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The fate of prostaglandin A_1-5,6-^3H in the rat

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The plasma concentrations, tissue distribution and excretion of prostaglandin A_1 (PGA_1) and related metabolites have been determined in rats, following the intravenous injection of a single dose of PGA_1-5,6-^3H. Urinary and faecal excretion accounted for averages of 25 and 43%, of the administered dose of PGA_1, respectively. Oxidative cleavage of the carboxyl side chain of PGA_1 appeared to be a major metabolic pathway in the rat. PGA_1-5,6-^3H was deemed unsuitable for metabolism studies in man, in view of the significant loss of tritium label from the prostaglandin.

Antihypertensive and diuretic effects of intravenously infused prostaglandin A_1 (PGA_1) have been demonstrated in man (Carr, 1970; Westura, Kannegiesser & others, 1970; Fichman, 1971; Lee, McGiff & others, 1971a; Lee, Kannegiesser & others, 1971b). However, little has been published on the *in vivo* metabolism, distribution and excretion of PGA_1 in experimental animals or man. We have investigated the fate of PGA_1 in the rat, and evaluated the suitability of the commercially available radioactive PGA_1 (which is tritium-labelled on C-5 and C-6) for metabolism studies in man.

MATERIALS AND METHODS

Tritium-labeled PGA_1. PGA_1-5,6-^3H, purchased from New England Nuclear (specific activity 0·14 Ci mg^{-1}), was mixed with non-radioactive PGA_1. The specific activity of the diluted PGA_1-^3H administered to the rats was 188×10^6 d min^{-1} mg^{-1}. The purity was established by t.l.c. on: (a) untreated, pre-coated silica gel plates in ethyl acetate–acetic acid–2,2,4-trimethyl pentane–water (9:2:5:10) (organic phase), and (b) on ferric-chloride-impregnated silica gel plates in ethyl acetate–hexane–acetic acid (30:19:1) (Wickramasinghe & Shaw, 1973).

Animals. Male Upjohn Sprague-Dawley rats (~190 g average body weight), were housed in metabolism cages.

Preparation of dose. Absolute ethanol (0·4 ml) was added dropwise to dissolve the diluted PGA_1-^3H (see above) (0·774 mg). Sterile 0·5 M sodium chloride (2·6 ml) was added dropwise with mixing, taking care to avoid turbidity.

Drug administration. Each rat [lightly anaesthetized with Metofane (1,1-difluoro-2,2-dichloroethyl methyl ether) by inhalation] received a bolus injection of 0·2 ml containing 50 μg of PGA_1 ($9{\cdot}4 \times 10^6$ d min^{-1}) ($267 \pm 5\,\mu g\ kg^{-1}$), into the left femoral vein. Identical doses were delivered into volumetric flasks, diluted and counted.

Specimen collection

Blood samples (about 5 ml) were collected 1, 2, 5, 10, 15, 30, 60, 120, 240 or 600 min after dosing, using one rat for each sample; the carcasses were reserved for tissue analysis (see below). Samples (up to 15 min) were withdrawn from the exposed dorsal aorta of the rats (arterial blood); samples at 30 min and later were obtained by heart puncture. These were collected in heparinized tubes, centrifuged for 10 min at about 2000 rev min^{-1}, and the plasma stored at −18° until analysed (about 3 days later).

Timed *urine and faecal samples* were collected from two of the rats whenever an adequate sample was available during the 0–12 h after dosing. Thereafter, specimens were collected at 12 or 24 h intervals up to 312 h. Control urine and faecal samples were collected from each rat for 24 h before dosing. All samples were stored at −18°. The animals were killed and their carcasses frozen after specimen collection.

Sample preparation and analysis. Radioactivity counting of all samples was with a liquid scintillation spectrometer. Counting efficiencies for tritium were determined by the internal standard technique using appropriately labelled toluene standards.

Urine samples. Aliquots (0·5 ml) were counted in 18 ml diotol scintillation solvent [toluene–dioxane–methanol (35:35:21) containing naphthalene (73 g), 2,5-diphenyl oxazole (4·6 g) and 1,4-bis-2-(phenoxazolyl)benzene (0·08 g) $litre^{-1}$]. Separate 2 ml aliquots of the urine samples were lyophilized, and 0·5 ml aliquots of the trapped water were counted in diotol.

Faecal samples. Each sample was homogenized with five times its weight of water. Duplicate weighed aliquots of this homogenate were dried in cellophane sacs and then combusted by the Schoniger oxygen-

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flask technique. The resulting tritiated water was counted in diotol. Combustion efficiency was determined using homogenized faeces from control rats, and added PGA_1-^3H, with appropriate corrections.

Plasma samples. Aliquots (0·5 ml) in Cellophane sacs were air dried, combusted and counted in diotol. Tritiated water was determined by lyophilizing 1 ml plasma plus 1 ml water, and 0·5 ml of the trapped water was counted in diotol (18 ml).

Organ and tissue samples. The organs and tissues indicated in Table 2 were excised from the carcass of each rat used for blood collection at 2, 15, 60 and 600 min after administration of the PGA_1-5,6-^3H. Larger organs (wet weight >0·4 g) were homogenized with 4 times the weight of water. Aliquots in Cellophane sacs (~0·5 g) were air dried and combusted. The resultant tritiated water was counted in a solution consisting of 22·7 g PPO and 0·45 g POPOP/4·55 l toluene: biosolve BBS-3 (Beckman) (85:15). Smaller organs (e.g., eyes, thyroid) were air dried whole in Cellophane sacs and combusted. For tissues like fat, representative portions were homogenized and weighed aliquots combusted. Representative portions of washed, shaved skin were dried directly in sacs and combusted.

Rat carcasses for radioactive residues. The skin was removed from the two rats killed after collecting urine and faeces, and the entire remains were homogenized with four times the weight of water. Aliquots (~0·5 g) of the homogenate were dried, combusted and counted and combustion efficiencies were determined using control tissue. The tritiated water content of each carcass was determined by lyophilizing 2 ml aliquots of the homogenate and analysing the trapped water. The total water content of each carcass was assumed to be 70% of the total weight.

Representative portions (~50 mg) of shaved skin were dried and combusted as indicated above.

RESULTS AND DISCUSSION

PGA_1-5,6-^3H had to be examined before use in metabolism studies. In view of the high specific activity the PGA_1-5,6-^3H (0·14 Ci mg^{-1}) could not be analysed for any PGB_1 by conventional methods, e.g., ultraviolet spectroscopy. The thermal lability of PGA_1 and its derivatives makes gas chromatography with radioactivity monitoring unsuitable for assessing radiochemical purity. Thin-layer chromatography on untreated silica gel, in conjunction with radioactivity scanning indicated that most of the radioactivity was associated with the composite PGA_1-PGB_1 zone (both having similar mobilities). A small fraction (1·1%) of the radioactivity remained at the origin. This fraction recurred on extraction and rechromatography of the PGA_1-^3H zone, and therefore appeared to originate during chromatography. Thin-layer chromatography on ferric chloride-impregnated silica gel (a procedure which separates PGA, PGB and PGC; Wickramasinghe & Shaw, 1973), showed that on sequential rechromatography 0·7 to 2·7% of the radioactivity was associated with the PGB_1 zone. Since this fraction recurred on rechromatography of the extracted PGA_1 zone, it represents the extent of formation of PGB_1 from PGA_1 during chromatography and/or subsequent extraction. Thus PGA_1-5,6-^3H was deemed to be radiochemically homogeneous.

Plasma concentration and metabolism

The plasma concentrations of PGA_1 radioactivity after intravenous administration of the PGA_1-^3H, are given in Table 1. Peak plasma concentrations of radioactivity equivalent to 1·751 μg PGA_1 ml^{-1} were observed 1 min after drug administration (estimated lower limit of detection being 1 ng PGA_1 equivalents, based on a radioactivity count of twice the background count). The plasma PGA_1-^3H radioactivity disappearance half-life in rat was estimated by semilog plotting of PGA_1 related radioactivity concentrations against time which appeared to give a biphasic curve. The half-life from the initial portion was estimated to be 3·0 min and that from the terminal portion was 48·0 min. Radioactivity due to any tritiated water originally present in the plasma was determined separately, by lyophilizing aliquots of the plasma and counting the trapped water. When a sample of control rat plasma containing added PGA_1-^3H was lyophilized the trapped water had no radioactivity. Thus, tritiated water in the plasma of the PGA_1-5,6-^3H treated rats most probably results from the loss of the tritium label from C-5 due to β-oxidative cleavage of the carboxy side chain leading to the formation of nor-metabolic products of PGA_1. The loss of tritium from the α-carbon atom (*viz.* C-6) of the resulting tetra-nor carboxy acid by some equilibration process although possible, has not been observed in the case of similar steroid acids during *in vivo* biotransformations (Varma, Wickramasinghe & Caspi, 1969) implying that no unlabelled PGA_1 metabolites would be expected to result from the metabolism of PGA_1-5,6-^3H by known prostaglandin metabolism pathways. A measure of the appearance of tritiated water in the plasma of the PGA_1-5,6-^3H treated rats may thus reflect the extent of formation of the nor-metabolic products of PGA_1 by β-oxidative cleavage of the

Table 1. *Plasma content of PGA_1-^3H- and metabolites-related radioactivity in rats (I) given an intravenous injection of PGA_1-5,6-^3H (267 $\mu g\ kg^{-1}$).*

Time after admin. (min)	I[a] (ng equiv ml^{-1})	^3H_2O[b] (% of total plasma radioact.)
1	1751	0·7
2	1441	1·9
5	653	3·5
10	198	15·4
15	114	27·4
30	52	40·9
60	30	46·4
120	14	75·0
240	12	79·7
600	12	82·9

[a] Excluding ^3H_2O. [b] Derived from PGA_1-5,6-^3H by the metabolic oxidation of the carboxy side chain.

carboxy chain. 30 min after drug infusion, as much as 41% (at 2 h, 75%) of total radioactivity in the plasma was tritiated water (Table 1). Apparently oxidative cleavage of the carboxy side chain of PGA_1 is a major metabolic pathway in the rat.

Tissue distribution

Most of the available information on the tissue distribution of exogenously administered prostaglandins relates to PGE_1 and $PGF_{2\alpha}$ (Samuelsson, 1964; Hansson & Samuelsson, 1965; Green, Hansson & Samuelsson, 1967; Holmes & Horton, 1968; Nakano, 1970; Landolt, Shaw & others, 1974).

We therefore examined the distribution of PGA_1 (I) and its metabolites in the tissues of the rats treated with PGA_1-5,6-^3H. Since the metabolism of PGA_1 and its clearance with the metabolites from the plasma appeared to occur rapidly, the sites of metabolism and (perhaps pharmacological activity) are likely to be those tissues which show relatively high concentrations of PGA_1 radioactivity shortly after drug administration.

Maximum tissue concentrations of PGA_1-related radioactivity were found as early as 2 min after administration, and then decreased in most organs examined (except the spleen and the reservoirs of excreta, e.g., the bladder and intestine) (see Fig. 1 and Table 2). As with PGE_1 (Samuelsson, 1964; Hansson & Samuelsson, 1965; Nakano, 1970) and $PGF_{2\alpha}$ (Green & others, 1967), maximum concentrations of PGA_1-related radioactivity were found in the kidneys (Table 2) but the concentration of PGA_1 related material in the kidney relative to that in the liver was higher. Thus, the kidney to liver radio-

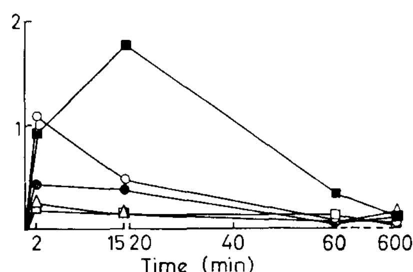

FIG. 1. Tissue uptake of radioactivity in rats, after administration of a single i.v. dose of PGA_1-5,6-^3H (267 $\mu g\ kg^{-1}$; 9·4 × 10^6 d min^{-1} rat^{-1}). ○—○ Heart, ■—■ spleen, △—△ eye, □—□ brain, ●—● adipose tissue. y axis—% Dose g^{-1} of tissue found.

activity concentration ratio in the PGA_1-treated rats was 1·6 times that in PGE_2-treated rats (Samuelsson, 1964). In contrast to PGE_1 for which urinary excretion was the major route of elimination (Samuelsson, 1964; Hansson & Samuelsson, 1965), biliary (faecal) excretion is the major route of elimination of the intravenously administered PGA_1 related radioactivity in rats. Hence the relatively higher early concentrations of PGA_1-related radioactivity found in the kidneys suggests them to be an important site for the metabolism (and/or pharmacological activity) of PGA_1 especially since it is rapidly metabolized and excreted. This is supported

Table 2. *Tissue concentrations of PGA_1-related radioactivity in rats at 2, 15 min, 1 and 10 h after administration of a single intravenous dose of PGA_1-5,6-^3H (267 $\mu g\ kg^{-1}$; 9·4 × 10^6 d min^{-1} rat^{-1}).*

Tissue	% dose g^{-1} tissue[a] (time after dosing) 2 min	15 min	1 h	10 h
Liver[c]	2·27	1·52	0·25	0·24
Kidney[c]	4·74	2·66	0·18	0·12
Thyroid[b]	2·78	1·05	0·73	1·15
Adrenal[b]	2·31	1·10	0·17	0·28
Blood (plasma)[d]	2·85	0·23	0·06	0·02
Spleen[c]	0·93	1·78	0·34	0·12
Lung[c]	1·30	0·43	0·12	0·06
Brain[c]	0·19	0·14	0·13	0·03
Eye[b]	0·25	0·14	0·07	0·17
Adipose tissue[d]	0·43	0·38	0·06	0·09
Heart[c]	1·09	0·48	0·09	0·05
Skin[d]	0·00	0·26	0·13	0·39
Bladder[b]	0·28	14·14	3·14	0·11
Small intestine[c]	0·57	0·66	3·75	2·33
Large intestine[c]	0·35	1·10	0·42	6·42
Stomach[c]	0·17	0·23	0·05	0·12

[a] Based on wet weight of tissue. [b] Whole organ combusted. [c] Homogenized and an aliquot combusted. [d] Representative portion combusted.

by the work of Attalah, Payakkapan & Lee (1974), who demonstrated that the kidney cortex was capable of extensively metabolizing the closely related PGA_2. The observations of Golub, Zia & others (1974), are not in agreement since they suggest the liver to be the major site of PGA_1 metabolism.

The PGA_1-related radioactivity concentration in the lung tissue was significantly lower than that in plasma (Table 2); the converse was true with PGE_1 (Samuelsson, 1964; Hansson & Samuelsson, 1965).

Other than kidneys and liver, significant concentrations of PGE_1-related radioactivity were reported to have been found only in connective tissue, lung and uterus (Samuelsson, 1964; Hansson & Samuelsson, 1965; Nakano, 1970). In contrast, significantly high concentrations of PGA_1-related radioactivity (higher than or equivalent to plasma) were found in the thyroid and adrenal gland (Table 2); the concentrations in the thyroid, adrenals and plasma were higher than that found in the liver. These relatively high concentrations of PGA_1 (and/or related metabolites) observed in the thyroid and the adrenal gland may relate to the hormonal role suggested for PGA_1 (Golub & others, 1974). The PGA_1-related material appears to persist in the thyroid gland since relatively higher radioactivity concentrations were present even 10 h after administration (being 57 times that in the plasma at this time interval) (Table 2). This observation may relate to the postulate that certain prostaglandins may play an important role in TSH (thyrotropin) stimulation of cyclic AMP formation and hormonogenesis in the thyroid gland (Burke, 1970; Burke, Kowalski & Babiarz, 1971; Burke & Sato, 1971; Kowalski, Sato & Burke, 1972; Sato, Szabo & others, 1972; Yu, Chang & Burke, 1972; Burke, 1974). The relatively high concentrations of PGA_1-related radioactivity in the adrenal tissue may be associated with its postulated role in the stimulation of aldosterone secretion (Finchman & Horton, 1973; Fichman, Littenberg & others, 1973).

A significant concentration of PGA_1-related radioactivity (higher than that found in the lung) was also observed in the spleen (see Fig. 1 and Table 3). The maximum was reached at 15 min. Lower concentrations of PGA_1-related radioactivity were also found in heart, adipose tissue, eye, brain, stomach and skin (see Fig. 1 and Table 2). The high concentrations of PGA_1-related radioactivity found in the intestines (small and large) and the urinary bladder, particularly at the later times (see Table 2), probably reflect the progress of biliary and urinary excretion, respectively.

Table 3. *Recovery of PGA_1-^3H radioactivity from rats treated with an intravenous dose of PGA_1-^3H (267 $\mu g\ kg^{-1}$).*

	% of i.v. administered dose excreted		
Mode of excretion	Rat 1	Rat 2	Mean
Urine[a]	25·7	25·0	25·4
Faeces	50·1	36·8	43·5
^3H_2O in Urine	5·8	7·0	6·4
^3H_2O lost in respiration[b]	5·8	7·0	6·4
^3H_2O in carcass[c]	2·6	1·8	2·2
Total ^3H_2O	14·2	15·8	15·0
Total radioactivity	89·0	77·6	83·8

[a] Excluding ^3H_2O.
[b] Estimated from ^3H_2O in urine.
[c] Determined experimentally by analysis of carcass.

Urinary and faecal excretion

After the intravenous administration of PGA_1-^3H to two rats, urinary excretion accounted for a mean of 32% of the radioactive dose, during a 13-day collection (see Table 3). Faecal excretion similarly accounted for an average of 43% of the administered radioactive dose (see Table 3). Analysis of urine samples indicated the presence of tritiated water. A total of 6·4% (mean) of the radioactive dose was present as tritiated water in the urine. An approximately equivalent fraction of the radioactive dose may be assumed to have been expired as tritiated water during respiration. After 13 days the carcasses were analysed for ^3H_2O and radioactive PGA_1 residues. Tritiated water corresponding to a mean of 2·2% of the administered PGA_1-^3H dose was found (Table 3). No nonvolatile PGA_1-related radioactive residues were present. Thus, a total of 84% (mean) of the administered radioactive dose was accounted for. The graphical representation of cumulative urinary, faecal and combined urinofaecal excretion data (Fig. 2 shows typical curves) clearly indicate that the excretion of PGA_1-^3H radioactivity was nearly complete by the second day.

From the large fraction of the administered PGA_1-^3H radioactivity recovered in the faeces (43%), it is apparent that biliary excretion is a major route of elimination of PGA_1 and/or related metabolites. The PGA_1-^3H urinary excretion profile in rats is compatible with that observed in a man by Professor Samuelsson (private communication). In the latter instance, cumulative urinary excretion during 21 h after the PGA_1 accounted for 29% (25% after 5 h; 27% after 12 h) of a subcutaneously administered dose of PGA_1-5,6-^3H(10 μg).

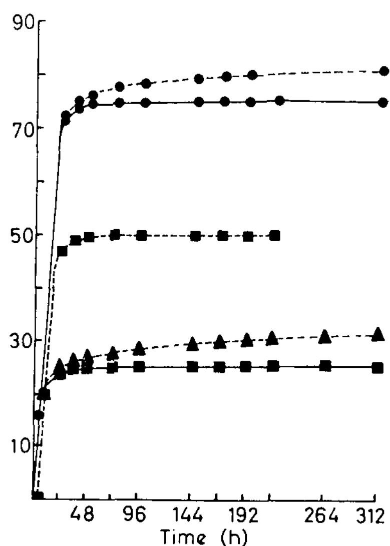

FIG. 2. Typical curves for cumulative excretion of radioactivity (represented by rat 1) after single dose (267 μg kg^{-1}) i.v. administration of PGA$_1$-5,6-^{3}H. ●— —● total urine and faeces including ^3H_2O, ●—● total urine and faeces excluding ^3H_2O, ■- -■ faeces, ▲- -▲ urine including ^3H_2O, ■—■ urine excluding ^3H_2O. y axis—% of administered dose.

The urinary and faecal excretion half-times were estimated graphically and the initial values are summarized in Table 4. Although the combined urinary and faecal excretion half-times may not be of pharmacokinetic significance, they indicate the rate of elimination of total radioactivity.

Table 4. *Excretion half-times of PGA$_1$-^{3}H radioactivity*[a] *in rats treated with an intravenous dose of PGA$_1$-^{3}H* (267 μg kg^{-1}) I—Initial half time (h). II—Interval (h).

Rat	Urine		Faecal		Total	
No.	I	II	I	II	I	II
1	7·4	0–30	4·8	0–43	20	0–192
2	9·6	0–38	6·7	0–48	22	0–192

[a] Tritiated water excreted has been excluded.

In view of the significant loss of tritium label from PGA$_1$-5,6-^{3}H during metabolism (as indicated by the relatively large quantity of resultant tritiated water), this material with the tritium label at C-5 and C-6 was deemed unsuitable for metabolism studies in man.

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