spectrum from that of the former sample (I_a), mp 254—256° (decomp.). This article reports that 6-glucuronide possesses two different structures in a solid state.

The IR spectrum of I_a showed a strong peak at 1750 cm^{-1} which suggested that I_a should exist in the structure possessing a non-ionized carboxyl group shown in Fig. 1. On the other

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1) Location: *Katakasu, Fukuoka.*
2) H. Yoshimura, K. Oguri and H. Tsukamoto, *Tetrahedron Letters*, **1968**, 483; *idem, Chem. Pharm. Bull.* (Tokyo), **16**, 2114 (1968).
3) H. Yoshimura, K. Oguri and H. Tsukamoto, *Biochem. Pharmacol.*, **18**, 279 (1969).
4) H. Yoshimura, S. Îda, K. Oguri, E. Hasegawa and H. Tsukamoto, Meeting of Kyushu Branch, Pharmaceutical Society of Japan, Fukuoka, July 1968.

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