radioactivity remaining is much higher than in the controls.

The inhibitory effect of angiotensin II on noradrenaline or metaraminol uptake was also manifested in the present experiments in lung tissue. Inhibition was greater than that obtained in perfused rabbit hearts (Peach, Bumpus & Khairallah, 1969) in which maximal inhibition of metaraminol uptake was about 50%. In the present experiments angiotensin II inhibited the uptake of [^{3}H]metaraminol in lung to about 70% in the doses used. From the washout experiments the rate of removal of radioactivity was followed. The loss of radioactivity from lung is rapid at the beginning of the washout, thereafter there is slow disappearance and in controls declined to about zero during the first 5 min of washout. In this case the radioactivity remaining after 20 min of washout was about 1%. When angiotensin II was present in the medium during the washout period the amount of radioactivity remaining was much higher, i.e. about 45%. The initial slope of the disappearance curves can be used for the determination of the half-life of [^{3}H]metaraminol, i.e. the time in which the amount of radioactivity drops to 50% was 1–2 min for control, and about 10 min for angiotensin II.

The present study has demonstrated that angiotensin II inhibits the uptake of [^{3}H]metaraminol in rat lung. The findings obtained in washout experiments indicate the possibility of the existence of two different sites at which metaraminol is bound in the lung tissue.

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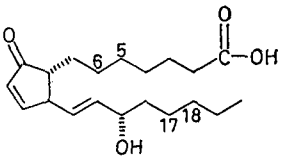
REFERENCES

DAVILA, D. & DAVILA, T. (1975a). *Archs int. Pharmacodyn. Thér.*, **214**, 53–61.
DAVILA, T. & DAVILA, D. (1975b). *Ibid.*, **215**, 336–344.
DAVILA, D. & KHAIRALLAH, P. A. (1970). *Ibid.*, **185**, 357–364.
DUNNETT, C. V. (1955). *J. Am. Stat. Ass.*, **50**, 1096–1121.
PALAIC, DJ. & KHAIRALLAH, P. A. (1967). *Biochem. Pharmac.*, **16**, 2291–2298.
PEACH, M. J., BUMPUS, F. M. & KHAIRALLAH, P. A. (1969). *J. Pharmac. exp. Ther.*, **167**, 291–299.
PEACH, M. J., CLINE, W. H. JR., DAVILA, D. & KHAIRALLAH, P. A. (1970). *Eur. J. Pharmac.*, **11**, 286–292.

The fate of prostaglandin A_1-17,18-^{3}H in the dog

A. J. WICKREMA SINHA*, S. R. SHAW, *Drug Metabolism Research, The Upjohn Company, Kalamazoo, MI 49001, U.S.A.*

An earlier communication described the metabolic fate of the commercially available PGA_1-5,6-^{3}H in the rat (Wickrema Sinha & Shaw, 1977). This material which bears the tritium-label on C-5 and C-6 of the prostaglandin was deemed unsuitable for metabolism studies in man, due to the significant loss of its tritium label during metabolism (Wickrema Sinha & Shaw, 1977). The loss of tritium was attributed to β-oxidative cleavage of the carboxy side chain of the prostaglandin. Consequently, radioactive PGA_1 bearing the tritium label on C-17 and C-18 was synthesized (Hsi, 1977) and the fate of the PGA_1-17,18-^{3}H was investigated in the dog.



Each of four fasted Beagle dogs (two males and two females; average weight 9 kg) received a bolus injection of 2 ml of a solution of PGA_1-17,18-^{3}H (specific activity 0·153 Ci mg^{-1}) in 0·15 M sodium chloride with 0·1% sodium bicarbonate, into the left jugular vein. The bolus contained 335 μg of PGA_1 ($113·4 \times 10^6$ d min^{-1}) (38 μg kg^{-1}). Plasma, urine and faeces samples were collected and analysed (Wickrema Sinha & Shaw, 1977). The titriated water content of the urine and plasma were determined (Wickrema Sinha & Shaw, 1977).

Peak plasma concentrations of radioactivity equivalent to 516 ng of PGA_1 ml^{-1} were observed 5 to 10 s after administration of the prostaglandin, with a second peak at 10 to 15 min after drug administration (see Table 1). The second peak may correspond to the appearance of metabolites of PGA_1 in the plasma, or the release of PGA_1-^{3}H related material from a tissue depot. The initial plasma PGA_1-^{3}H radioactivity disappearance half-life in dog was estimated to be 1 min, suggesting rapid clearance of intact PGA_1. The half-life of 70 min associated with a terminal portion of the disappearance curve probably corresponds to the clearance of the metabolites of PGA_1.

The tritiated water found in the time interval plasma samples of the PGA_1-17,18-^{3}H treated dog is presented in Table 1. The appearance of tritiated water was indicative of the loss of the tritium label from C-18 due to ω-oxidation of PGA_1 (or one of its metabolites) followed by β-oxidative cleavage of the two terminal

* Correspondence.

Table 1. *Blood content of PGA_1-^3H related radioactivity in dog 2 given an intravenous injection of PGA_1-17,18-^3H (38 $\mu g\ kg^{-1}$).* (I) Time after admin. (min); (II) PGA-^3H and related metabolites* (ng equivalents ml $^{-1}$ plasma); (III) ^3H_2O† (% of total plasma radioactivity); (IV) % of whole blood radioactivity localized in washed cellular fraction‡.

I	II	III	IV
0·08–0·17	516	0·5	16·2
1·5	133	1·4	14·3
2·5	83	2·4	20·4
4·0	39	4·7	20·9
7·0	42	4·6	21·0
10	55	3·0	14·9
15	48	4·2	15·3
30	26	6·3	17·7
69	19	7·9	19·2
120	9	13·7	16·8
180	4	28·9	0
240	3	34·2	0
360	2	44·9	0
600	1	70·0	0

* Excluding ^3H_2O.
† Resulting from the metabolic oxidation of the lower alkyl carbon chain of PGA_1.
‡ Sedimented by centrifugation during the isolation of plasma.

carbon atoms, in a manner analogous to that observed in the metabolism of $PGF_{2\alpha}$. A measure of the upper carboxy chain β-oxidative cleavage process in the case of PGA_1-5,6-^3H (as measured by the appearance of ^3H_2O) was reported earlier (Wickrema Sinha & Shaw, 1977). Comparison of the quantities of tritiated water derived from the metabolism of PGA_1-^3H (~15% from PGA_1-5,6-^3H with ~1% from PGA_1-17,18-^3H), suggested that the β-oxidative cleavage of the upper carboxy side chain may be a much more important metabolic pathway than the ω-oxidation and β-oxidative cleavage of the lower alkyl chain of PGA_1.

Circulating prostaglandins have been shown to be bound chiefly to serum albumin (Raz, 1972; Attallah & Schussler, 1973). Recently, Smith, Kocsis & others (1973) and Cagen, Pisano & Fales (1974) reported that PGA_1 is taken up by the red-cells in human blood, metabolized and released as more polar, inactive metabolites, based on observations from *in vitro* incubations. We, therefore examined the localization of PGA_1-^3H radioactivity in the cellular fractions from blood samples from the PGA_1-17,18-^3H treated dog. The sedimented cellular fraction from each heparinized blood sample was washed, disrupted and analysed for radioactivity (see Table 1). A more or less constant fraction (~17%) of the PGA_1-^3H related radioactivity was localized in the cellular fraction, the concentration profile following that in the plasma. No special concentration of PGA_1-^3H similar to that observed in kidney tissue (Wickrema Sinha & Shaw, 1977) was apparent in the red blood cells at the early time intervals when the prostaglandin was being extensively metabolized. Thus, although some metabolism of the administered PGA_1-^3H may occur in the red blood cells, these cells did not seem to be a major site for the metabolism of this prostaglandin in the dog. Evidence from a tissue distribution study earlier indicated that the kidney may be the major site of *in vivo* metabolism (Wickrema Sinha & Shaw, 1977).

Table 2. *Recovery of PGA_1-^3H radioactivity from dogs given an intravenous injection of PGA_1-17,18-^3H (38 $\mu g\ kg^{-1}$).*

Mode of excretion or recovery	% of administered dose				
	Dog 1 ♀	Dog 2 ♀	Dog 3 ♂	Dog 4 ♂	Mean with s.d.
Urine*	45·70	39·33	50·52	50·85	46·60 s.d. 5·39
Faeces	49·67	60·03	43·58	43·74	49·25 s.d. 7·72
^3H_2O lost in urine	0·52	0·44	0·82	0·35	0·53 s.d. 0·20
^3H_2O lost in respiration†	0·52	0·44	0·82	0·35	0·53 s.d. 0·20
Total recovery	95·88	99·80	94·92	94·94	96·39 s.d. 2·32

* Excluding ^3H_2O.
† Estimated from ^3H_2O in urine.

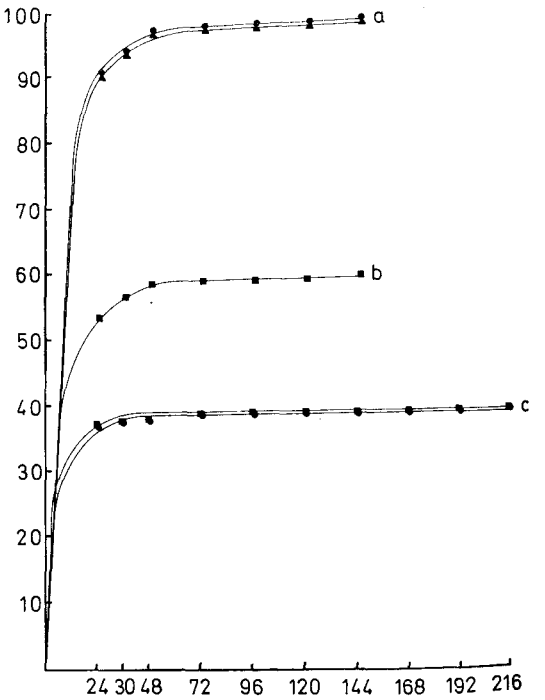


FIG. 1. Typical curves for cumulative excretion of radioactivity (represented by dog 2) after single dose intravenous administration of PGA_1-17, 18-^3H (38 $\mu g\ kg^{-1}$). a—▲ Total (urine and faeces excluding ^3H_2O), ● total (urine and faeces including ^3H_2O). b—■ Faeces. c—■ Urine (including ^3H_2O), ● urine (excluding ^3H_2O). Ordinate: % of administered dose. Abscissa: Time (h).

Table 3. *Excretion half-times of PGA_1-^3H radioactivity* in dogs treated with an intravenous dose of PGA_1-17,18-^3H (38 μg kg^{-1}). (I) half-time (h) and (II) interval (h).*

Dog no.	Urine		Faeces		Total	
	I	II	I	II	I	II
1	10·0 66·5	0– 48 48–192	14·8	0–120	36·0	0–144
2	25·9 168·0 10·1	0– 72 72–144 144–192	29·2	0–120	35·6	0–144
3	3·9 60·0	0– 24 24–168	25·6	0–120	36·0	0–120
4	1·7 50·4	0– 24 24–168	21·6	0–120	26·0	0–120

* Tritiated water excreted has been excluded.

After the intravenous administration of PGA_1-^3H to four dogs, urinary excretion accounted for an average of 47% of the radioactive dose (see Table 2). Faecal excretion similarly accounted for an average of 49% of the administered dose. Excretion of the PGA_1-^3H related radioactivity in the urine and faeces was almost complete in 48 h after drug administration (Fig. 1 shows typical curves). An average of 0·5% of the radioactive dose administered was present as tritiated water in the urine (see Table 2). An approximately equivalent fraction may be assumed to have been expired as tritiated water during respiration.

Both urinary and biliary excretion appear to be equally important routes for the elimination of exogenous PGA_1 and related metabolites in the dog (see Table 2). In this respect, the dog appears to differ from the rat (Wickrema Sinha & Shaw, 1977), in which biliary (faecal) excretion was the major route of elimination of exogenous PGA_1 and related metabolites. Comparison of these excretion profiles with those in man (Wickrema Sinha & Shaw, 1977) suggested that man may resemble the rat more closely than the dog in this respect.

The urinary, faecal, and the combined urinary and faecal excretion half-times (which indicate the rate of elimination of total radioactivity from the body) are summarized in Table 3.

Thus, the tritium label in PGA_1-17,18-^3H is metabolically stable, and this material is suitable for use in metabolism studies in man.

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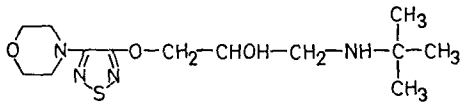
REFERENCES

ATTALLAH, A. & SCHUSSLER, G. C. (1973). *Prostaglandins*, **4**, 479–484.
CAGEN, L. M., PISANO, J. J. & FALES, H. M. (1974). *Fedn Proc. Fedn Am. Socs. exp. Biol.*, **33**, 576.
HSI, R. S. P. (1977). *J. Labelled Compds Radiopharm.*, in the press.
RAZ, A. (1972). *Biochem. J.*, **130**, 631–636.
SMITH, J. A., KOCSIS, J. J., INGERMAN, C. & SILVER, M. J. (1973). *Pharmacologist*, **15**, 208.
WICKREMA SINHA, A. J. & SHAW, S. R. (1977). *J. Pharm. Pharmac.*, **29**, 99–103.

The disposition of timolol in man

P. VERMEIJ*, M. EL SHERBINI-SCHEPERS†, P. A. VAN ZWIETEN†, *Pharmacy, Andreas Ziekenhuis, Th. de Bockstraat &, Amsterdam, The Netherlands and †Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, The Netherlands*

Timolol, 1-(*t*-butylamino)-3-(4-morpholino-1,2,5-thiadiazol-3-yl)-oxy-2-propanol] (I), is a relatively new β_1- + β_2-adrenoceptor blocking drug, resembling propanolol and sotalol in that it has no intrinsic sympathicomimetic activity (Waal-Manning, 1976). Unlike propranolol, it is devoid of local anaesthetic properties and on a molar base it is about 10× more potent (Waal-Manning, 1976; Achong, Piafsky & Ogilvie, 1976).



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Little is known on the disposition of timolol, and the pharmacokinetic data are scarce and even controversial (Tocco, de Lunn & Duncan, 1975a; Tocco, Duncan & others, 1975b). For these reasons we have examined the pharmacokinetic behaviour of the drug in healthy volunteers.

Three female and 2 male volunteers, ages 22–35 years, 52–85 kg, participated. They did not receive any other drug, took a light breakfast and moved around freely.

Initially, they ingested an aqueous solution of timolol maleate (1·0 mg timolol base ml^{-1}), to give 0·1 mg timolol base kg^{-1}, about 2 h after breakfast. In other experiments, doses up to 0·4 mg kg^{-1}, were also given. Blood samples were taken at regular intervals. Subsequently, in a second series of experiments, 6 weeks later, the same subjects received 3·7 mg timolol given as the commercial preparation†. The

* Correspondence.

† Blocadren (MSD). Tablets containing 10 mg timolol maleate (= 7·4 mg timolol).