METABOLISM AND INTERCONVERSION

OF

PROSTAGLANDINS

by

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TABLE OF CONTENTS

DECLARATION

I declare that this thesis has been composed by me and that the work described in it is my own.

SUMMARY

The partial purification of prostaglandin A isomerase from cat plasma by a 3-stage process comprising fractional protein precipitation by the addition of solid ammonium sulphate, ion exchange chromatography on DEAE Sephadex and gel chromatography on Sephadex G-200 is described. This procedure resulted in a 16 to 40 fold purification. Two spectroscopic methods for the assay of prostaglandin A isomerase have been developed. The first measured the formation of prostaglandin B_1 at pH 8.5 whereas the second measured formation of prostaglandin C_1, which during the course of this study was discovered by R.L. Jones, to be the true product of the enzyme. The pH and temperature characteristics of the enzyme were determined using both assays. The effect of substrate concentration and enzyme concentration on the rate of enzymatic isomerisation of prostaglandin A_1 were studied using the second assay. The enzyme had a pH optimum of between 7.5 and 9.5 and was maximally active at a temperature of $45^{\circ}C$. It was inactivated by temperatures of about $55^{\circ}C$ or above. An approximate K_m of prostaglandin A isomerase was found to be $5.5 \times 10^{-5}M$. The enzyme has been detected in the plasma of rabbit, cat, dog and rat but not of man, guinea pig, sheep or ox. The richest source is rabbit plasma.

Although prostaglandins of the A series have been extracted from various sources, a route by which these compounds are synthesised in tissues has not yet been reported. Since they are formed readily from prostaglandin E by dehydration, the existence of a prostaglandin E dehydrase was postulated. A search for this enzyme has been made in some organs and tissues which might synthesise prostaglandin A. Of these, rat kidney did convert prostaglandin E_1 to less polar material but this could not be identified as prostaglandin A_1. The biosynthesis

of prostaglandin A from endogenous and exogenous fatty acids by male reproductive tract from the domestic fowl, the baboon and man was examined. Subsequently, these tissues were incubated with prostaglandin E but in no case could evidence be obtained that prostaglandins of the A series are formed, either from fatty acid precursors or from the corresponding prostaglandin E. Thus the presence of endogenous prostaglandin A (as reported by others previously) in semen and male reproductive tract of the domestic fowl could not be confirmed.

The presence of prostaglandin E_2, but not prostaglandin A_2, was demonstrated conclusively in baboon seminal vesicles by argentous thin layer chromatography, ultraviolet spectroscopy and combined gas chromatography-mass spectrometry.

There have been reports that prostaglandin E_1 is metabolised by human plasma to a compound which co-chromatographs with prostaglandin A_1. Careful repetition of this work failed to substantiate these claims and in addition did not provide even tentative evidence that human plasma contains a prostaglandin E dehydrase.

These results raise once again the question of the extent to which prostaglandins of the A series occur artefactually in tissue extracts. The evidence for and against this conclusion is discussed fully.

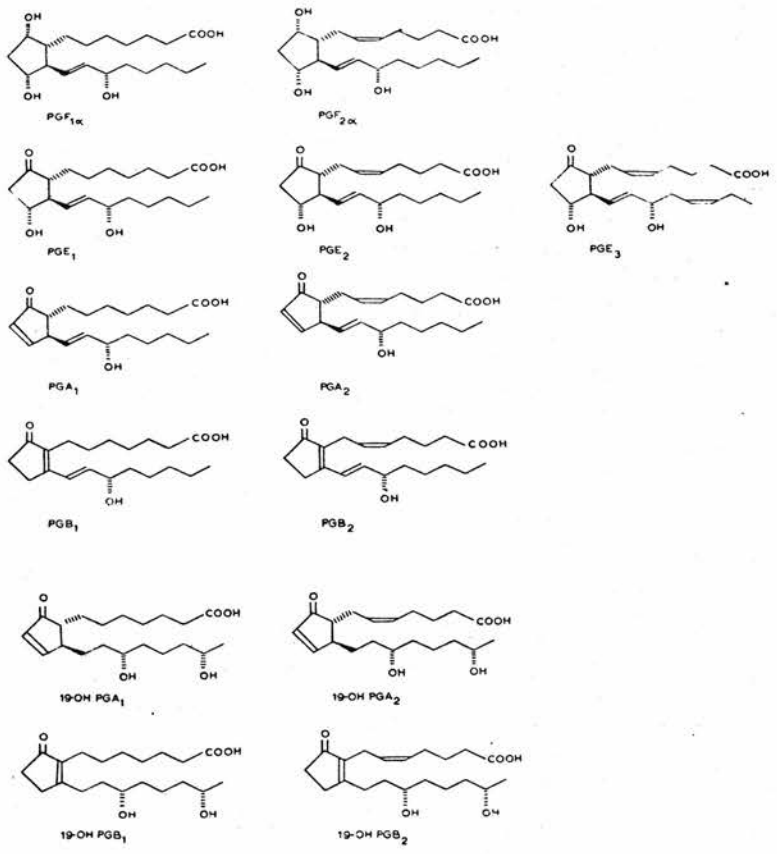
INTRODUCTION

Historical

The smooth muscle stimulating and depressor activities of human semen observed independently by Goldblatt (1935) and von Euler (1936) were attributed to "prostaglandin". Subsequent analysis of human semen (Bergström and Samuelsson, 1962; Samuelsson, 1963; Bygdeman, 1964; Hamberg and Samuelsson, 1965; Bygdeman and Samuelsson, 1966a; 1966b; Hamberg and Samuelsson, 1966a) has led to the isolation and identification of a total of 13 different prostaglandins. Their structures are shown in Figure 1, page 4. It can be seen that prostaglandins are 20 carbon unsaturated fatty acids. Prostaglandins of the A, E and F series differ from each other in the substituents on the cyclopentane ring; 19-OH prostaglandins are hydroxylated at C-19. The subscript 1, 2 or 3 denotes the degree of unsaturation of the side chains. Prostaglandins A's are potent depressor agents, but have little activity on non-vascular smooth muscle (Bergström, Carlson and Öro, 1967; Pike, Kupiecki and Weeks, 1967; Weeks, Chandra-Sekhar and DuCharme, 1969; Horton and Jones, 1969). Thus the depressor activity and some of the smooth muscle stimulating activity of human semen is accounted for by prostaglandins of the A, B, E and 19-OH series. Hydroxylation of A prostaglandins at C-19 reduces their biological potency slightly (Bygdeman and Hamberg, 1967; Jones, 1970a) and isomerisation of the 10,11 double bond to the 8,12 position reduces depressor activity considerably. Smooth muscle stimulating activity is not affected by hydroxylation at C-19 (Bergström et al., 1967; Jones, 1970a). The 19-OH prostaglandin B's have very low depressor and smooth muscle stimulating potencies (Jones, 1970a). Whereas the E prostaglandins are

Figure 1

Structures of 13 naturally occurring prostaglandins.



potent depressor (Bergström et al., 1967; Pike et al., 1967; Weeks et al., 1969; Nakano, 1969) and smooth muscle stimulating agents (Pike et al., 1967; Bergström, Carlson and Weeks, 1968; Weeks et al., 1969), prostaglandins of the F series are inactive on vascular smooth muscle in some species and are pressor agents in others (Bergström et al., 1968). Like the E prostaglandins, those of the F series are active on non-vascular smooth muscle (Bergström et al., 1968).

The E and F prostaglandins have also been found in ram semen (Bygdeman and Holmberg, 1966). The concentrations of prostaglandins E_1, E_3, $F_{1\alpha}$ and $F_{2\alpha}$ are similar to those in human semen, that of prostaglandin E_2 is lower whilst A, B and 19-OH prostaglandins have not been detected. "Vesiglandin", extracted from the semen and seminal vesicles of the monkey, "Macacus rhesus", has depressor but very little smooth muscle stimulating activity (von Euler, 1936), and may therefore be accounted for by the A, B or 19-OH prostaglandins. It is of interest that prostaglandin-like activity in semen of some other species examined is very much lower than in the sheep or the human. Goat semen contains prostaglandin-like activity (Eliasson, 1959) but activity could not be detected in the semen from horse, dog, ox (Eliasson, 1959) or rabbit (Horton and Thompson, 1964).

Another source from which prostaglandin A and prostaglandin E have been extracted and identified is the kidney. Depressor activity of homogenates of rabbit kidney is attributed to 3 renomedullary acidic lipids named compound I, compound II and medullin (Lee, Hickler, Saravis and Thorn, 1963). Medullin has been identified as prostaglandin A_2 by combined gas chromatography-mass spectrometry (Lee, Crowshaw, Takman, Attrep and Gougoutas, 1967). Compound II was identified as prostaglandin E_2 and compound I as prostaglandin $F_{2\alpha}$. Three biologically active lipids which co-chromatograph with

prostaglandins A_2, E_2 and $F_{2\alpha}$ have been extracted from dog renal medulla (Crowshaw, McGiff, Strand, Lonigro and Terragno, 1970). The same prostaglandins have been detected in canine renal venous blood during ischaemia (McGiff, Crowshaw, Terragno and Lonigro, 1970) and increased levels were found in rabbit renal venous blood during renal nerve stimulation (Davis and Horton, 1972). The prostaglandins detected in renal venous blood are released from the kidney. However it is not certain whether prostaglandin A_2 extracted from the kidney and from renal venous blood is biosynthesised by the kidney, or whether it is an extraction artefact. Dehydration of prostaglandin E_1 to prostaglandin A_1 in 90% acetic acid at 60°C (Daniels, Hinman, Johnson, Kupiecki, Nelson and Pike, 1965), at pH 1-2 and room temperature for 60 minutes (Strong and Bohr, 1967) and during extraction with mineral acids (Nugteren, Beerthuis and van Dorp, 1966a) occurs. Non-enzymatic formation of prostaglandin A is therefore likely to occur during extraction of prostaglandin E. The extent to which prostaglandin E is dehydrated can be modified by altering the extraction procedure used (Schneider, Pike and Kupiecki, 1966; Strong and Bohr, 1967). Thus Strong and Bohr (1967) did not confirm the presence of medullin in renal medullary extracts shown by Lee et al. (1963). Using a double labelling technique, Schneider et al. (1967) showed that non-enzymatic dehydration of prostaglandin E could account for prostaglandin A extracted after incubating sheep seminal vesicle prostaglandin synthetase with dihomo-γ-linolenic acid (Daniels et al., 1965). Although a proportion of the A, B and 19-OH prostaglandins in human semen may also be an artefact, 3 findings indicate that most of these prostaglandins are enzymatically derived. The same extraction procedure does not produce detectable amounts of these prostaglandins from E prostaglandins in ram semen (Bygdeman and Holmberg, 1966);

in human semen there are amounts of 19-OH prostaglandins A and B equal to or greater than those of prostaglandin E, yet there are no 19-OH prostaglandin E compounds present from which they could be non-enzymatically formed (Bygdeman and Samuelsson, 1966b); the amounts of prostaglandin A and prostaglandin B are not reduced (Bygdeman, Svanborg and Samuelsson, 1969) in cases of male infertility of no known cause in which the prostaglandin E level is low.

Pharmacology

A comprehensive study of the pharmacology of the A and B prostaglandins and their 19-OH derivatives has been made (Jones, 1970a). This study shows that the biological activity of prostaglandin A on several isolated smooth muscle preparations, including those of the female reproductive tract, is considerably less than that of the corresponding prostaglandin E. Sedation in the cat and the chick produced by actions of A prostaglandins on the central nervous system is only slightly less than that found (Horton and Main, 1965a) for E prostaglandins. However intra-arterial doses of A and E prostaglandins are equipotent on the cat blood pressure, and in inhibiting cat salivary secretion and pentagastrin-induced gastric acid secretion in the rat. A comparison of the biological activities of 19-OH prostaglandin A_1 and 19-OH prostaglandin A_2 with those of prostaglandins A_1 and A_2 shows that hydroxylation at C-19 reduces the potency of prostaglandin A on all systems tested. Isomerisation of the 10,11 double bond to the 8,12 position also reduces the biological activity of prostaglandin A_1 on several non-vascular smooth muscle preparations and on vascular smooth muscle. These results extended other reports that prostaglandin A_1 is less active than prostaglandin E_1 on isolated

non-vascular smooth muscle (Daniels et al., 1965; Lee, Covino, Takman and Smith, 1965; Bygdeman, Hamberg and Samuelsson, 1966; Bergström et al., 1967; Bygdeman and Hamberg, 1967; Pike, 1967) but equiactive on vascular smooth muscle (Lee et al., 1965; Bergström et al., 1967; Pike et al., 1967). In addition, it has been shown that prostaglandin A_1 has less potency than prostaglandin E_1 in inhibiting adenosine diphosphate (ADP) induced platelet aggregation (Kloeze, 1969; Weeks et al., 1969). Prostaglandin A, unlike prostaglandin E, does not inhibit basal or noradrenaline induced lipolysis *in vitro* (Daniels et al., 1965; Bergström et al., 1967; Pike et al., 1967; Weeks et al., 1969) or *in vivo* (Steinberg and Pittman, 1966). Recently another difference between the actions of prostaglandin A_1 and prostaglandin E_1 has been found. Prostaglandin E_1 but not prostaglandin A_1 injected into the 3rd ventricle of unanaesthetised cats and rabbits induces hyperthermia (Milton and Wendlandt, 1971).

Physiology

Many physiological roles have been postulated for F and E prostaglandins because of their ubiquitous distribution and potent, diverse pharmacological effects (Horton, 1969). Fewer speculations have been made about the A prostaglandins.

Prostaglandin release from several organs in response to chemical and nervous stimulation occurs (see Horton, 1972). However the half life of prostaglandins of the E and F series in the circulation is very short (Hamberg and Samuelsson, 1971). Much of the biological activity of these prostaglandins is lost during one passage through the lungs. Therefore prostaglandins E and F are unlikely to be circulating hormones. The A and 19-OH prostaglandins are not inactivated in the lungs (Horton and Jones, 1969) and are therefore

more likely to be possible circulating hormones than E or F prostaglandins.

There is evidence for a circulating natriuretic hormone produced in response to increased extracellular volume (de Wardener, Mills, Clapham and Hayter, 1961; Lichardus and Pearce, 1966; Johnston, Herzog and Lauler, 1967). This hormone has not been identified nor is its source known although there is some evidence that it may originate in the kidney (Johnston, Davis, Howards and Wright, 1967). However it is reported to induce natriuresis in response to saline loading without altering the glomerular filtration rate or the systemic blood pressure (de Wardener et al., 1961). There is also evidence from cross circulation experiments that it may not have a long half life in the systemic circulation (de Wardener et al., 1961). Prostaglandins E_1, E_2 and A_1 induce natriuresis in the absence of changes in glomerular filtration rate and systemic blood pressure (Herzog, Johnston and Lauler, 1968). Prostaglandins E_2 and A_2 have been detected in renal venous blood during renal ischaemia (McGiff, Crowshaw, Terragno and Lonigro, 1970) and after renal nerve stimulation (Davis and Horton, 1972). Prostaglandin A_2 has a longer half life in the circulation of the rat and cat than prostaglandin E_2 because it is not biologically inactivated in the lungs (Horton and Jones, 1969; McGiff, Terragno, Strand, Lee, Lonigro and Ng, 1969), although some loss of activity occurs in the hepatic circulation (Horton and Jones, 1969). On the basis of these findings it has been suggested (Lee, 1967; Horton, 1972) that the circulating natriuretic hormone may be prostaglandin A_2.

Prostaglandin A_2 may also participate in the hypotension produced by the kidney independently of its excretory function (Lee, 1967; 1972; 1973). Administration of extracts of renal medulla to

a binephrectomised rat was shown to prevent hypertension which would normally occur (Muirhead, Jones and Stirman, 1960). Two renomedullary lipids with depressor activity have been extracted from rabbit renal medulla (Lee et al., 1963) and named compound II and medullin, which have been identified as prostaglandin E_2 and prostaglandin A_2 respectively (see page 5). Release of a prostaglandin of the A series from tissue other than the kidney has not been demonstrated.

Male infertility of no known cause is correlated with a low level of E prostaglandins in semen but the concentration of other prostaglandins is not reduced. The function of human seminal A, B and 19-OH prostaglandins, which are not extraction artefacts, is unknown. Therefore it is possible that E prostaglandins in human semen are important for fertility but their role in aiding conception is also unknown.

Biosynthesis

Biosynthesis of prostaglandins of the E and F series has been studied using homogenates or acetone powder preparations of ovine or bovine seminal vesicles (see Horton, 1972). Prostaglandins E_1, E_2, E_3, $F_{1\alpha}$ and $F_{2\alpha}$ have been extracted from ram semen (Bygdeman and Holmberg, 1966), and from ram vesicular glands (Bergström, Dressler, Ryhage, Samuelsson and Sjövall, 1962a; Bergström, Ryhage, Samuelsson and Sjövall, 1962b; Bergström, Ryhage, Samuelsson and Sjövall, 1963). The structures of prostaglandins E_1, $F_{1\alpha}$ and $F_{1\beta}$ (Bergström et al., 1963; Samuelsson, 1963) showed a similarity to those of some 20 carbon fatty acids essential in the diet. Conversion of dihomo-γ-linolenic acid to prostaglandins E_1 and $F_{1\alpha}$ (Bergström, Danielsson, Klenberg and

Samuelsson, 1964a; van Dorp, Beerthuis, Nugteren and Vonkeman, 1964a), arachidonic acid into prostaglandin E_2 (Bergström, Danielsson and Samuelsson, 1964b; Bergström et al., 1964a; van Dorp, Beerthuis, Nugteren and Vonkeman, 1964b) and 5,8,11,14,17-eicosapentaenoic acid into prostaglandin E_3 by ram seminal vesicle glands has been demonstrated. Synthesis of prostaglandins E_1 and $F_{1\alpha}$ from dihomo-γ-linolenic acid of bovine vesicles (Kupiecki, 1965), and by acetone powders of this tissue (Wallach, 1965) have also been shown. It is now established that this synthesis proceeds via a common cyclic endoperoxide intermediate (Samuelsson, Granström and Hamberg, 1967). This intermediate is isomerised to prostaglandins of the E series and reduced to prostaglandins of the F series (Hamberg and Samuelsson, 1973; Nugteren and Hazelhof, 1973).

It has been suggested (Hamberg and Samuelsson, 1967) that human seminal A prostaglandins may be biosynthesised by enzymatic dehydration of E prostaglandins. However attempts to demonstrate biosynthesis even of prostaglandin E and prostaglandin F from essential fatty acids by human male reproductive tract tissue have been unsuccessful (van Dorp, 1966). Human seminal vesicles were incubated with ^{3}H-dihomo-γ-linolenic acid. Even though conversion of this precursor to prostaglandin E_1 by sheep seminal vesicles under the same conditions was 70%, the conversion to prostaglandins obtained using human tissue was less than 0.5%. The identity of the radioactive prostaglandins formed in this low yield by human tissue was not reported. Thus there is as yet no evidence to support the suggestion that an enzyme which dehydrates prostaglandins of the E series is involved in biosynthesis of A prostaglandins in human semen.

Biosynthesis of prostaglandin $F_{2\alpha}$ and prostaglandin E_2 by rabbit renal medulla occurs from endogenous precursors (Crowshaw, 1971)

and from ^{3}H-arachidonic acid (Hamberg, 1969). Synthesis of prostaglandin E_2 from ^{3}H-arachidonic acid has also been demonstrated in canine renomedullary interstitial cells (Muirhead, Germain, Leach, Pitcock, Stephenson, Brooks, Brosius, Daniels and Hinman, 1972). Although prostaglandin A_2 has been extracted from renal interstitial cells, biosynthesis of prostaglandin A_2 has not been demonstrated conclusively because the proportion of prostaglandin A_2 derived non-enzymatically from prostaglandin E_2 was not estimated. It is possible that prostaglandin A_2 biosynthesis in the kidney may be via enzymatic dehydration of prostaglandin E_2.

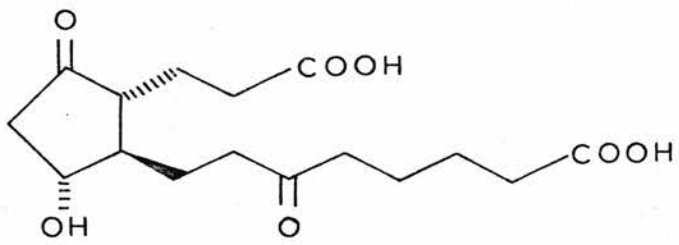
Metabolism and Interconversion

The structures of the principal urinary metabolites of man (Hamberg and Samuelsson, 1971), guinea pig (Hamberg and Samuelsson, 1972) and rat (Gréen, 1971) show that there are species differencies in the metabolism of prostaglandins. Figure 2, page 13, shows the structures of the major urinary metabolites of prostaglandin E_1 or E_2 in man and in the guinea pig. It can be seen that in both compounds oxidation at C-15, reduction of the 13,14 double bond and two steps of β-oxidation have occurred. The human metabolite has resulted from ω-oxidation whereas the guinea pig metabolite has been produced by reduction of the oxo group at C-9. The 9-hydroxyl group formed is in the β configuration.

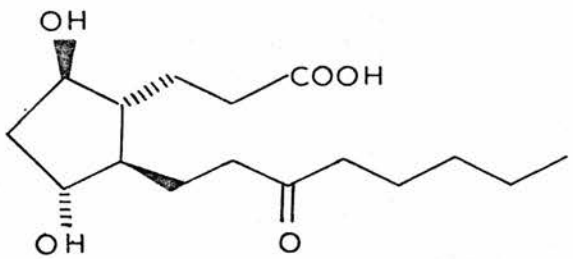
The enzymes responsible for oxidation at C-15 and reduction of the 13,14 double bond are ubiquitous in tissues of the swine (Ångård and Samuelsson, 1970). Oxidation at C-15 is catalysed by a 15-OH prostaglandin dehydrogenase (Ångård and Samuelsson, 1966) which has been partially purified from bovine (Saeed, 1970), and swine (Ångård

Figure 2

Structures of the principal urinary metabolites of man and the guinea pig.



human metabolite



guinea pig metabolite

and Samuelsson, 1966) lung, and from human placenta (Jarabak, 1971). It was the first prostaglandin metabolising enzyme to be partially purified. Studies on partially purified preparations show that it is NAD^+ dependent (Ängård and Samuelsson, 1966) and stereospecific for prostaglandins with the (S) configuration at C-15 (Ängård and Samuelsson, 1966; Shio, Ramwell, Andersen and Corey, 1970). Prostaglandins of the F, E and A, but not the B series, are substrates (Nakano, Ängård and Samuelsson, 1969; Vonkeman, Nugteren and van Dorp, 1969). Dinor and tetranor prostaglandins are poor substrates (Nakano et al., 1969). It is probable that biological inactivation of E prostaglandins in the pulmonary circulation (Ferreira and Vane, 1967; Horton and Jones, 1969; McGiff et al., 1969) is the result of the action of 15-OH prostaglandin dehydrogenase in the lungs. The metabolites of prostaglandin E released into the blood from the lungs have not been identified but 15-oxo dihydro prostaglandin E_1 has been obtained after incubation of the particle free fraction of guinea pig lung homogenates with prostaglandin E_1 or prostaglandin E_2 (Ängård and Samuelsson, 1964; 1965). Oxidation of the hydroxyl group at C-15 reduces the depressor potency of prostaglandin E_1 (Nakano, 1972) and its potency on isolated smooth muscle preparations (Ängård, 1966; Pike et al., 1967).

In guinea pig lungs (Ängård and Samuelsson, 1964) and liver (Hamberg and Samuelsson, 1971a), reduction of the 13,14 double bond of prostaglandin E_1 as well as oxidation at C-15 has also been demonstrated. A second metabolite, 13,14-dihydro prostaglandin E_1, has been identified from particle free fractions of guinea pig lung (Ängård and Samuelsson, 1964; 1965) and liver (Hamberg and Samuelsson, 1971a) homogenates. Although the configuration of the OH group at C-15 is the same in both prostaglandin E and 13,14-dihydro

prostaglandin E, it has been shown *in vivo* (Hamberg and Samuelsson, 1971a) that 13,14-dihydro prostaglandin E is not formed directly from prostaglandin E by reduction of the 13,14 double bond, but that reduction is preceded by oxidation of the 15-OH group. The oxo group formed is then reduced stereospecifically. These two reactions are catalysed by a 15-oxo prostaglandin 13,14 reductase (Ångård and Larsson, 1971). The sequence of reactions has been confirmed *in vitro* in swine lung, liver and kidney homogenates (Ångård and Larsson, 1971). Formation of 15-oxo and 15-oxo dihydro prostaglandins are major initial steps in the *in vivo* metabolism of prostaglandin E_1 and prostaglandin E_2 in man (Hamberg and Samuelsson, 1971b). The half life of ^{3}H-prostaglandin E_1 or E_2 in the systemic circulation is about 2 minutes. Four minutes after injection of ^{3}H-prostaglandin E_1 or E_2, large amounts of 15-oxo dihydro prostaglandin are present. This compound has a longer half life in the blood than prostaglandin E.

Subsequent steps in the metabolism of prostaglandin E in man are β and ω-oxidation. Evidence for β-oxidation of prostaglandin E_1 has been obtained in the perfused rat liver preparation (Dawson, Jessup, McDonald-Gibson, Ramwell and Shaw, 1970). Prostaglandin E_1 and 15-oxo 13,14-dihydro prostaglandin E_1 are good substrates for the β-oxidation system in rat liver mitochondria (Hamberg, 1968). Attempts to demonstrate *in vitro* ω-oxidation of prostaglandins of the E series have been less successful. A maximum conversion of only 3% of prostaglandin E_1 to 19-OH prostaglandin E_1 was obtained by rat liver microsomes (Israelsson, 1969). Hydroxylation at C-20 and hydroxylation of 15-oxo 13,14-dihydro prostaglandin E_1 could not be demonstrated.

There are *in vivo* studies of prostaglandin $F_{2\alpha}$ metabolism in human females in which C_{18}, C_{16} and C_{14} urinary metabolites

Granström and Samuelsson, 1971; Granström, 1971) and metabolites in blood (Beguin, Bygdeman, Gréen, Samuelsson, Toppozada and Wiqvist, 1972; Granström, 1972) have been identified. Although it has been observed that A prostaglandins are resistant to biological inactivation in the pulmonary, but not the hepatic circulation in cats (Horton and Jones, 1969), there are no reports of identification of metabolites from in vivo metabolism of prostaglandin A. In vitro enzymatic isomerisation of prostaglandin A_1 to prostaglandin B_1 by cat blood (Jones, 1970a; 1970b; Polet and Levine, 1971), hydroxylation of prostaglandin A_1 and prostaglandin B_1 at C-19 and C-20 by guinea pig liver microsomes (Israelsson, 1969) and β-oxidation by rat liver mitochondria (Hamberg, 1968) have been demonstrated. There is a difference between metabolism of prostaglandin A_1 and prostaglandin B_1 by the mitochondria. Prostaglandin A_1 is partially converted to a mixture of dinor and tetranor prostaglandin A_1 but prostaglandin B_1 is almost completely converted to dinor prostaglandin B_1. Another difference between prostaglandin A and prostaglandin B metabolism in vitro is that prostaglandins A_1 and A_2 are substrates for partially purified 15-OH prostaglandin dehydrogenase, prostaglandins B_1 and B_2 are not (Änggård and Samuelsson, 1966; Nakano et al., 1969). It is intriguing that although prostaglandin A_1 and prostaglandin A_2 are substrates for partially purified 15-OH prostaglandin dehydrogenase in vitro, they are not biologically inactivated in vivo by this enzyme in the lungs.

Metabolism of prostaglandin E_2 in the rat is of interest as prostaglandin F-type and prostaglandin B-type of compound are amongst urinary metabolites of prostaglandin E_2 (Gréen, 1971). The presence of these metabolites is evidence for the conversion of prostaglandin E into prostaglandins of the F and B series. Interconversion of prostaglandins is also demonstrated by the presence of a 5β OH group

in the major urinary metabolite of the guinea pig, shown in Figure 2, page 13. Reduction of the 9-oxo group of prostaglandin E_2 occurs in the particle free fraction of guinea pig homogenates but the hydroxyl group formed is 9α. It is not known if conversion of prostaglandin E_2 to prostaglandin $F_{2\alpha}$ by guinea pig liver takes place in vivo or whether $F_{2\alpha}$ is converted to $F_{2\beta}$ before excretion in the urine. Conversion of prostaglandin E_2 to prostaglandin $F_{2\alpha}$ by an enzyme associated with sheep erythrocytes has been demonstrated (Hensby, 1974). There is also a report of prostaglandin-9-oxo-reductase in rat tissues which converts prostaglandin E_2 to a compound with affinity for an antibody specific to prostaglandin $F_{2\alpha}$ (Leslie and Levine, 1973).

The presence of prostaglandin B-type compounds amongst rat urinary metabolites of prostaglandin E_2 suggests that, in the rat, conversion of prostaglandin E to prostaglandin B also occurs. It is possible that this conversion is achieved by enzymatic dehydration of prostaglandin E to prostaglandin A by a prostaglandin E dehydrase, and subsequent isomerisation to prostaglandin B by a prostaglandin A isomerase.

When the work to be described in this thesis was started, a prostaglandin A isomerase had been reported in cat plasma (Jones, 1970b). Preliminary studies on a partial purification procedure for the enzyme and on its pH and temperature characteristics had also been described (Jones, 1970a). In the work presented in this thesis, these preliminary observations have been extended. The species distribution of prostaglandin A isomerase has been studied, new assay procedures have been developed and the enzyme has been sufficiently purified to enable its characteristics to be investigated in greater detail.

In view of the possibility discussed above that A prostaglandins are formed from E prostaglandins by a dehydrase, a search was

undertaken for such an enzyme system. A prostaglandin E dehydrase may participate in the biosynthesis of prostaglandin A by the kidney, and may account for prostaglandin A found in renal venous blood (McGiff et al., 1969; Davis and Horton, 1972). Such an enzyme may also be involved in the biosynthesis of human seminal prostaglandins of the A and B series (Hamberg and Samuelsson, 1967). There are reports that human plasma metabolises prostaglandin E_1 to less polar material which co-chromatographs with prostaglandin A_1 (McDonald-Gibson, McDonald-Gibson and Greaves, 1972a). Therefore human plasma may be yet another source of prostaglandin E dehydrase. For these reasons the possible metabolism of prostaglandin E by kidney, male reproductive tract tissue and plasma has been investigated.

SECTION I

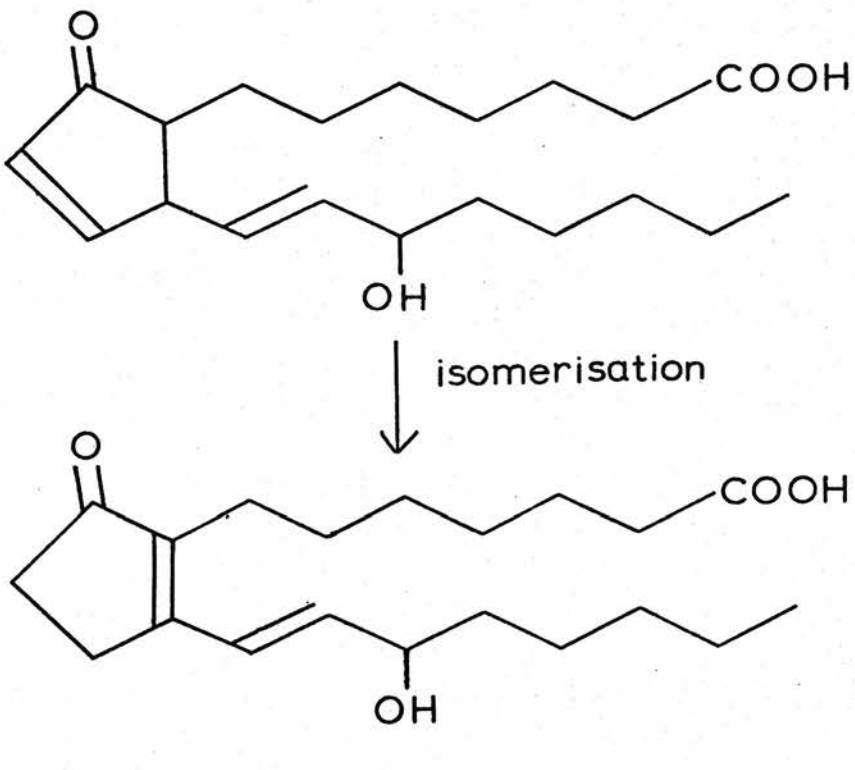
PARTIAL PURIFICATION AND PROPERTIES OF A PROSTAGLANDIN A ISOMERASE

Introduction

During an investigation into the pharmacology of prostaglandin A_1, loss in depressor activity, as assayed on the kitten blood pressure (Horton and Jones, 1969), was observed after incubation of prostaglandin A_1 with cat blood at 37°C (Jones, 1970a). This was initially attributed to metabolism to the biologically less potent isomer, prostaglandin B_1 (Figure 3, page 20). This compound was detected in cat plasma after incubation of the plasma with prostaglandin A_1 for 1 hour. Its identification as the methyl ester-trimethylsilyl ether derivative was achieved by combined gas chromatography-mass spectrometry (Horton, Jones, Thompson and Poyser, 1971). Isomerisation of prostaglandin A to prostaglandin B does occur easily under alkaline conditions (Bergström et al., 1963). However when cat blood was heated at 70°C for 5 minutes before incubation with prostaglandin A_1, prostaglandin A_1 was not isomerised during the subsequent incubation at 37°C. In addition, isomerisation of prostaglandin A_1 incubated with Krebs Henseleit solution at pH 7.4 did not take place. These two findings are strong evidence that isomerisation of prostaglandin A_1 in cat blood is enzymatic. Some preliminary work on purification of the enzyme from cat plasma and its pH and temperature characteristics have been reported (Jones, 1970a; 1970b; Horton et al., 1971). The enzyme has been named prostaglandin A isomerase. In the present investigation, attempts were then made to find a richer source of the enzyme, to purify it on a larger scale and to obtain a

Figure 3

Formation of prostaglandin B_1 from prostaglandin A_1 by cat blood.



PG A_1

PG B_1

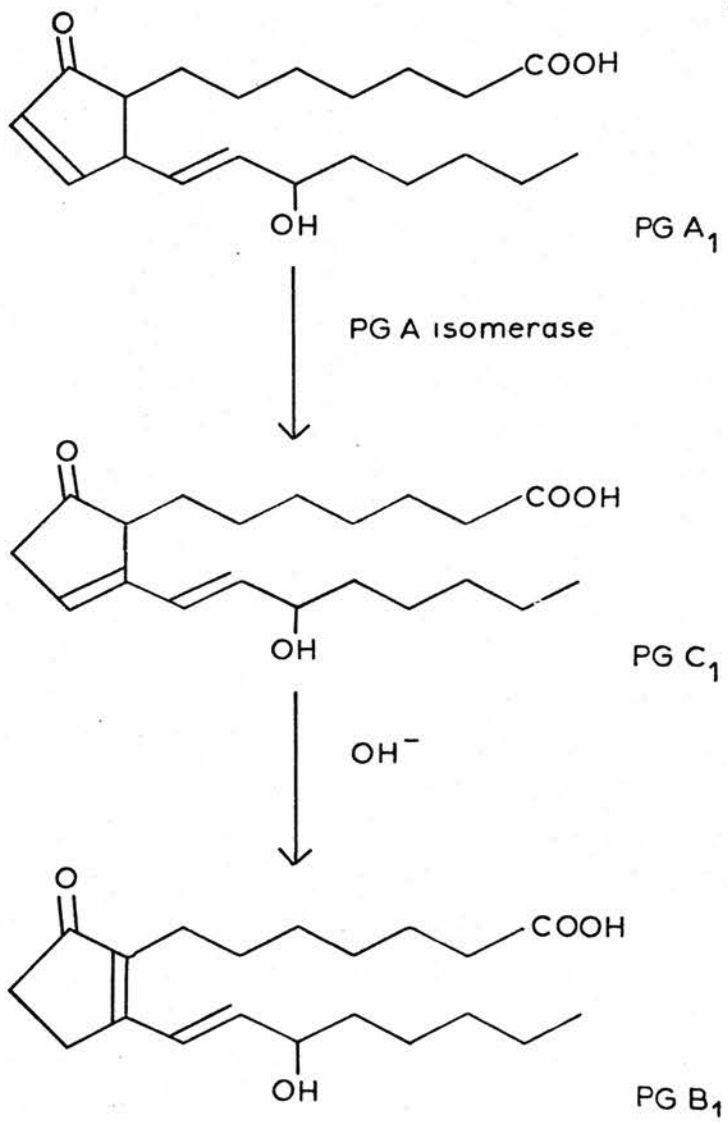
preparation pure enough to determine the enzyme's pH and temperature characteristics and its substrate specificity.

During the course of the investigation described in this thesis, it was found that the true product of the prostaglandin A isomerase is not prostaglandin B_1 but a compound intermediate in structure between prostaglandins A_1 and B_1, designated prostaglandin C_1. This compound is unstable, isomerising under alkaline conditions to prostaglandin B_1 (Jones, 1972a), Figure 4, page 22. Prostaglandin C_1 has an absorption maximum at 234 nm with shoulders at 228 nm and 242 nm. Its presence in incubation mixtures containing unpurified enzyme was masked due to large amounts of other compounds absorbing in this region of the ultraviolet spectrum.

Before it was apparent that prostaglandin B_1 is not the true product of the reaction, assays of the enzyme during the purification procedure and some of the initial work on the characteristics of the enzyme depended upon measurement of prostaglandin B_1 formation. The formation of prostaglandin B_1 could be affected not only by enzymatic activity but also by the stability of prostaglandin C_1 in the conditions used. Since assay conditions throughout the purification were constant, the measurement of prostaglandin B_1 formation can be regarded as sufficiently accurate for the estimation of enzymatic activity of the different purification fractions and for determining the degree of purification at the different stages. However the method was not suitable for examining the effects of temperature, pH and substrate concentration on enzyme activity nor for studying substrate specificities. Therefore it was necessary to use methods which measured the true product of the enzyme, prostaglandin C_1, and to repeat some of the early studies on pH and temperature characteristics of cat plasma prostaglandin A isomerase.

Figure 4

Enzymatic isomerisation of prostaglandin A_1 and subsequent chemical isomerisation of prostaglandin C_1.



Materials and Methods

Buffers

All isomerase assays were performed in buffered solutions of varying composition according to the pH required by the experimental procedures. These are shown in Table 1.

Table 1

Buffers used during studies on Prostaglandin A Isomerase

Experimental Procedure	pH	Buffer (0.1M)
Purification assays Initial temperature studies	8.5	Tris/HCl
Ion exchange chromatography Gel chromatography Effect of substrate concentration Effect of enzyme concentration Later temperature studies	7.0	Tris/HCl
Effect of pH	5.0 - 6.5	Citric acid/trisodium citrate
	7.0 - 8.5	Tris/HCl
	9.0 - 9.5	Sodium bicarbonate/ sodium carbonate

Preparation of Prostaglandin A_1 solution used for Prostaglandin A isomerase assays

A stock solution was prepared from solid prostaglandin A_1 generously supplied by Dr. J.E. Pike of Upjohn Company and was standardised by estimation of prostaglandin B_1 following treatment of a small part of it with 0.1 M potassium hydroxide in methanol (Andersen, 1969). The prostaglandin was transferred to a 25 ml

pear-shaped flask and was dissolved in 10 ml methanol. Approximately 20 µg was removed in 20 µl and added to 1.98 ml methanol in a 3 ml silica cell, pathlength 1 cm. The spectrum between 325 nm and 200 nm was recorded against 2.2 ml methanol using a Pye Unicam SP.800 spectrophotometer with a programme controller. An 0.20 ml volume of N KOH, prepared by dissolving 5.6 g potassium hydroxide pellets in 100 ml methanol, was added and the solution mixed. Spectra were recorded immediately after addition of potassium hydroxide and at 5 minute intervals thereafter until no further increase in absorbance (λ_{max}^{MeOH} prostaglandin B_1 = 278 nm) occurred. This showed that conversion of prostaglandin A_1 to prostaglandin B_1 was complete. Prostaglandin B_1 was calculated from the absorbance as follows :-

OD_{278} for 10 µg/ml prostaglandin B_2 = 0.81

[ϵ_{278} for prostaglandin B_1 = 27,200 (Andersen, 1969)]

Therefore prostaglandin B_1 present in 2.2 ml MeOH

$$\frac{OD_{278} \times 10 \times 2.2}{0.81} = x\ \mu g$$

Therefore prostaglandin A_1 in 20 µl of original solution is x µg

Therefore prostaglandin A_1 in 10 ml of original solution is

$$\frac{x \times 10{,}000}{20}\ \mu g$$

$$= 500\ x\ \mu g$$

In the preliminary work on the enzyme (Jones, 1970) the substrate concentration in the reaction mixture of 15µmolar was below the

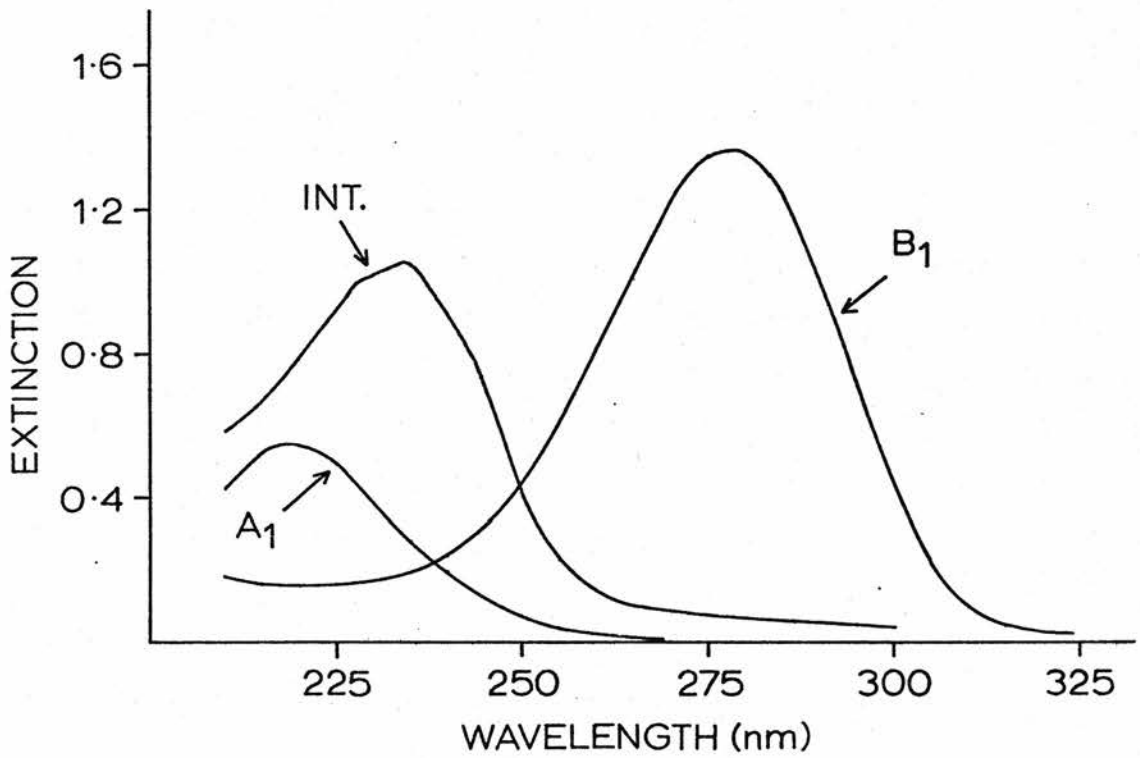
saturating substrate concentration for the enzyme. The value of this concentration was unknown, due to limited supplies of prostaglandin A_1, but it was hoped that one of 66 µmolar (22.3 µg/ml) approximated to it. This concentration in the reaction volume of 3 ml required the addition of 100 µl of stock prostaglandin A_1 solution 668 µg/ml. The stock solution was prepared from the standardised solution containing x µg prostaglandin A_1 by evaporation to dryness at 45°C in vacuo and addition of $\frac{x}{668}$ ml of 10% ethanol in distilled water. A little carefully added sodium bicarbonate was required in addition to ethanol to dissolve this high concentration of prostaglandin in water.

Prostaglandin A isomerase assays: The formation of prostaglandin B_1 and prostaglandin C_1 was measured by ultraviolet spectroscopy using a Pye Unicam SP.800 spectrophotometer. The enzymatic reaction was carried out in 3.0 ml silica cells, pathlength 1.0 cm, enclosed in an externally thermostatically controlled constant temperature holder at 25°C.

Assay method number 1 Prostaglandin C_1 estimation: After addition of 22 µg/ml prostaglandin A_1 to partially purified isomerase diluted in pH 7.0 0.1 M Tris/HCl buffer, a characteristic chromophore (λ_{max} 234 nm with shoulders at 228 nm and 242 nm) appears. Residual prostaglandin A_1 (λ_{max} 217 nm) contributes significantly to the absorbance at 234 nm. At 242 nm this contribution is less, and that of prostaglandin B_1 (λ_{max} 283 nm) still negligible (Figure 5, page 26). Therefore prostaglandin C_1 formation was estimated by absorbance changes at 242 nm which were proportional to prostaglandin isomerase activity (see Results and Discussion Section, page 44). Readings were made against 3.0 ml partially purified isomerase diluted 1 in 10 with 0.1 M Tris/HCl buffer pH 7.0. After equilibration at 25°C for

Figure 5

Absorption spectra in 0.1 M Tris/HCl buffer of prostaglandin A_1, prostaglandin C_1 (intermediate) and prostaglandin B_1.



5 minutes, prostaglandin A_1 was added to 2.9 ml diluted prostaglandin A isomerase and mixed for 15 seconds. The absorbance at 242 nm was then read continuously for 10 minutes and recorded on a potentiometric recorder. The initial reaction rate was measured by drawing a tangent to the line obtained and expressed as the change in absorbance per minute.

Assay method number 2 Prostaglandin B_1 estimation: The pH of the assay used on crude plasma and during purification was 8.5. The prostaglandin C_1 formed by the enzyme isomerises to prostaglandin B_1 at this pH. Prostaglandin B_1 formation was measured by the increase in absorbance at 283 nm. After temperature equilibration for 5 minutes, 2 µmoles prostaglandin A_1 was added to 2.9 ml diluted enzyme solution, 2 minutes later and at 5 minute intervals thereafter, spectra were recorded between 325 nm and 250 nm against 3.0 ml diluted enzyme solution. A lag period of about 5 to 10 minutes occurred before the rate of increase in absorbance at 283 nm became linear. Enzyme activity could therefore not be assayed by the initial reaction rate. Instead, the time taken for an increase in 0.08 absorbance units was used. This was found to vary linearly with the amount of isomerase, at times greater than 20 minutes. If the required absorbance change occurred in less than 20 minutes, the solution was diluted further and the assay repeated. A solution whose protein concentration was above 1 mg/ml could not be assayed due to absorbance of light by the protein at 280 nm. Protein concentrations of dilutions of crude plasma or fractions obtained during the early stages of purification, were always checked before assay by recording the spectra of the diluted solutions between 325 nm and 250 nm against air. The solutions were diluted further if their absorbance at 280 nm was greater than 1.0.

Comparisons of isomerase activities were made from the specific activities of the enzyme solution expressed as milliunits of isomerase per milligram of protein. One unit of isomerase has been defined as the amount of isomerase catalysing the formation of 1 µmole prostaglandin B_1 under the following conditions :-

Volume	3 ml
Buffer	0.1 M Tris/HCl
pH	8.5
Temperature	25°C
PGA_1 concentration	0.66 µmoles/ml

Amounts of isomerase were calculated from the time taken for an absorbance change 0.08 optical density units as follows :-

ϵ_{283} PGB_1 = 27,210 OD units

The rate of change in optical density (Δ OD) due to the formation of 1 µmole PGB_1 in a 3 ml cell, pathlength 1 cm, is

0.00907 OD units per minute

Therefore the rate of change in optical density produced by 1 mU isomerase in a 3 ml cell, pathlength 1 cm, is

0.00907 OD units per minute

Therefore the time taken for a change in optical density of 0.08000 OD units to occur in the presence of 1 mU isomerase in 3 ml is

$$\frac{0.08000}{0.00907} \text{ minutes}$$

= 8.82 minutes

The time taken for a change in optical density of 0.08000 OD units to occur in the presence of 1 mU isomerase in 1 ml is

$$\frac{8.82}{3} \text{ minutes}$$

= 2.94 minutes

Therefore the concentration of isomerase in a diluted solution of enzyme is

$$\frac{2.94}{\text{Time for } \Delta \text{ OD } 0.08} \text{ mU/ml}$$

Therefore the concentration of isomerase in the original undiluted solution of enzyme is

$$\frac{2.94 \times \text{dilution}}{\text{Time for } \Delta \text{ OD } 0.08} \text{ mU/ml}$$

Protein concentrations of crude plasma and of bulked enzymatically-active fractions from each stage of the purification procedure were estimated by the method of Lowry (Lowry et al.,1951), using

bovine serum albumin as standard, but those of individual fractions by absorbance at 280 nm. A protein solution whose concentration is 1 mg/ml produces an absorbance of 1.0 optical density units.

Estimation of prostaglandin A isomerase content of plasma of various mammalian species

In an attempt to find a richer source of the enzyme than cat plasma for large scale purification, plasmas from several mammalian species were screened for prostaglandin isomerase activity, using assay method number 2. Blood was obtained from guinea pig, rat, dog and rabbit in the laboratory and from pig, sheep and ox at the slaughter house. It was collected into a citrate anticoagulant (sodium citrate 2.20 g, citric acid monohydrate 0.80 g, glucose 2.45 g, water 100 ml), 15 ml anticoagulant/100 ml blood, and centrifuged at 1200 g at 4°C for 15 minutes. The plasma was then separated.

Rabbit plasma was the best potential source of the enzyme. The effectiveness of protein precipitation by heat, extremes of pH and the addition of acetone as initial purification procedures were therefore investigated.

Comparison of different methods of protein precipitation as initial purification procedures

A 2.7 kg rabbit was anaesthetised by the intravenous injection of 1.8 ml pentobarbitone sodium (60 mg/ml). Blood was collected into citrate anticoagulant from the abdominal aorta whilst Krebs solution was infused into a femoral vein in an attempt to increase the volume of blood collected by maintaining the venous return. Plasma was obtained as previously described and divided into three

portions for protein precipitation by low pH, by heat and by addition of cold acetone.

Protein precipitation by low pH: The pH of 26 ml rabbit plasma was lowered by careful addition of 10% w/v citric acid solution, the pH being measured on a Pye model 79 pH meter.

Protein precipitation by heating: 26 ml plasma was heated in a water bath maintained at 58°C till the temperature was 55°C. The plasma was then transferred to another water bath maintained at 55°C. The temperature was kept at 55°C for 10 minutes, with constant mixing. The plasma was then cooled in ice and the precipitate removed by centrifugation at 1200 *g* at 4°C for 10 minutes. The supernatant was poured off and a portion removed for prostaglandin isomerase assay by estimation of prostaglandin B_1. The process was repeated at 58°C and 63°C to achieve further protein precipitation. However no further precipitation occurred at these temperatures; higher temperatures were not used as the enzyme was known to be unstable above 65°C (Jones, 1970a).

Protein precipitation by addition of cold acetone: Acetone is the most effective organic solvent for selective protein precipitation used as a first step in enzyme purification (Askonas, 1951). Since most enzymes are labile in organic solvents, the risk of enzyme denaturation during precipitation is reduced by adding the acetone very slowly with good mechanical stirring, and at a low temperature.

Rabbit plasma was dialysed at 4°C for 21 hours against 10 volumes of 0.02 M citrate buffer to obtain the low electrolyte concentration required for acetone precipitation (Askonas, 1951). During preliminary experiments, acetone was added to the plasma from a 0.5 ml pipette

down the side of a 50 ml glass centrifuge tube immersed in an ice bath, in amounts equal to one tenth of the original volume of plasma. During and for 5 minutes after each addition the plasma was stirred continuously, after which the precipitate was centrifuged at 1200 g for 5 minutes at 0°C. The supernatant was decanted back into the centrifuge tube for the addition of more acetone, whilst the precipitate was dissolved immediately in 15 ml Tris buffer pH 8.5 to dilute the acetone prior to prostaglandin isomerase assay.

Subsequent acetone precipitation experiments were carried out at -7°C as some loss in isomerase activity was shown by the incomplete recoveries of activity, although protein precipitation was almost complete. The low temperature was maintained by an acetone/dry ice freezing mixture stirred with an air current. Acetone from a 100 ml measuring cylinder was added at 2 ml/min by displacement by air pumped into the cylinder by means of a Watson-Marlow peristaltic pump. Plasma was contained in an aluminium beaker. Sodium citrate was added to the suspension of protein in plasma to a concentration of 0.01 M which enabled the precipitates obtained with acetone concentrations of 40% and above to be centrifuged down at 1200 g. Incomplete separation of precipitate and supernatant occurred during the preliminary experiments when citrate was omitted, probably because the electrolyte concentration of the suspension was low (Askonas, 1951). Acetone precipitation experiments were carried out on several batches of rabbit plasma to define the percentage composition of acetone producing a fraction with the greatest fold purification (expressed as the ratio of the specific activity of the fraction and the dialysis solution). During some of these experiments, dialysis was replaced by gel chromatography on a Sephadex G-25 column 60 x 3.0 cm. Elution was with citrate buffer 0.02 M pH 6.5 at 2.0 ml per minute. The column eluant

containing protein was collected. Prostaglandin A isomerase was assayed (method number 2). 200 ml of the eluant following was also collected and retained.

Two observations indicated that some loss of prostaglandin A isomerase had occurred during dialysis and gel chromatography. Fold purification of the enzyme from dialysed plasma was lower than that from undialysed plasma. In addition the specific activity of dialysed plasma was lower than that of undialysed plasma. The effect of recombining the protein containing-eluant from the Sephadex G-25 column and the eluant following it on the prostaglandin A isomerase activity of the protein solution was examined. It was thought that inactivation of the enzyme in rabbit plasma during dialysis or gel chromatography might be attributable to the separation of protein and some small molecule activator.

Effect of recombination of prostaglandin A isomerase and non-protein eluant from gel filtration on enzyme activity: The 48% to 52% acetone fraction obtained from the fourth acetone precipitation experiment (see Results and Discussion Section, page 56) was used as the source of prostaglandin isomerase. The non-protein eluant was screened for protein content and for prostaglandin A isomerase activity. 0.1 ml portions of undiluted eluant were added to 2.8 ml of the prostaglandin A isomerase solution diluted in Tris/HCl buffer pH 8.5 1 in 100 or 1 in 200. The lag period occurring before the linear increase in absorbance at 283 nm and the rate of this increase were compared with those obtained during assay of only the enzyme at the same dilution. Although fractional protein precipitation by acetone at $-7^{\circ}C$ produced some fold purification over the dialysis solution or protein eluant from the gel filtration column, inactivation of

prostaglandin A isomerase by dialysis and gel chromatography reduced its overall efficiency as a preliminary step in purification. The specific activity of enzymatically active fractions was no higher than that of the original rabbit plasma. It was decided not to use acetone in the purification of prostaglandin isomerase and also to revert to cat plasma as a source of the enzyme. An attempt to improve the earlier purification procedure (Jones, 1970a) was made.

The partial purification of prostaglandin A isomerase from cat plasma comprised fractional precipitation by ammonium sulphate, ion exchange chromatography on DEAE Sephadex and gel chromatography on Sephadex G-200. Gel chromatography on Sephadex G-25 was used to remove ammonium sulphate prior to ion exchange chromatography on Sephadex DEAE A-50. The enzyme solution obtained after this stage was concentrated for gel chromatography on Sephadex G-200 by a method using Sephadex G-50 (Fischer, 1969). Blood was obtained from the abdominal aorta of a cat anaesthetised with pentobarbitone sodium solution (40 mg/kg). Blood was collected into $\frac{1}{10}$ volume of citrate anticoagulant. It was centrifuged at 1200 g for 15 minutes at 4°C to separate plasma from cells. Plasma from different animals was pooled, the volume measured and 0.25 ml removed for prostaglandin A isomerase assay and protein estimation.

Fractional precipitation by ammonium sulphate

Ammonium sulphate saturated fractions were obtained by adding solid ammonium sulphate to plasma kept cool in an ice bath. The amount appropriate to the initial plasma volume for a given percentage saturation by weight of ammonium sulphate, was found from the nomogram

of Dixon (Dixon, 1953). The proteins were allowed to precipitate for 15 minutes whilst the suspension was continuously stirred. If was then centrifuged at 1200 g at 4°C for 10 minutes after which the supernatant was removed for addition of more ammonium sulphate. The precipitated proteins were dissolved in a small volume of 0.1 M phosphate buffer pH 7.0. 0.25 ml portions of each fraction were dissolved in Tris buffer and assayed for prostaglandin A isomerase activity (assay method number 2). Fractions with a specific activity greater than that of plasma were bulked. This pooled fraction was also assayed. Proteins were determined by the method of Lowry (Lowry et al., 1951).

Preparation of gel chromatography and ion exchange columns

Sephadex G-25 was allowed to swell in an excess of 0.1 M Tris/HCl buffer pH 7.0 for 3 days. After the gel had settled the clear supernatant was removed and the remaining gel made into a slurry with fresh buffer, care being exercised to avoid formation of air bubbles. This slurry was poured into a perspex chromatography column (3.0 cm x 75 cm) down the side of a glass rod, and the gel allowed to settle under gravity whilst buffer was run through the bed at a flow rate of 1 ml per minute for 12 hours. The column was then ready for use. Flow rate could be adjusted by a screw clip on the outflow tubing. A constant pressure head was maintained adding buffer by means of a Watson-Marlow peristaltic pump.

Sephadex G-200 was allowed to swell for 3 days in 0.1 M Tris/HCl buffer, pH 7.0, containing 1.0 M NaCl to reduce the aggregation of protein molecules in the partially purified enzyme solution whose ionic strength had been reduced by removal of small molecules earlier

in the purification. The slurry was poured down the side of a glass rod into a perspex column obtained from Pharmacia Chemical Company. This column was a closed system so that the flow rate was altered only by the rate at which eluant was pumped through the column by the peristaltic pump. The bed was allowed to settle for 24 hours whilst the rate of flow of buffer was 0.5 ml per minute.

DEAE Sephadex A-50 was allowed to swell in 0.1 M Tris/HCl buffer pH 7.0, containing sodium chloride to increase the ionic strength to 0.1. This reduces the bed shrinkage which occurs during column elution with solutions of low ionic strength. The swelling process was continued for 3 days with 3 changes of buffer. Equilibration was complete when the pH of the gel was constant at 7.0. The slurry, free of air bubbles, was poured down the side of a glass rod into a glass column containing 1/4" glass wool and 1/4" clean sand to act as a bed support. After the gel had been allowed to settle under gravity for 5 minutes, Tris/HCl buffer pH 7.0 was allowed to flow through the column at a rate of 1 ml per minute for 24 hours. The pH's of the inflow and outflow fluid were compared to check that equilibration of the gel was complete.

Desalting of ammonium sulphate fraction by gel chromatography on Sephadex G-25

Ammonium sulphate had to be removed from the pooled fraction obtained from the fractional precipitation to achieve a protein solution of the low ionic strength required if the ion exchange column was to be eluted by increasing the ionic strength of the solution passing through it. Gel chromatography using a coarse grade of Sephadex for group separation was chosen as a quick method. The sample

was loaded with a peristaltic pump on to the surface of a Sephadex G-25 column (bed dimension 60 x 3.0 cm) and was eluted with Tris/HCl buffer pH 7.0 at a flow rate of 5 ml per minute. The coloured protein band was collected as one fraction. Its protein content was measured and the prostaglandin isomerase level assayed. Specific activities of the protein solution before and after gel chromatography were the same, indicating that no loss of enzyme activity occurred during the process.

Ion exchange chromatography on DEAE Sephadex A-50

The protein containing salt free eluant (volume varying from 50 to 150 ml) from the Sephadex G-25 column was loaded on to the surface of a DEAE Sephadex A-50 column at 1 ml per minute. It was eluted with 15 ml of starting buffer followed by a sodium chloride in Tris buffer gradient obtained from a starting buffer containing 0.05 M NaCl and a limiting buffer containing 0.6 M NaCl. Ten minute fractions were collected and the protein content of alternate fractions measured. Volumes of 0.25 ml from fractions which, from the results of the preliminary experiment were likely to contain prostaglandin A isomerase, were diluted in Tris buffer pH 8.5 and assayed. Those showing significant purification were pooled, assayed and concentrated for gel chromatography on Sephadex G-200.

Concentration of pooled fractions from ion exchange chromatography on DEAE Sephadex A-50

It was necessary to reduce the volume of the sample to be applied to the Sephadex G-200 column because poor resolution is obtained if the sample size constitutes too large a proportion of the bed volume (Fischer, 1969).

Sephadex G-50 was swollen in the pooled fraction (volume 40 ml) 1.20 g Sephadex per 10 ml protein solution for 10 minutes. The Sephadex beads take up water and small molecules in preference to large molecules. After packing into a sinter funnel containing a Whatman No.1 filter disc moistened with Tris/HCl buffer pH 7.0, ionic strength 1.0, centrifugation at 70 x g at 4°C for 10 minutes forced the solution out of the gel; a concentrated protein solution of original salt content was thus obtained.

Gel chromatography on Sephadex G-200

12 ml concentrate (protein concentration 10 mg/ml) was loaded with a sample applicator on to the surface of a Sephadex G-200 column (bed dimensions 75 cm x 2.0 cm). The column was eluted with 0.1 M Tris/HCl buffer pH 7.0 containing 1.0 M sodium chloride for 24 hours at a flow rate of 0.5 ml per minute. Twenty minute fractions were collected. The protein concentration of every fourth fraction (undiluted) was measured by the method of Lowry (Lowry et al., 1951), since enough dilution had occurred to reduce the protein concentration to below the threshold for estimation by extinction at 280 nm. Fractions showing significant purification were pooled. The specific activity of the partially purified isomerase preparation obtained was determined. 5.0 ml portions of the enzyme solution were ampouled and stored at -20°C to minimise bacterial contamination and loss of activity. Single ampoules of prostaglandin A isomerase were thawed and used for investigation of some properties of the enzyme. Once an ampoule had been opened, its contents were used, without refreezing, on the same day or the day after. Any storage was at 4°C.

Effect of pH on prostaglandin A isomerase activity

Buffers pH 5.0 to 10.7 were made up from 0.1 M stock solutions. Citrate buffer was used for pH 5.0 to 6.5, Tris/HCl for pH 7.0 to 8.5 and bicarbonate for pH 9.0 to 10.7. Prostaglandin A isomerase solution was diluted 1 in 10 in the appropriate buffer and assayed initially by measurement of prostaglandin B_1 formation (assay method number 2). After the discovery that the true product of the reaction is prostaglandin C_1, the investigation was repeated using the assay based on prostaglandin C_1 formation (assay method number 1). Non-enzymatic conversion to prostaglandin A_1 to prostaglandin B_1 was followed over the pH range by measuring prostaglandin B_1 formation in the absence of prostaglandin A isomerase. The stability of the enzyme at acid and alkaline pH was examined. The enzyme was diluted in the appropriate buffer and after 10 minutes the pH was adjusted to 9.0 with solid sodium bicarbonate or acid and the enzyme assayed by method number 2. When the work was repeated with assay method number 1, the pH was adjusted to 7.0 after exposure of the enzyme to acid or alkaline pH.

Effect of temperature on prostaglandin A isomerase activity

3.0 ml diluted (1 in 10) prostaglandin A_1 isomerase in the reference cell and 2.9 ml of enzyme in the reaction cell were equilibrated in the constant temperature enclosure of the spectrophotometer for 15 minutes. 0.1 ml substrate was added to the reaction cell and prostaglandin A_1 isomerase assayed by method number 1 or method number 2. The temperature stability of the enzyme was tested. The diluted enzyme was incubated at a given temperature for 10 minutes, cooled rapidly to 25°C in an ice bath and assayed at 25°C by method number 1.

Effect of substrate concentration of prostaglandin A isomerase activity

Prostaglandin A isomerase diluted 1 in 10 in Tris/HCl buffer pH 7.0 was assayed by method number 1 using prostaglandin A_1 concentrations in the reaction cell from 1.6×10^{-6}M to 2.6×10^{-5}M. Substrate was added in 100 µl from solutions prepared by serial dilutions of a stock prostaglandin A_1 solution 1.8 mg per ml in methanol.

Results and Discussion

Prostaglandin A isomerase assays

Measurement of prostaglandin C_1 formation at pH 7.0 (assay method number 1): The ultraviolet spectral changes produced by the conversion of prostaglandin A_1 to prostaglandin C_1 by prostaglandin A isomerase are shown in Figure 6, page 42. Absorbance at 242 nm corresponding to a shoulder on the spectrum of prostaglandin C_1, indicated appearance of prostaglandin C_1 as described in the Materials and Methods Section, page 26. Figure 7, page 43, shows this increase continuously recorded for 11 minutes. The initial rate of increase was used to measure the rate of prostaglandin C_1 production. Its linear relationship with the amount of isomerase is illustrated in Figure 8, page 44. Enzyme activity assayed by this method was always expressed as the rate of increase in absorbance at 242 nm and shows that measurement of this chromophore is a valid assay for the enzyme.

When the prostaglandin A_1 concentration for assays used during purification of the enzyme was chosen, it was hoped, but not known, that it was near to the saturating substrate concentration for the enzyme. If this were not so, changes in rate of appearance of product might be attributable to alterations in substrate concentration as well as variations in enzyme activity. It can be seen from Figure 19, page 79, that when the prostaglandin A_1 concentration was 0.22 µmole in 3 ml (6.6×10^{-5}M) the rate of isomerisation was not independent of substrate concentration, but nevertheless any fluctuations in substrate concentration would produce little alteration in enzyme activity. The most likely source of such differences in the amount of substrate added to the enzyme would be the addition of 100 µl portions from different batches of stock solution. The final concentration of these

Figure 6

Changes in the absorption spectrum of prostaglandin A_1 (absorption maximum in Tris buffer 223 nm) occurring after the addition of prostaglandin A isomerase.

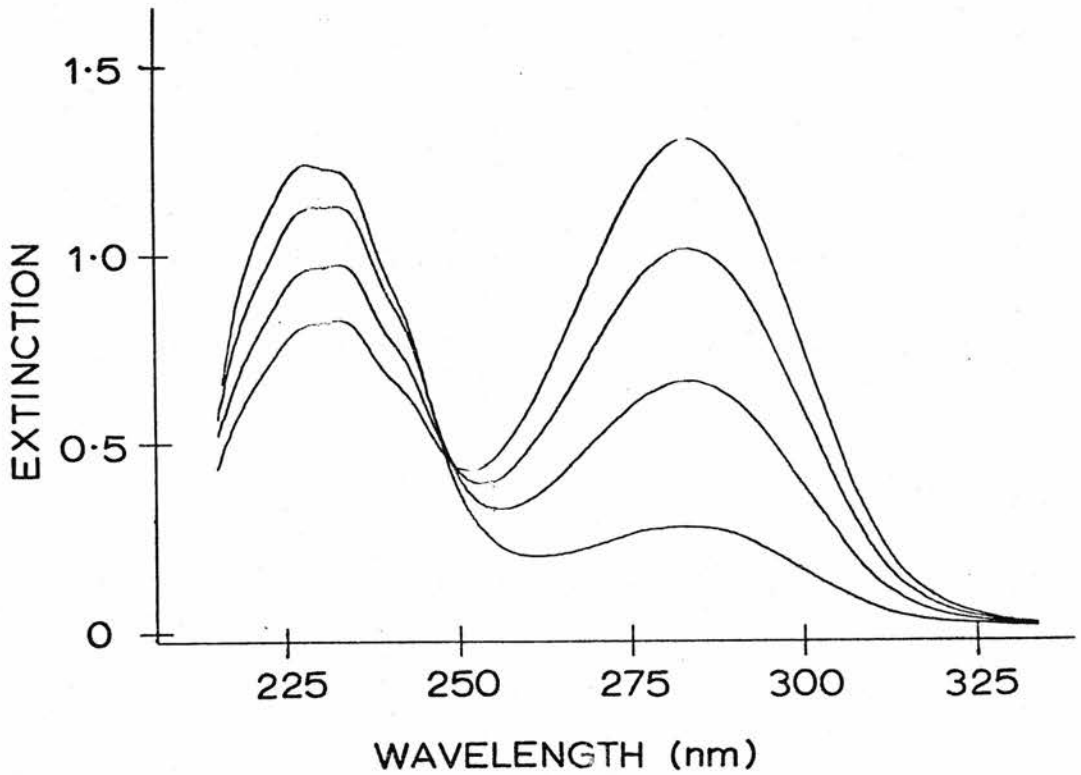
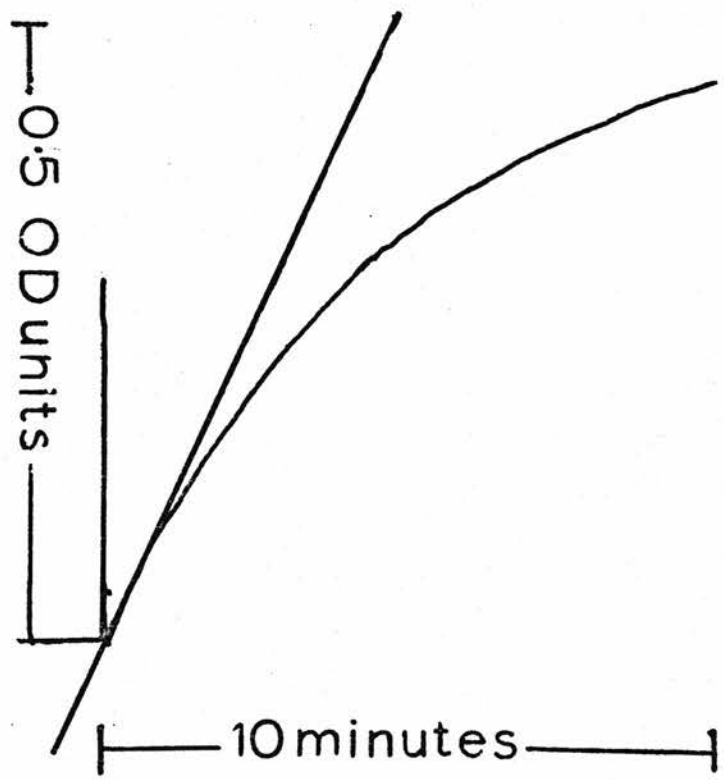


Figure 7

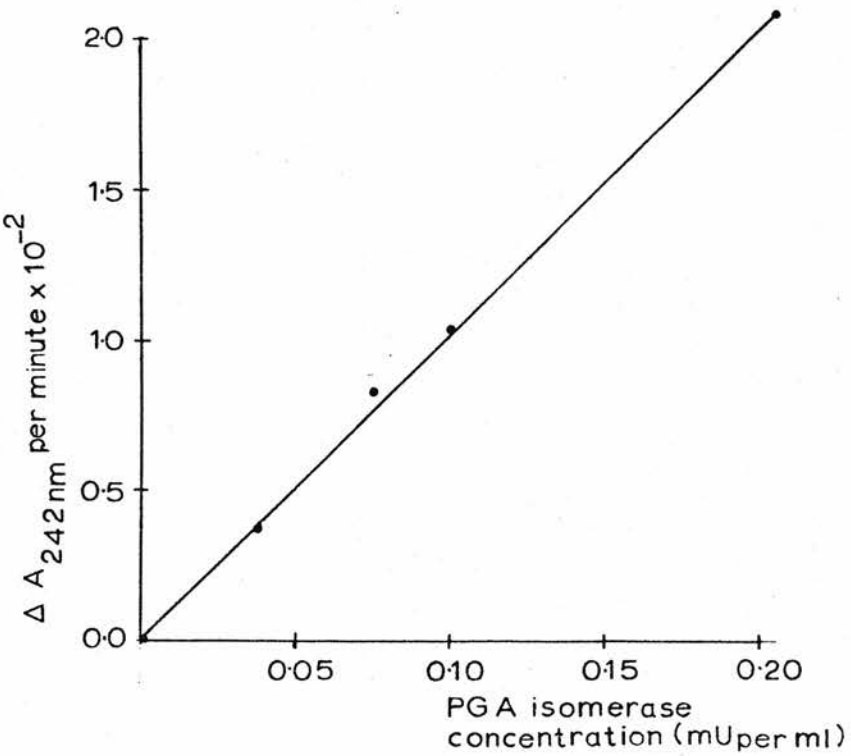
Increase in absorbance at 242 nm continuously recorded.



The tangent to the curve is also shown and was used to estimate the initial rate of increase in absorbance.

Figure 8

Prostaglandin A isomerase concentration (abscissa) plotted against the initial rate of increase in absorbance at 242 nm (ordinate).



might vary slightly because of pipetting errors made whilst adding the required volume of 10% aqueous ethanol. Solutions were always stoppered when not in use and stored at -20°C so that changes in concentration produced by evaporation of solvent from any one stock solution were very small. However the linear relationship between absorbance increase and prostaglandin A_1 concentration suggests that the amounts of substrate added were consistent.

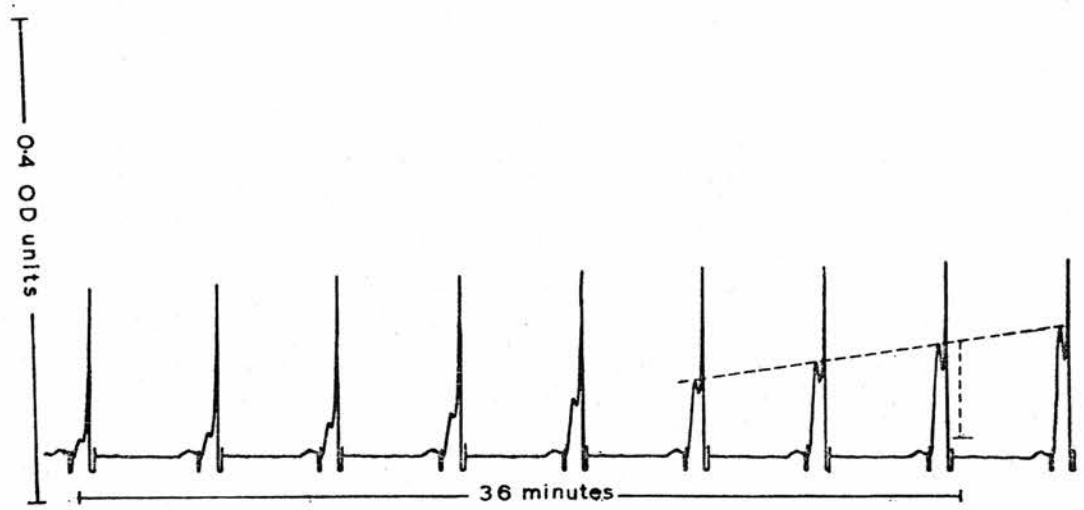
This assay, as well as measuring the true product of the reaction dependent only on enzyme activity, had the additional advantage of speed. A record of absorbance increase with time from which the enzyme concentration was calculated, could be obtained in 5 minutes (Figure 7, page 43).

Measurement of prostaglandin B_1 formation at pH 8.5 (assay method number 2): Spectra of 0.2 µmoles prostaglandin A_1 solution recorded between 325 nm and 250 nm immediately after addition to diluted enzyme in Tris buffer solution and at 5 minute intervals thereafter are shown in Figure 9, page 46. Increase in absorbance at 283 nm does not become linear in this example of the assay until after a lag period of about 15 minutes. The time taken for production of a fixed amount of prostaglandin B_1 (1 µg/ml = 0.08 OD units) is therefore used to measure prostaglandin A isomerase activity. The relationship between this time and the amount of enzyme is shown in Figure 10, page 47. At times longer than 20 minutes it is linear and is therefore a valid assay for the enzyme. Assays in which the change occurred in less than 20 minutes were always repeated at a higher dilution.

This assay, used initially before the discovery of prostaglandin C_1, was not ideal because measurement of enzymatic activity could not be determined from the initial reaction rate. Moreover

Figure 9

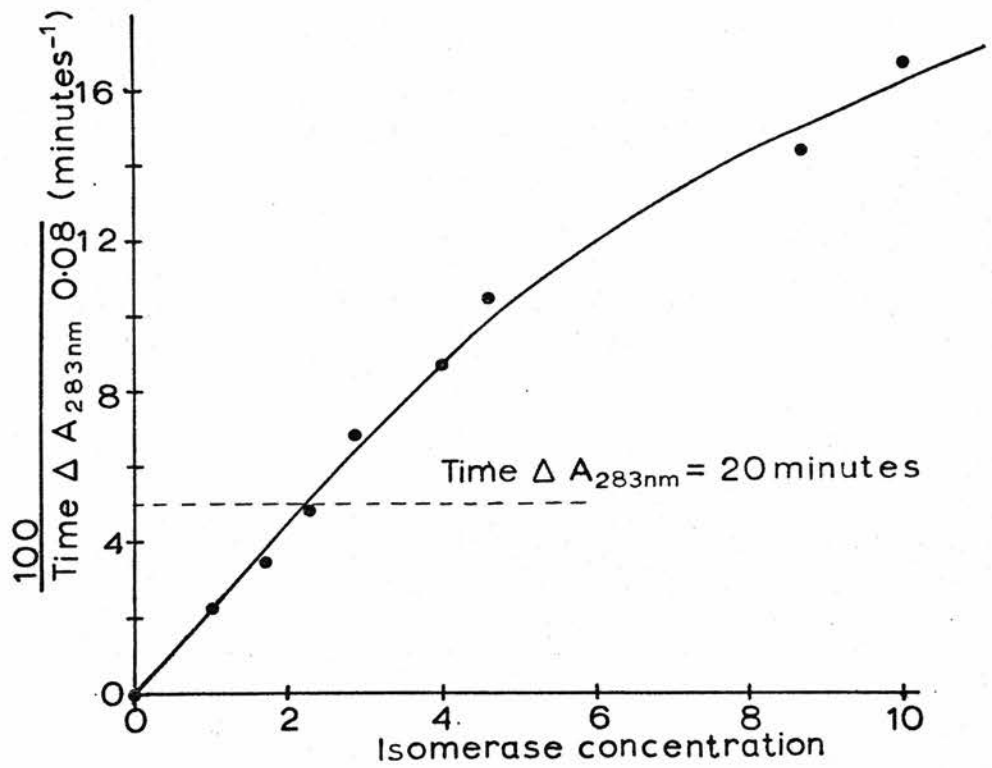
Spectra between 325 nm and 250 nm recorded immediately after addition of prostaglandin A isomerase to prostaglandin A_1 and every 5 minutes thereafter.



The time for an increase in absorbance at 283 nm of 0.08 OD units (vertical broken line) is read off the trace.

Figure 10

Prostaglandin A isomerase concentration (abscissa) plotted against a rate factor of the time taken for an increase in absorbance at 283 nm of 0.08 OD units to occur (ordinate).



prostaglandin B_1 formation is secondary to enzymatic activity. During the lag period observed before an increased absorbance at 278 nm occurs (Figure 5, page 26), prostaglandin C_1 is formed by enzymatic isomerisation of prostaglandin A_1 (Figure 5, page 26) but no prostaglandin B_1 is detected until the prostaglandin C_1 concentration has reached a sufficiently high level for chemical conversion to prostaglandin B_1 to occur at an appreciable rate.

Preliminary experiments (Jones, personal communication) indicate that the situation is different when prostaglandin A isomerase concentrations are high. Levels of the enzyme comparable to those in plasma can be obtained by bonding it on to a Sepharose column down which the substrate flows. The rate of prostaglandin B_1 formation at pH 8.5 is higher than can be accounted for by spontaneous breakdown of prostaglandin C_1 and occurs at pH 7.0 at which prostaglandin C_1 is stable. This suggests that enzymatic conversion of prostaglandin C_1 to prostaglandin B_1 occurs at high prostaglandin A isomerase levels and thus very probably in undiluted plasma.

Appearance of prostaglandin B_1 measured by radioimmunoassay has been used in another laboratory (Polet and Levine, 1971) to assay prostaglandin A isomerase activity. A compound with a greater affinity for an antibody directed towards prostaglandin B_1 than prostaglandin A_1 has been measured at pH 7.4. When the high prostaglandin isomerase levels in undiluted sera or sera of low dilutions are assayed, it is possible that this compound is prostaglandin B_1 or a mixture of prostaglandin B_1 and prostaglandin C_1. However the compound measured at high dilutions of sera is more likely to be prostaglandin C_1 which has been shown as the product of the isomerisation at lower enzyme levels (Jones, 1972). Cross reactivity of the antibody between prostaglandin A_1 and prostaglandin B_1 is 10%

(Levine and van Vunakis, 1970; Polet and Levine, 1971) which compares well with specificities of other antibodies raised towards prostaglandin A (Raz, 1973; Frailey, Child and Bartholomew, 1973). However there is no available data about the cross reactivity between prostaglandin C_1 and prostaglandin B_1 which are even more similar structurally than are prostaglandin A_1 and prostaglandin B_1. It is therefore very unlikely that an antibody directed towards prostaglandin B_1 would not bind with prostaglandin C_1 so that a serological prostaglandin A isomerase assay would measure both compounds. This would distort the results of assays in conditions in which the rate of non-enzymatic prostaglandin B_1 formation was comparable with that of enzymatic isomerisation of prostaglandin A_1 to prostaglandin C_1; for example, if the assay is performed at alkaline pH below that at which prostaglandin isomerase is still active. Significant levels of prostaglandin C_1 would be achieved and maintained whilst concentration dependent conversion to prostaglandin B_1 accelerated by the greater instability of prostaglandin C_1 at high pH, would occur. Failure to distinguish between these two compounds is a possible explanation for the apparent continuous increase in enzymatic activity up to pH 10 to 11 (Polet and Levine, 1971). The pH profile obtained with no decrease in activity at alkaline pH usually ascribed to changes in ionic groups on the enzyme and to protein denaturation (Dixon and Webb, 1958), is unusual. The lack of assay specificity does not explain differences in the heat stability of the enzyme obtained by Polet and Levine (Polet and Levine, 1971).

Prostaglandin A isomerase levels in the blood plasma of various mammalian species

Estimates of prostaglandin A isomerase levels in the blood plasma of the nine mammalian species examined, are summarised in Table 2.

Table 2

Prostaglandin A isomerase levels in the blood plasma of various mammalian species

Species	Isomerase level mU/mg protein ± S.E.	No. of determinations
Rabbit	1.17 ± 0.28	8
Cat	0.27 ± 0.06	8
Pig	0.24 ± 0.06	3
Dog	0.18 ± 0.09	3
Rat	0.068 ± 0.014	3
Ox	None detected	2
Sheep		3
Guinea pig		3
Human		3

Assays on plasma in which no isomerase could be detected were performed on the lowest dilution in which the protein concentration was less than 1.2 mg/ml. The spectra between 325 nm and 250 nm were recorded for 1 hour during which time there was no change in absorbance at 283 nm. The standard change of 0.08 units would be produced in 1 hour by 0.049 mU/ml of isomerase (Materials and Methods Section, page 29).

At the highest protein concentration permitted by the assay, the specific activity of the isomerase solution would be 0.040 mU/mg protein so that isomerase levels in human, guinea pig, sheep and ox plasma are lower than this. The enzyme may be absent from some or all of the plasmas of these species. Assays used in this laboratory have less sensitivity but more specificity than that used elsewhere (Polet and Levine, 1971). Nevertheless both sets of results have thresholds of the same order.

Plasma prostaglandin A isomerase levels are not correlated with the occurrence of prostaglandins of the B-type. These have been identified only in human seminal plasma (Hamberg and Samuelsson, 1966) and yet prostaglandin A isomerase is absent from or present in only very small amounts in human plasma. Isomerase levels in human tissues other than plasma were not examined in this study but the enzyme was not detected (Polet and Levine, 1971) in various tissue homogenates. The 10,000 *g* fraction, from high speed centrifugation, containing the prostaglandin synthetase complex (Bergström et al., 1964a; 1964b; van Dorp et al., 1964a; 1964b; Schneider et al., 1966; Änggard, Bohman, Griffin III, Larsson and Maunsbach, 1972; Larsson and Änggård, 1973), prostaglandin dehydrogenase and prostaglandin reductase (Änggård and Samuelsson, 1964; Änggård and Samuelsson, 1966; Änggård and Larsson, 1971; Jarabak, 1971; Larsson and Änggård, 1973), ω-hydroxylating enzymes (Israelsson, Hamberg and Samuelsson, 1969) and β-oxidising enzymes (Hamberg, 1969), was used. Tissues investigated were liver, kidney, heart, lung, spleen, stomach and small intestine of the cat; liver, kidney, heart, lung, spleen and brain of the rabbit; and prostate and seminal vesicles of the rat. All three species have easily detectable plasma levels of prostaglandin A isomerase.
It would seem that plasma prostaglandin A isomerase activity is unrelated to tissue enzyme activity and unlikely to occur by enzyme leakage from a tissue.

Comparison of different methods of protein precipitation as initial purification procedures

Protein precipitation by low pH: The pH of 26 ml of rabbit plasma was reduced from 6.6 to 3.9, further reduced to 3.0 and finally to 2.5. No precipitation occurred at any of these values. It was concluded that protein precipitation by acidification could not be effective as an initial stage in the partial purification of prostaglandin A isomerase from rabbit plasma.

Protein precipitation by heat: Specific activities of supernatant separated from precipitates obtained by heating plasma for 10 minutes at 55°C, 59°C and 63°C are shown in Table 3.

Table 3

The effectiveness of heat denaturation as a preliminary purification procedure

Temperature	Protein conc. undiluted supernatant (mg/ml)	Enzyme in undiluted supernatant (mU/ml)	Specific activity of supernatant mU/mg protein
55°C	43	54.0	1.25
59°C	41	42.4	1.06
63°C	50	51.2	1.02
Unheated plasma	46	56.4	1.23

It was concluded that heat denaturation of rabbit plasma proteins did not increase the specific activity of rabbit plasma prostaglandin isomerase and was not a suitable preliminary purification step.

Protein precipitation by the addition of cold acetone:

The specific activities of fractions obtained when cold acetone was added to plasma to a concentration of varying percentages of the total volume are shown in Tables 4 to 7.

Table 4

Acetone precipitation - experiment 1. Temperature 0°C.
Acetone added down the side of a 50 ml glass centrifuge tube

Sample	Enzyme in undiluted solution (mU/ml)	Protein concentration undil. sol. (mg/ml)	Specific activity (mU/mg protein)	Total enzyme (mU)	Total protein (mg)
Dialysis fluid	37.40	32.0	1.16	440	384
9% acetone fraction	0.35	2.6	0.13	5	39
17% acetone fraction	0.88	3.7	0.23	13	56
22% acetone fraction	0.49	2.8	0.17	7	42
28% acetone fraction	0.62	3.8	0.63	36	58
33% acetone fraction	0.63	5.0	0.13	10	75
37% acetone fraction	0.60	4.2	0.15	6	42
39% acetone fraction	5.60	7.1	0.55	56	103

Total protein precipitated	393 mg
Recovery of protein	102%
Total enzyme recovered	133 mU
Recovery of enzyme	30%

The poor recovery of prostaglandin isomerase activity after fractional precipitation of rabbit plasma proteins compared with complete recovery of protein suggested that a temperature of 0°C was not low enough to prevent denaturation of the enzyme with consequent loss in activity. Precipitation was therefore repeated at -7°C. The results obtained are shown in Table 5.

Table 5

Acetone precipitation - experiment 2. Temperature -7°C

Sample	Enzyme in undiluted solution (mU/ml)	Protein in undiluted solution (mg/ml)	Specific activity (mU/mg protein)	Total enzyme (mU)	Total protein (mg)
Plasma	66.0	44.0	1.50	5900	3950
Dialysis fluid	44.50	44.0	1.01	4000	3950
10% acetone fraction	0.51	7.2	0.07	5	720
19% acetone fraction	3.25	14.3	0.22	81	370
26% acetone fraction	4.06	21.0	0.19	101	530
33% acetone fraction	6.20	22.0	0.27	154	550
40% acetone fraction	7.50	5.70	1.32	190	142
50% acetone fraction	19.50	12.0	1.63	2550	1560

Total protein precipitated 3870 mg
Recovery of protein 96%

Total enzyme activity recovered 2790 mU
Recovery of enzyme activity 73%

The closer agreement between recoveries of protein and prostaglandin A isomerase activity suggested that there was less denaturation of the enzyme, and consequent reduction of enzymatic activity at -7°C.

Experiments using smaller increases in the % composition of acetone in the plasma-acetone mixture between 40% and 50% were carried out at -7°C. The results are shown in Tables 6 and 7.

Table 6

Acetone precipitation - experiment 3. Temperature -7°C

Sample	Enzyme in undiluted solution (mU/ml)	Protein in undiluted solution (mg/ml)	Specific activity (mU/mg protein)	Total enzyme (mU)	Total protein (mg)
Plasma	31.0	33.0	0.94	2258	2400
Dialysis fluid	19.1	30.0	0.64	1470	2225
0-30% acetone fraction	4.7	17.0	0.28	380	1360
30-35% acetone fraction	1.06	4.0	0.26	15	56
35-40% acetone fraction	4.9	13.0	0.38	37	97
40-44% acetone fraction	20.08	29.0	0.72	560	780
44-48% acetone fraction	0.81	6.1	0.13	17	130
52%	No isomerase activity detected after 15 minutes	Protein absorbance obscured by acetone			

Citrate was added to the plasma-acetone mixture after each fractionation to a concentration of 0.01 M to increase the electrolyte concentration to enable separation of precipitate and supernatant at acetone concentrations above 50%

Total protein recovered	2430 mg
Recovery of protein	108%
Total prostaglandin A isomerase activity recovered	1010 mU
Recovery of prostaglandin A isomerase activity	69%

Table 7

Acetone precipitation - experiment 4. Temperature -7°C.
Dialysis replaced by gel filtration on Sephadex G-25

Sample	Enzyme in undiluted solution (mU/ml)	Protein in undiluted solution (mg/ml	Specific activity (mU/mg protein)	Total enzyme (mU)	Total protein (mg)
Plasma	147	50.0	2.94	27600	9500
Protein containing eluant from Sephadex G-25 column	45.5	27.0	1.70	15200	9018
0-40% acetone fraction	N O T A S S A Y E D				
40-44% acetone fraction	18.0	36.0	2.0	6300	3150
44-48% acetone fraction	31.5	28.0	1.12	3800	3390
48-52% acetone fraction	13.6	4.90	2.80	1500	532

The fold purification obtained by precipitation of protein with different final concentrations of acetone in the four acetone precipitation experiments performed are shown in Table 8.

Table 8

Fold purification obtained by protein precipitation with acetone

Experiment	% acetone fraction	Fold purification relative to plasma	Fold purification relative to dialysis fluid or Sephadex column eluant
1	37 - 39	Plasma not assayed	0.47
2	33 - 40	0.88	1.30
	40 - 50	1.08	1.60
3	35 - 40	0.40	0.59
	40 - 44	0.77	1.13
	44 - 48	0.14	1.17
4	40 - 44	0.68	1.17
	44 - 48	0.41	0.72
	48 - 52	0.95	1.64

An increase in the final acetone concentration of the acetone-plasma mixture from 40% to 50% precipitates protein with the highest specific activity in terms of prostaglandin A isomerase. There is no advantage in producing fractions of 40% to 44%, 44% to 48% and 48% to 52% since the fold purification of the precipitates obtained by adding acetone to final concentrations within these limits was inconsistent.

Effect of recombination of prostaglandin A isomerase and non-protein eluant from gel filtration on enzyme activity

Table 9

Protein content and prostaglandin A isomerase activity of non-protein eluant

Absorbance at 283 nm of undiluted solution	0.62 OD units
Protein concentration of non-protein eluant	0.62 mg/ml
Volume of non-protein eluant	530 ml
Total protein in non-protein eluant	330 mg
Prostaglandin A isomerase activity:	
Enzyme in undiluted eluant	0.6 mU/ml
Specific activity	0.97 mU/mg protein
Total enzyme in non-protein eluant	320 mU

Table 10

Effect of non-protein eluant on the activity of prostaglandin A isomerase (48% to 52% acetone fraction from rabbit plasma)

Eluant dilution	Undiluted		Undiluted	
Enzyme dilution	1 in 200		1 in 100	
Volume of eluant	0.1 ml	-	0.1 ml	-
Length of lag period	19	23	17	17
Linear rate of absorbance increase Δ OD/min.	0.008	0.005	0.008	0.005
Time taken for Δ absorbance of 0.08 OD units min.	20.0	28.0	20.0	30.5

It was concluded that during gel filtration of rabbit plasma prostaglandin A isomerase is separated from some activating factor. It is possible that this factor is the small amount of prostaglandin A isomerase found in the "protein free" eluant. The enzyme thus added (0.02 mU/ml) would be detectable. The linear rate of increase in absorbance would be 0.007 OD units per minute (see Materials and Methods Section, page 29), and the standard absorbance change of 0.08 OD units would occur in about 2 hours.

Partial purification of prostaglandin A isomerase from cat plasma

Fractional precipitation by ammonium sulphate: Fractional precipitation of proteins in cat plasma was achieved by producing fractions of the percentage saturation shown in Table 11. The initial plasma volume was 126 ml. Table 12 shows the fold purification and yields of enzyme obtained for each fraction.

Table 11

Fractional precipitation of proteins in cat plasma

Saturation with ammonium sulphate (%)	Mass ammonium sulphate added (g)	Volume of phosphate buffer dissolving protein precipitate (ml)
0 - 50	38.0	80
50 - 55	4.4	10
55 - 60	4.4	10
60 - 65	4.4	10
65 - 70	4.4	precipitate failed to separate during centrifugation

Table 12

Purification of prostaglandin A isomerase from cat plasma by fractional ammonium sulphate precipitation

Saturation with ammonium sulphate (%)	Total protein	Protein (% total in plasma)	Enzyme (mU)	Enzyme (% total in plasma)	Specific activity (mU/mg protein)	Fold purification
Plasma	8640	100	5260	100	0.61	1.0
0 - 50	4150	48.0	1280	23	0.37	0.6
50 - 55	215	2.5	530	10.0	2.45	4.0
55 - 60	1040	12.0	1980	38.0	1.90	3.2
60 - 65	1100	12.8	485	9.0	0.44	0.7

The fractions saturated with ammonium sulphate 50% to 55% and 55% to 60% were pooled. Specific activity of the solution before and after gel chromatography on Sephadex G-25 was compared. Table 13 shows that loss of prostaglandin A isomerase activity did not occur during this process.

Table 13

	Volume (ml)	Total protein (mg)	Total enzyme (mU)	Specific activity (mU/mg protein)
Before gel chromatography	58	1040	2160	2.08
After gel chromatography	143	930	2500	2.70

Ion exchange chromatography on DEAE Sephadex A-50

Separations of proteins obtained by three different sodium chloride gradients during the preliminary experiments are shown in Figure 11, pages 62 and 63.

Figure 12, page 64, shows the separation obtained using a larger column eluted with one of these NaCl gradients.

Gel chromatography on Sephadex G-200

Gel chromatography on Sephadex G-200 of proteins in the pooled fractions obtained from the ion exchange chromatography procedure shown in Figure 12, page 64, are depicted in Figure 13, page 65.

Summary of the partial purification of prostaglandin A isomerase

The 3 stage partial purification of prostaglandin A isomerase from cat plasma is summarised in Table 14. Details of the ion exchange chromatography and gel chromatography are in Figures 12 and 13, pages 64 and 65 respectively.

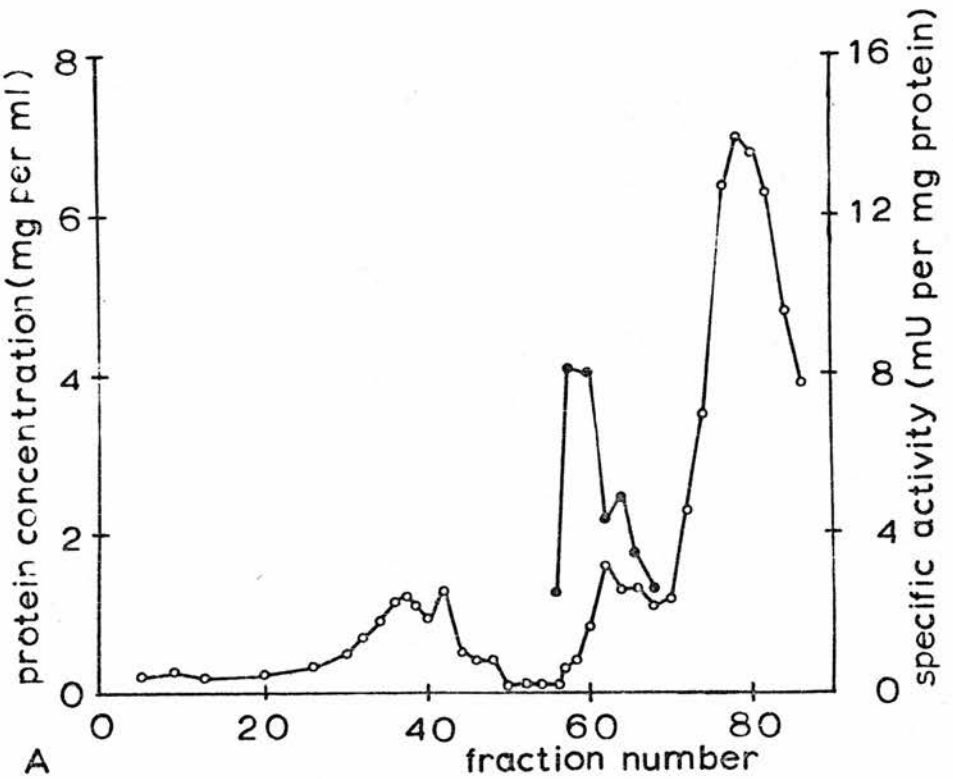
Table 14

Summary of partial purification of prostaglandin A isomerase

Sample	Total enzyme (mU)	Specific activity (mU/mg protein)	Fold purification	Yield (%)
Plasma	1920	0.15	1.0	100
$(NH_4)_2SO_4$ (45%-60%)	1420	0.62	4.1	73
DEAE Sephadex	40	0.96	6.4	2.1
Sephadex G-200	30	7.14	47.6	1.6

Figure 11

Separation of proteins in the 50%-60% saturated ammonium sulphate fractions of cat plasma on DEAE Sephadex A-50 eluted with 3 different NaCl gradients.



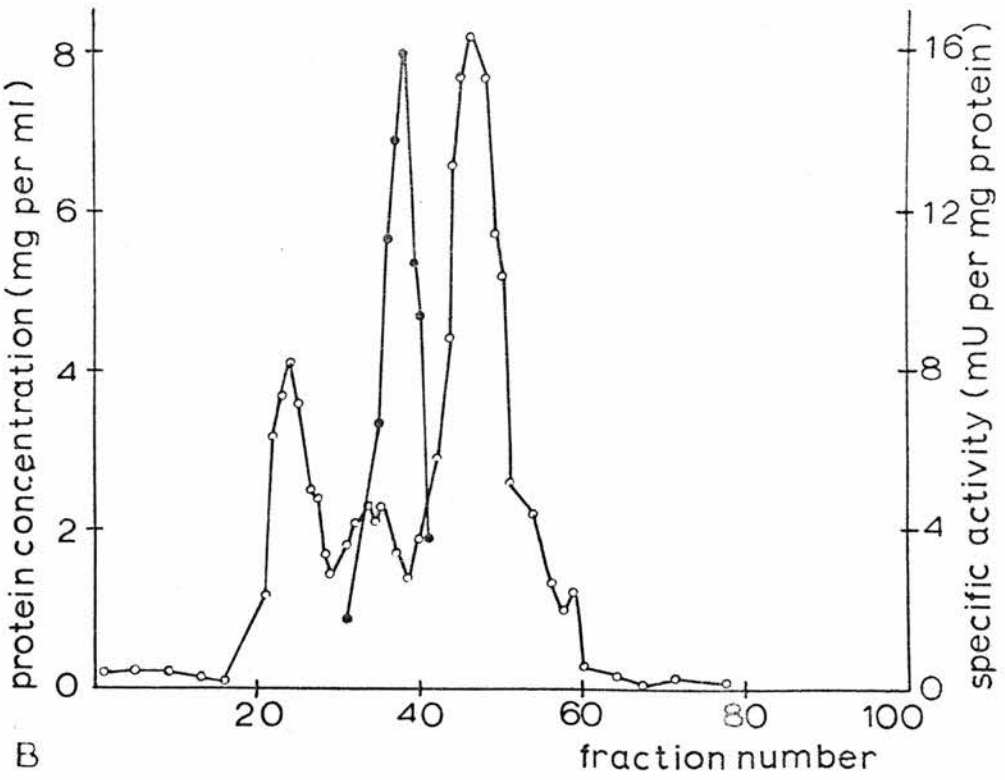
Loading buffer and starting buffer: 0.1 M Tris/HCl pH 7.0 containing 0.05 M NaCl. Limiting buffer: 0.1 M Tris/HCl pH 7.0 containing A 0.4 M NaCl, B 0.6 M NaCl, C 0.8 M NaCl.

Flow rate: 0.2 ml/minute. Fractions collected: 15 minutes.

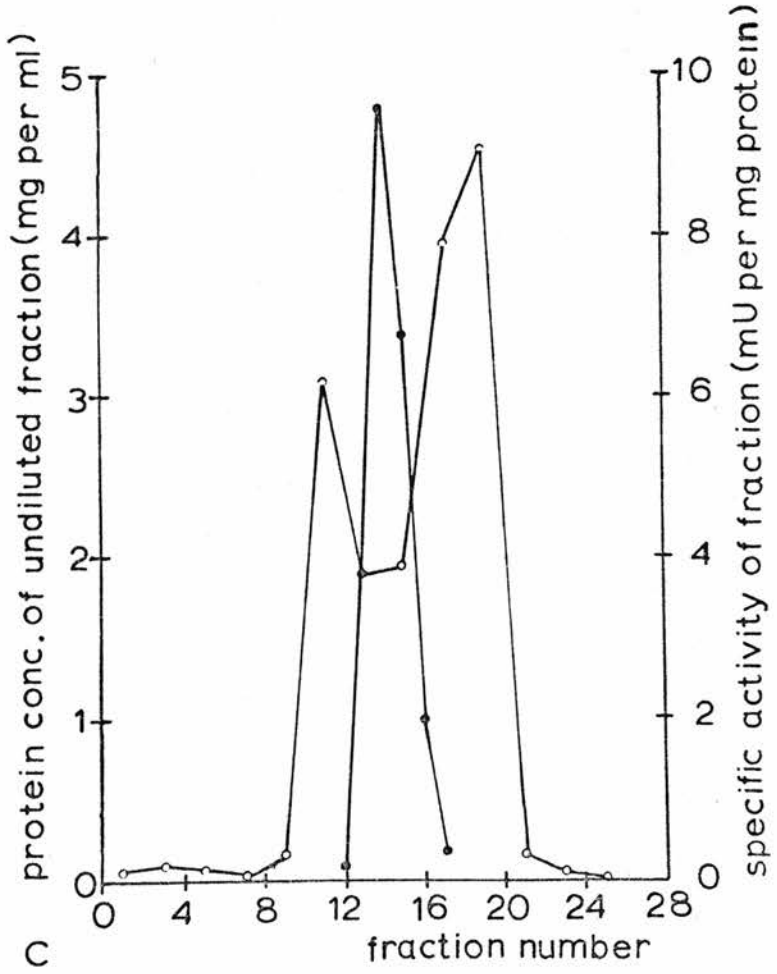
Column dimensions: 10 mm x 120 mm.

O——O : protein concentration

●——● : specific activity of prostaglandin A isomerase


protein concentration (mg per ml)
specific activity (mU per mg protein)
fraction number
0
2
4
6
8
0
4
8
12
16
20
40
60
80
100

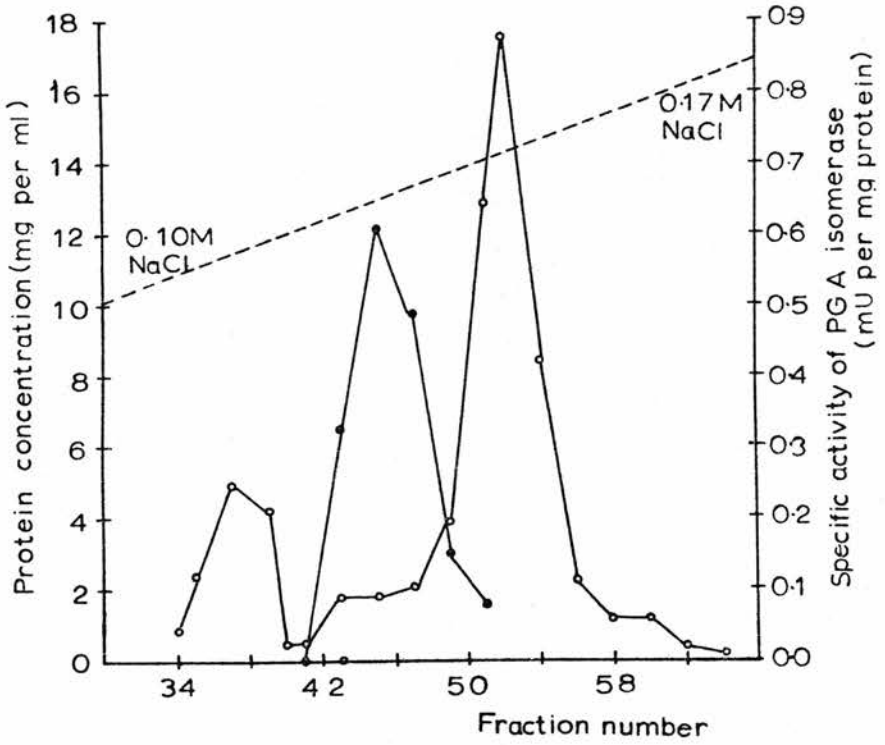
B


protein conc. of undiluted fraction (mg per ml)
specific activity of fraction (mU per mg protein)
fraction number
0
1
2
3
4
5
0
2
4
6
8
10
0
4
8
12
16
20
24
28

C

Figure 12

Separation of proteins in the 45%-60% saturated ammonium sulphate fraction of cat plasma on DEAE Sephadex A-50 eluted with NaCl gradient B.



Flow rate: 1 ml/minute

Fractions collected: 15 minutes

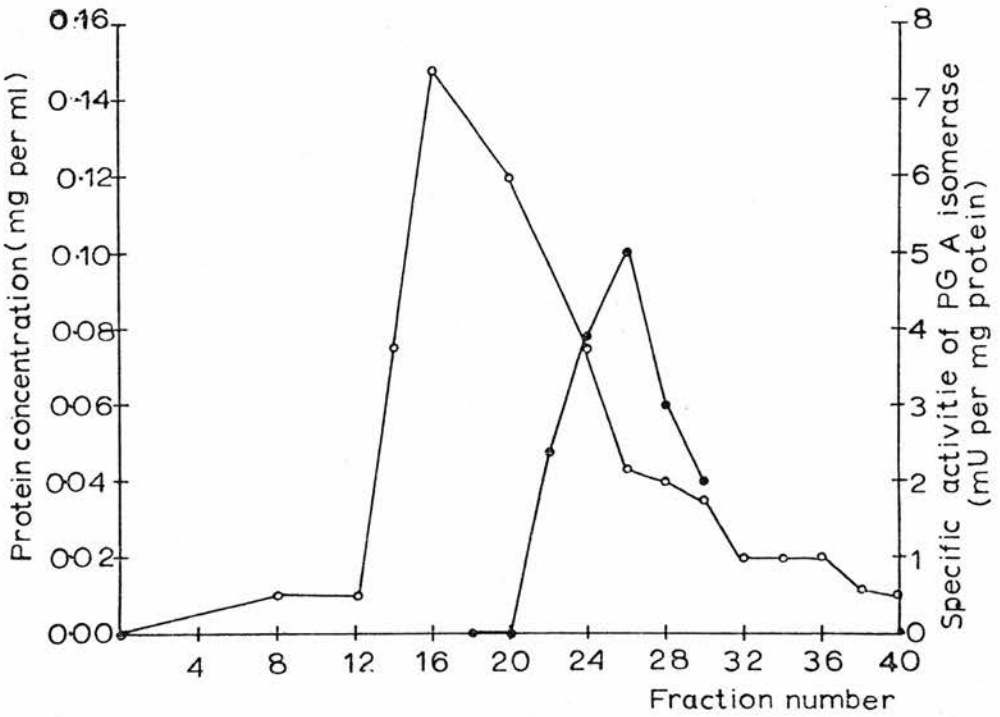
Column dimensions: 30 mm x 1000 mm

O——O : protein concentration

●——● : specific activity of prostaglandin A isomerase

Figure 13

Elution profile on Sephadex G-200 of pooled fractions from ion exchange chromatography on DEAE Sephadex A-50.



Buffer: 0.1 M Tris/HCl pH 7.0 containing 1.0 M NaCl

Flow rate: 0.5 ml/minute

Fractions collected: 20 minute fractions

Column dimensions: 20 mm x 750 mm

O——O : protein concentration

●——● : specific activity of prostaglandin A isomerase

Three other partial purifications of the enzyme were carried out with the fold purification relative to plasma and the yields shown in Table 15.

Table 15

Yield and fold purification obtained from partial purification of prostaglandin A isomerase from cat plasma

Batch Number	Yield (% of plasma content)	Fold purification (relative to plasma = 1)
1	8.5	14.6
2	9.5	18.0
3	2.1	48.0

Thus good yields are not compatible with high fold purification. Details of yields and purification obtained at different stages of the purifications of these three batches are shown in Table 16.

Table 16

Yields and fold purification obtained during the partial purification of three batches of prostaglandin A isomerase

Batch	I		II		III	
Specific activity of plasma (mU/mg protein)	0.16		0.170		0.365	
Sample	Yield (%)	Fold purification	Yield (%)	Fold purification	Yield (%)	Fold purification
$(NH_4)_2SO_4$	59.0	2.35	65.0	1.3	28.0	1.1
DEAE Sephadex	10.0	5.0	20.5	7.3	20.0	4.6
Sephadex G-200	8.5	14.6	9.5	18.0	27.0	48.0

Similar details of the partial purification of a fourth batch of the enzyme which has been described in more detail are in Table 14, page 61. The greatest fold purification always occurred during gel chromatography on Sephadex G-200, seemingly at the expense of a high yield. Gel chromatography was also where the greatest variation in loss of enzyme protein took place with resulting low yield.

Of the other known prostaglandin metabolising enzymes: the widely distributed 15-hydroxy prostaglandin dehydrogenase and 15-keto prostaglandin reductase (Ångård and Larsson, 1971; Ångård, Larsson and Samuelsson, 1971), the liver mitochondrial β-oxidising system (Hamberg, 1969), and the guinea pig liver ω-oxidising system (Israelsson et al., 1969), only 15-hydroxy prostaglandin dehydrogenase has been partially purified. Purification has been achieved from swine lung (Ångård and Samuelsson, 1966), from bovine lung (Saeed and Roy, 1972) and from human placenta (Jarabak, 1971). Ångård and Samuelsson and Saeed and Roy were unable to assay enzymatic activity of lung homogenates but obtained 11 fold purification, with 31% yield and 10 fold purification with 55% yield respectively of enzyme preparations obtained after the first step of the purification procedure. Jarabak was able to obtain 19 fold purification but only a 7% yield of enzymatic activity in the placenta homogenate.
Thus in all 3 purifications, significant purification was achieved at the expense of yield. A simple 2 stage procedure comprising ammonium sulphate and acetone precipitation (Saeed and Roy, 1972), produced an enzyme preparation of high specific activity and good yield only because the initial specific activity of the tissue homogenate was high. It was 10 times higher than that of human placenta (Jarabak, 1971) and about 5 times higher than that of swine lung (Ångård and Samuelsson, 1966). The fold purification was comparable with purification obtained

from swine lung and half that obtained from human placenta, where the yield was lowest. When rabbit plasma was found to have high specific activities of prostaglandin A isomerase compared to other species, it was hoped that the initial large amounts of enzyme would compensate for the low yields which would be obtained in a purification procedure in which the final fold purification is high. Thus a preparation of high specific activity of which only small amounts would be required for studies of its properties would be obtained from a simple, effective procedure comprising 3 or 4 stages. The poor purification (1.8) obtained of rabbit plasma prostaglandin A isomerase after ammonium sulphate precipitation and ion exchange chromatography due to the loss of activity occurring during dialysis, indicated that an attempt to improve the existing purification procedure for cat plasma would be more profitable than devising a totally new procedure for rabbit plasma. Effects of smaller ammonium sulphate fractions, different sodium chloride elution gradients and flow rates during ion exchange chromatography, and alterations in the flow rate during gel chromatography improved the fold purification relative to the ammonium sulphate fraction from 16 (Jones, 1970a) to 6.6 - 48. The enzyme preparations obtained had specific activities high enough for measurable absorbance changes at 283 nm to be obtained with a protein concentration in the reaction cell of about 14 µg/ml. This low level of material absorbing at the lower end of the spectrum enabled absorbance measurements between 200 and 250 nm to be made, and thus allowed for observation of changes in the prostaglandin A_1 spectrum (λ_{max}^{MeOH} 217 nm) leading to the discovery and isolation of prostaglandin C_1 (Jones, 1972).

Subsequent experiments using prostaglandin A isomerase successfully purified about 25 fold from rabbit plasma indicate that,

for this species at least, a very pure enzyme preparation is not an advantage. Inactivation of the enzyme at low protein concentrations, shown by a non-linear relationship between amount of enzyme and reaction rate, occurs. Linearity of this relationship (and thus enzyme activity) is restored by adding small amounts of bovine serum albumin (Jones, personal communication). Loss of activity during dialysis and gel chromatography observed during preliminary attempts to devise a purification procedure (page 57) may have occurred from protein loss. The eluant from the Sephadex G-25 column, which was collected after prostaglandin A isomerase, was not protein free. Though it had some enzyme activity (page 58), the presence of other proteins which might also contribute to the enzyme activation it produced, cannot be excluded. Continuing protein loss during purification might reduce the apparent fold purification obtained.

It is apparent that the purification obtained of cat plasma prostaglandin A isomerase compares well with the results of 3 purification procedures for prostaglandin dehydrogenase. Nevertheless, still further purification is required for detailed study of the enzyme's properties. In particular, the possibility that prostaglandin A isomerase is an isoenzyme. The K_m of rabbit plasma prostaglandin A isomerase for prostaglandin A_2 is considerably lower than the K_m of the cat plasma enzyme for prostaglandin A_1 (see Results Section, page 82). This suggests that the cat and rabbit plasma enzymes may be different. Preparative gel electrofocussing would be a useful additional step after Sephadex G-200 in the present purification procedure, since good purification without low yields have been reported with this technique (Vesterberg, 1971).
In addition, electrofocussing enables an enzyme's isoelectric point to be determined. Thus results from electrofocussing could distinguish

cat plasma prostaglandin A isomerase and the rabbit plasma enzyme by a difference in their isoelectric points. This would be strong evidence that prostaglandin A isomerase is an isoenzyme.

Some properties of prostaglandin A isomerase

Effect of pH on prostaglandin A isomerase activity: The pH dependence of prostaglandin A isomerase activity assayed by methods number 1 and number 2 are shown in Figures 14 and 15 respectively, pages 71 and 72. The effect of pretreatment with acid and alkali on the activity of the enzyme assayed by methods number 1 and number 2 is shown in Figure 16, page 73.

The effect of measuring the prostaglandin B_1 formation, which is not a primary function of prostaglandin A isomerase activity but also dependent on the stability of the product of the reaction, is clearly shown in differences in pH activity curves measured by assay methods number 1 and number 2 (Figure 14, page 71, and Figure 15, page 72, respectively). The lack of enzymatic activity below pH 7 shown in the apparent pH activity curve (Figure 15) compared with considerable activity at pH 5 to 7 depicted in the pH activity curve in Figure 14, occurs because prostaglandin C_1 is stable below pH 7 and no isomerisation to prostaglandin B_1 takes place (Jones, 1972). At pH above 7, enzyme activity is detected by assay method number 2. An increased prostaglandin B_1 formation with increased pH is measured, occurring from the combination of increased prostaglandin A isomerase activity and increased spontaneous breakdown of prostaglandin C_1 to prostaglandin B_1. The chemical conversion of prostaglandin C_1 to prostaglandin B_1 between pH 8.5 and 9.5 compensates for the loss in prostaglandin A isomerase activity which begins to occur at

Figure 14

Buffer pH (abscissa) plotted against initial rate of increase in absorbance at 242 nm (ordinate).

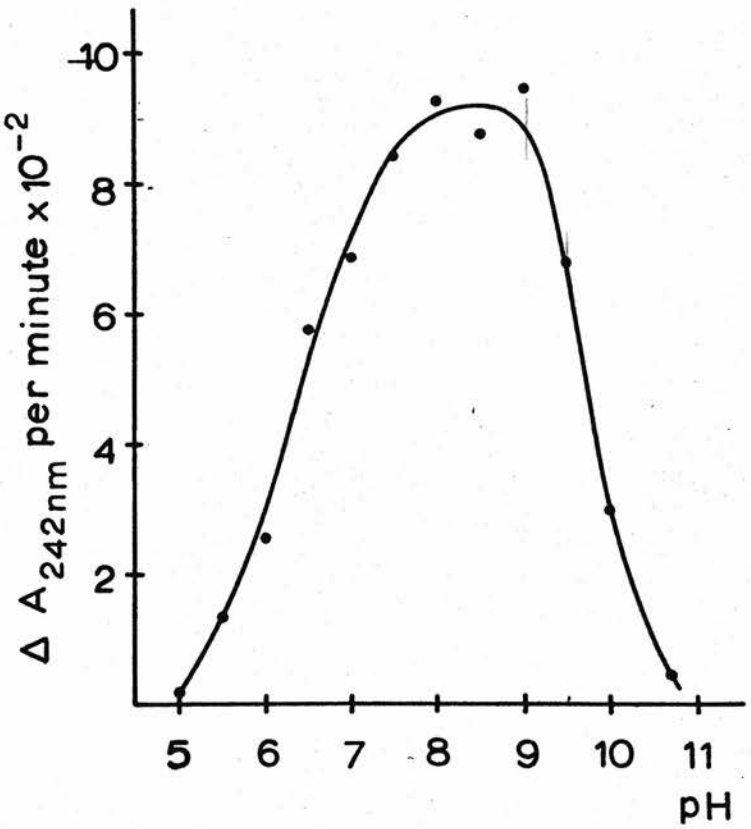
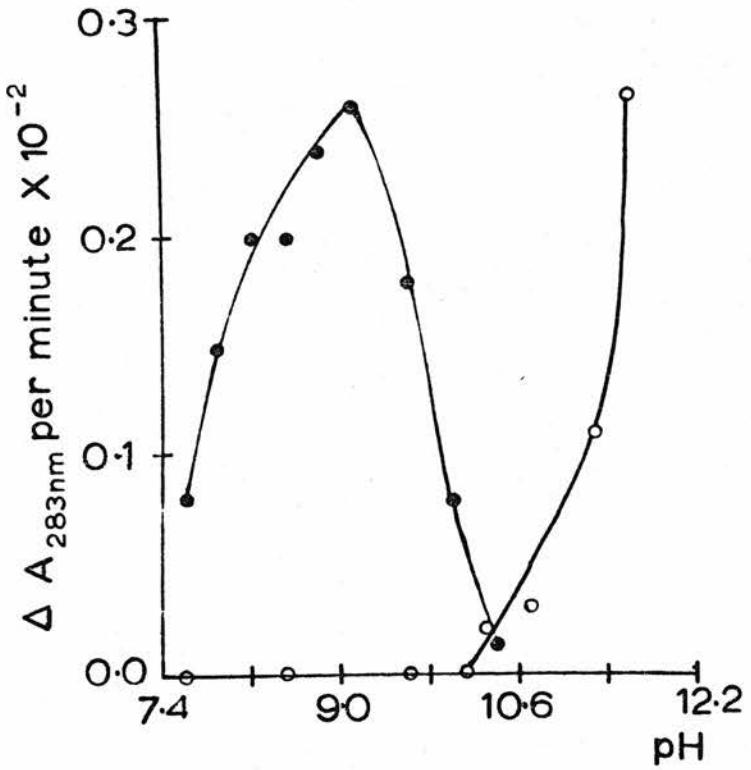


Figure 15

Buffer pH (abscissa) plotted against a rate factor of the time taken for an increase in absorbance at 283 nm of 0.08 OD units to occur (ordinate).

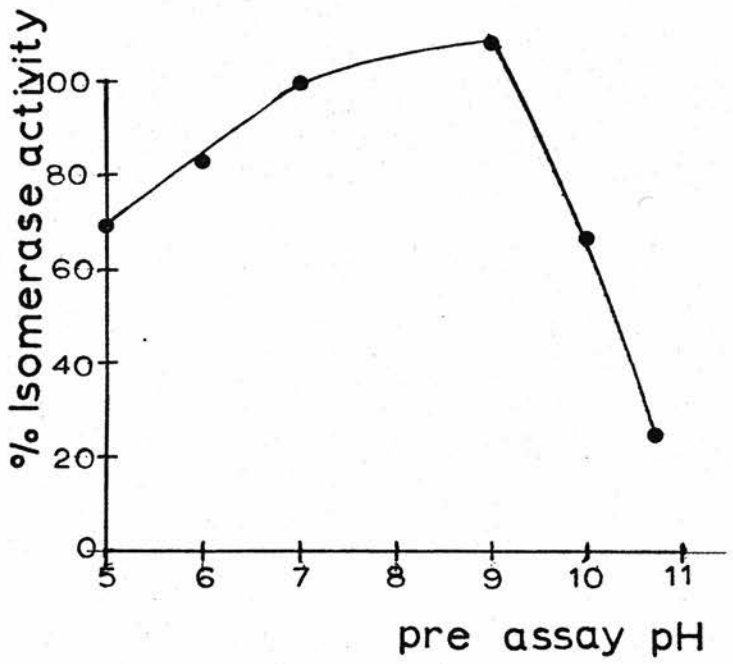


●——● prostaglandin A isomerase present

o——o prostaglandin A isomerase absent

Figure 16

Buffer pH during pre-incubation of prostaglandin A isomerase (abscissa) plotted against % maximum prostaglandin A isomerase activity measured by assay method number 1 (ordinate).



these pH's (Figure 14, page 71), so the apparent pH activity curve does not indicate enzyme inactivation until pH of about 9.5. At this pH the enzyme has in fact lost much of its activity, and sufficient prostaglandin C_1 to mask this inactivation, by its chemical breakdown, cannot be formed. Spontaneous base catalysed isomerisation of prostaglandin A_1 to prostaglandin B_1 (Bergström et al., 1963) does not contribute to apparent prostaglandin A isomerase activity measured by assay method number 2, as it cannot be detected below pH 10. It does not approach a rate comparable with the lowest apparent enzymatic rate detected until the pH is 10.5 to 11, when very little prostaglandin A isomerase activity is shown on the true (Figure 14, page 71) and the apparent (Figure 15, page 72) pH activity curves.

It has been demonstrated (Jones and Cammock, 1972) that prostaglandin A isomerase activity is affected by the buffer used, and that a continuous pH activity curve is not obtained when the range of pH's studied is provided by more than one buffer. Nevertheless the form of the profile obtained is the same as that in Figure 14, page 71, indicating a plateau of activity between pH 7 and 9 with a reduced activity at pH's 7 to 5 and 9 to 10.

Denaturation of the enzyme molecule below pH 7 and above pH 9 is the most probable cause of reduced enzymatic activity. Above pH 9 it is not reversible, below pH 7 it is more readily so (Figure 16, page 73). This confirms the acid stability of the enzyme noticed during preliminary work on partial purification (see page 52) and in another study (Levine and van Vunakis, 1972).

Effect of temperature on prostaglandin A isomerase activity:

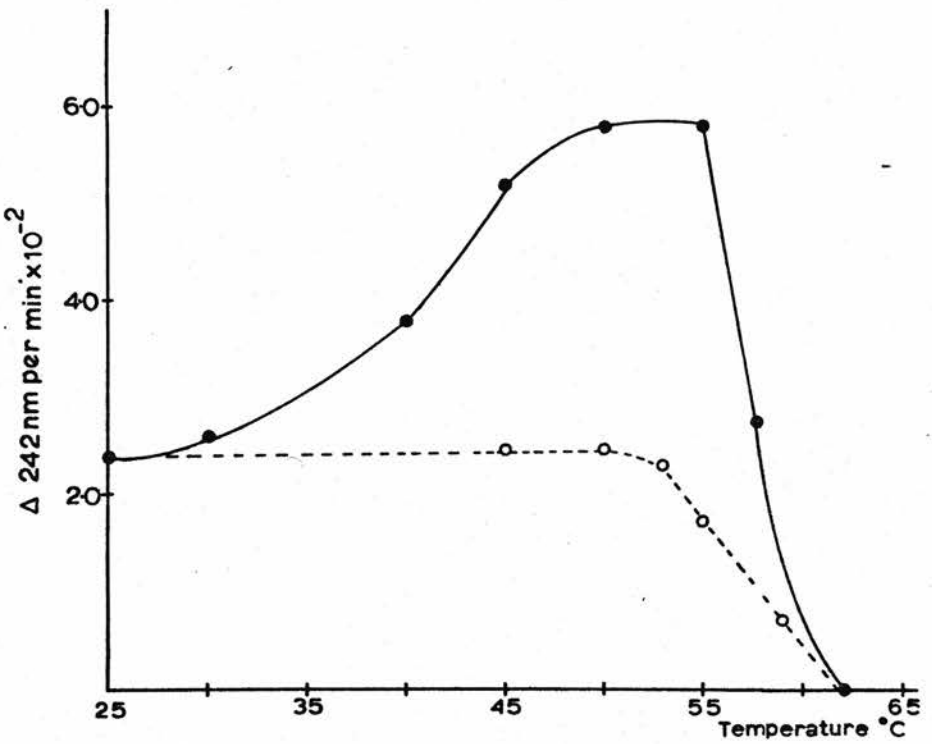
The temperature dependence of prostaglandin A isomerase activity and its heat stability assayed by method number 1 is depicted in

Figure 17, page 76. Figure 18, page 77, shows the temperature dependence of prostaglandin isomerase activity assayed by method number 2.

The faster rates of enzymatic prostaglandin A isomerisation with increased temperature is an example of the increased reaction rates of enzymatically catalysed and chemical reaction that occur at higher temperature (Dixon and Webb, 1965). However the rate of an enzymatically catalysed reaction does not continue to increase, since loss in enzyme activity takes place due to heat denaturation of enzyme protein. The levelling off of the rate of absorbance increase at 242 nm at a temperature of about 45°C indicates that the effects of heat denaturation on prostaglandin A isomerase are extensive enough to oppose the effect of increased temperature on the reaction rate (Figure 17, page 76). Heating the enzyme before assaying it at 25°C shows that irreversible heat denaturation begins between 50°C and 55°C. This difference in the estimate of the temperature below which prostaglandin A isomerase is stable might occur from a temperature instability of prostaglandin C_1 above 45°C, producing an apparent reduction in reaction rate. However there are no data on the heat stability of prostaglandin C_1 though it is much less stable than prostaglandin A_1 in alkali (Jones, 1973). Results from prostaglandin A isomerase assays at different temperatures and from pre-assay heat treatment are both compatible with complete heat denaturation at 62°C. The possible instability of prostaglandin C_1 at high temperatures is perhaps the reason for the reduced rate of prostaglandin B_1 formation starting at 37°C to 40°C, when the enzyme is assayed at different temperatures by assay method number 2, (Figure 18, page 77), and for an apparent inactivation at 58°C. Nevertheless the finding in that denaturation is complete between 58°C and 62°C,

Figure 17

Temperature dependence and stability of prostaglandin A isomerase studied with assay method number 1.

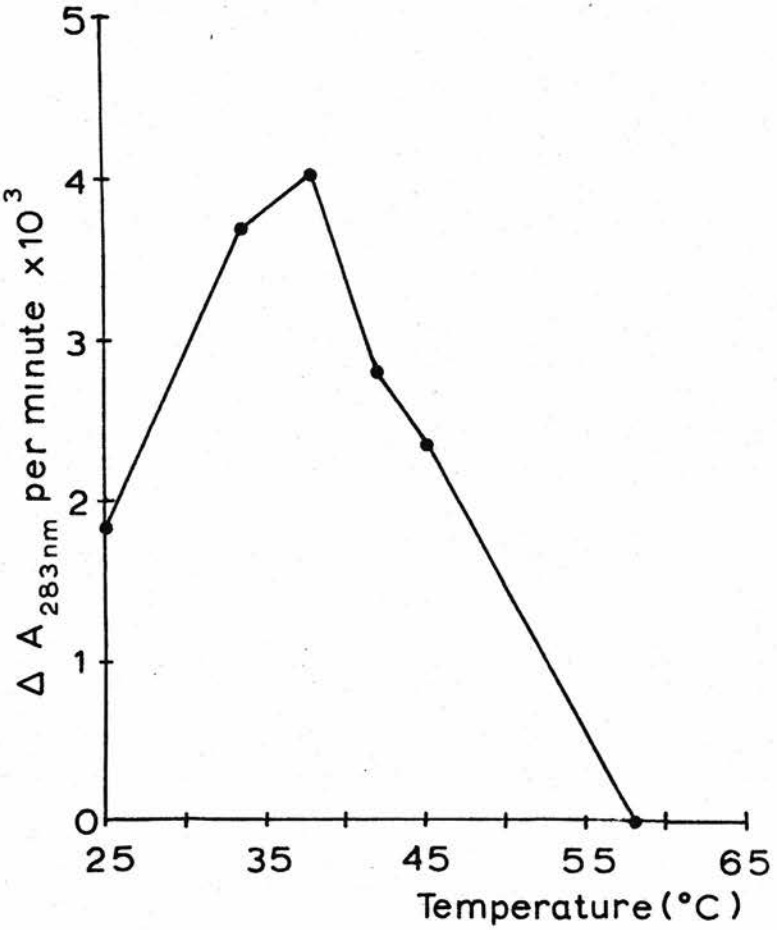


●——● Temperature at which the assay was performed.

o——o Temperature at which prostaglandin A isomerase was incubated before assay at 25°C.

Figure 18

Temperature dependence of prostaglandin A isomerase assayed by method number 2.



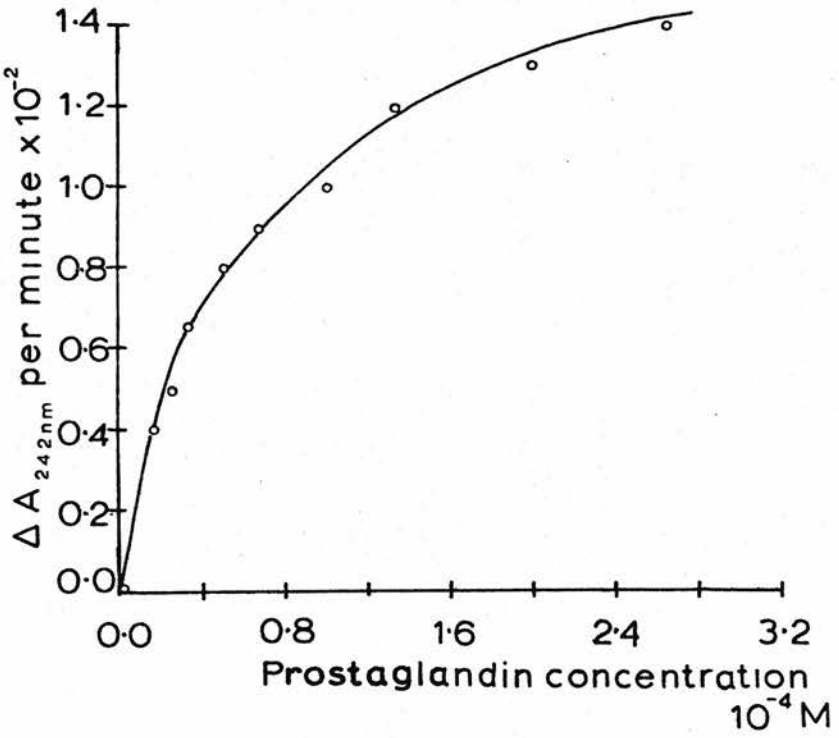
●——● Temperature at which the assay was performed.

whilst in agreement with results obtained from preliminary purification studies (page 52), do not agree with those of Levine and van Vunakis (Levine and van Vunakis, 1971) who failed to observe any loss in activity after pre-assay heating at 70°C for 30 minutes. This difference cannot be accounted for, even by the immunological method of assay.

Effect of substrate concentration on prostaglandin A isomerase activity: Figure 19, page 79, is the substrate concentration rate plot of prostaglandin A isomerase. It shows that the prostaglandin A_1 concentration (0.66×10^{-4}M = 22 µg/ml) does not approximate to the saturating substrate concentration at which the reaction rate, v, is maximal (equal to Vmax). Nevertheless it is sufficiently high for any apparent changes in enzyme activity due to fluctuations in substrate concentration, [S] , to be small. An approximate estimate of the saturating [S] for prostaglandin A isomerase using prostaglandin A_1 as substrate, made by extrapolating the substrate rate plot to the ordinate value corresponding to Vmax ($\Delta A_{242\ nm}$ 1.5×10^{-2} units) and obtaining [S] from the abscissa, is 4.4×10^{-4} M (146 µg/ml). The rate of reaction, v, at the substrate concentration used for the enzyme assay is 0.60 Vmax. An accurate extrapolation to the reaction velocities which would be obtained at high substrate concentrations is a major source of error in this method of estimating the saturating [S] . This graph may also be used to estimate approximately the Michaelis constant of the enzyme but is limited by the same error inherent in the assessment of Vmax. The relationship between v, [S] , and Vmax is described by the Michaelis-Menton equation (Dixon and Webb, 1958) :-

Figure 19

Prostaglandin A_1 concentration (abscissa) plotted against initial rate of increase in absorbance at 242 nm (ordinate).



$$v = \frac{Vmax \quad [S]}{Km + [S]}$$

Km is the Michaelis constant of the enzyme. By re-arranging the above equation, it is apparent that its empirical parameter is the substrate concentration when v is half Vmax :-

$$\frac{Vmax}{2} = \frac{Vmax \quad [S]}{Km + [S]}$$

$$Km + [S] = 2\,[S]$$

$$Km = [S]$$

Therefore an approximate estimate of Km is 4.80×10^{-5} M (Figure 19, page 79).

These errors in assessing Vmax and Km are eliminated in a double reciprocal plot (Figure 20, page 81) known as the Lineweaver-Burke plot (Dixon and Webb, 1965) :-

$$\frac{1}{v} = \frac{Km + [S]}{Vmax \quad [S]}$$

$$\frac{1}{v} = \frac{Km}{Vmax \quad [S]} + \frac{1}{Vmax}$$

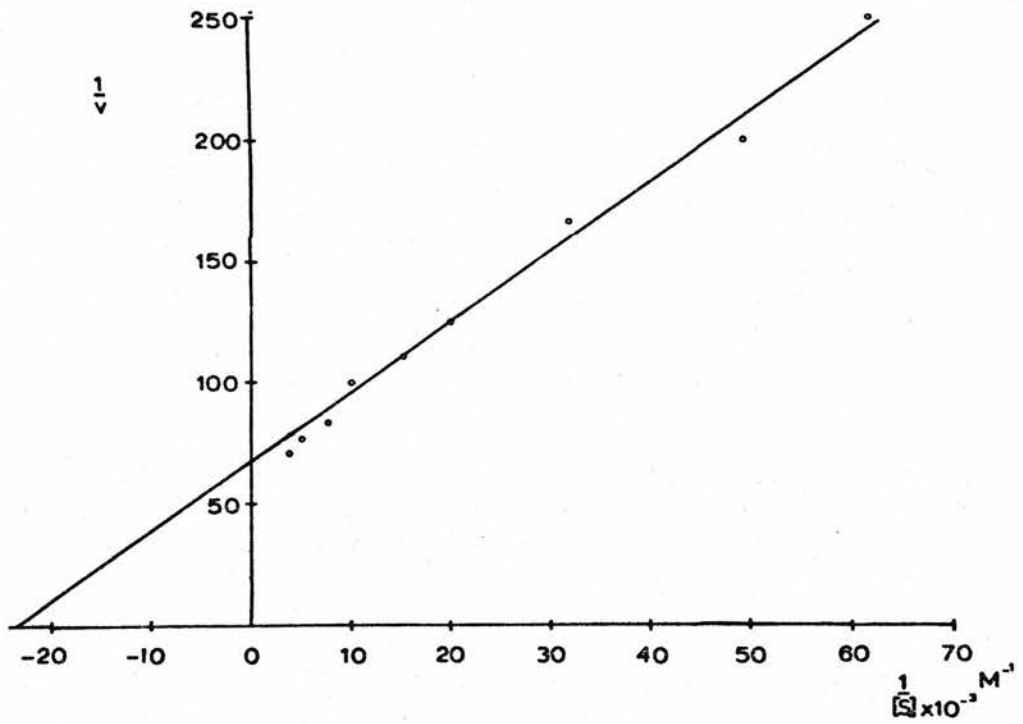
Thus when [S] = 0 the intercept on the ordinate is $\frac{1}{Vmax}$ and when v is 0 the intercept on the abscissa is $-\frac{1}{Km}$

$$\frac{1}{Vmax} = -\frac{Km}{Vmax} \cdot \frac{1}{[S]}$$

$$-[S] = Km$$

Figure 20

Double reciprocal rate plot of prostaglandin A isomerase.



The kinetic parameters for prostaglandin A isomerase obtained from Figure 20, page 81, are shown in Table 17. Those derived from the hyperbolic plot (Figure 19, page 79) are included for comparison. Errors in them occur from experimental errors in measuring initial absorbance increases and in obtaining the line of best fit through values of $\frac{1}{v}$ and $\frac{1}{[S]}$. Here the Lineweaver-Burke plot was drawn by eye.

Table 17

Kinetic parameters of prostaglandin A isomerase from cat plasma. Prostaglandin A_1 substrate

Parameter	Results obtained from Figure 20	Results obtained from Figure 19
Vmax	0.015 OD units per minute	0.015 OD units per minute
Km	5.55×10^{-5}M	4.75×10^{-5}M
Saturating [S]	-	4.40×10^{-4}M

K_m for prostaglandin A_1 for swine lung 15-OH prostaglandin dehydrogenase is 1.4×10^{-5} M (Nakano et al., 1969), and for the placental dehydrogenase 8.7×10^{-5} M (Jarabak, 1971). Thus the K_m for cat plasma prostaglandin A isomerase is of the same order. Since an enzyme's K_m is the substrate concentration at half the maximum velocity of the reaction (Dixon and Webb, 1965), the concentration in cat blood of prostaglandin A_1, which would be isomerised at half the maximum rate, would be about 1.5 µg/ml. In the cat, prostaglandin A_1 is depressor agent at a concentration 100 times lower than this (Horton and Jones, 1969). Nevertheless an appreciable rate of isomerisation to

prostaglandin C_1 may still occur in cat blood, even at this low concentration. Indirect evidence that it does comes from the biphasic depressor response to intraarterial doses of prostaglandin A_1 noticed by Kannegiesser and Lee (1971) and by Jones (1972b). The fall in blood pressure produced by prostaglandin B_1 is rapid, reaching maximum between 15 seconds and declining to $\frac{2}{3}$ maximum in 35 seconds. That due to prostaglandin C_1 is more prolonged; the maximum value does not occur until 45 seconds and does not decline to $\frac{2}{3}$ maximum until 135 seconds. However prostaglandin A_1 gives an initial rapid fall in which the maximum value occurs in 15 seconds, like prostaglandin B_1, which is followed by a more pronounced fall in which the maximum does not occur until 85 seconds (Jones, 1972b). It is possible that this biphasic action of prostaglandin A_1 is accounted for by an initial rapid fall due to prostaglandin A_1 followed by the more prolonged action of prostaglandin C_1 formed from prostaglandin A_1 in blood. There are as yet no reports of a similar biphasic response to A prostaglandins in species in which prostaglandin A isomerase has not been detected.

General Discussion

Since the first reports of prostaglandin A isomerase in cat plasma (Jones, 1970a; 1970b), the enzyme has been shown in plasmas of several species and though its presence has not yet been demonstrated in any other tissue, there are several situations in which its action may be implicated.

Prostaglandin B compounds amongst rat urinary metabolites of prostaglandin E_2

One of these is the formation of prostaglandin B-type compounds identified amongst rat urinary metabolites of prostaglandin E_2 (Gréen, 1971). They are all tetranor derivatives retaining the 13,14 diene and the 15-hydroxyl group of primary prostaglandins. It is suggested (Gréen, 1971) that some of the intravenously administered prostaglandin E_2 is initially β-oxidised to tetranor prostaglandin E which is a poor substrate for 15-OH prostaglandin dehydrogenase (Nakano et al., 1969; Vonkeman et al., 1969). Beta oxidation of prostaglandin has been demonstrated in rat liver (Hamberg, 1968) and reported in rat kidney and lung (Nakano and Morsy, 1971). In this way prostaglandin E_2 escapes oxidation of the 15-OH group by 15-OH prostaglandin dehydrogenase (Ångård and Samuelsson, 1966) and subsequent reduction by 15-oxo prostaglandin 13,14 reductase (Ångård and Larsson, 1971), both of which enzymes are widely distributed in animal tissue (Ångård and Larsson, 1971). The sequence of reactions following which produce tetranor prostaglandin B, 16-OH tetranor prostaglandin B, 15-OH tetranor prostaglandin B and 16-carboxylated tetranor prostaglandin B, is not clear.

Whilst the major urinary metabolite of prostaglandins E_1 and E_2 in man (Hamberg and Samuelsson, 1971) and also a major constituent of the rat urinary metabolites (Gréen, 1971) is 7α-hydroxy-5, 11-diketo-tetranor prosta-1, 16-dioic acid which is likely to be formed by ω-hydroxylation at the terminal methyl group, followed by oxidation (Robbins, 1968), little ω-oxidation of prostaglandin E_1 and none of 15-oxo-13,14-dihydro prostaglandin E_1 has been demonstrated in vitro (Israelsson et al., 1969). A guinea pig liver microsomal preparation hydroxylated 10% of prostaglandin A_1 and prostaglandin B_1 at C-19 and C-20 but only 3% of prostaglandin E_1 at C-19 only. No conversion was observed when 15-oxo-13,14-dihydro prostaglandin E_1 was substrate. Human seminal plasma which is the only source from which 19-hydroxylated prostaglandins have been isolated (Hamberg and Samuelsson, 1965) is devoid of hydroxylated prostaglandin E compounds but contains large amounts of 19-hydroxylated prostaglandins A_1, A_2, B_1 and B_2. These two facts would suggest that though prostaglandins of the E series may not be substrates for the fatty acid ω-oxidising system in human male reproductive tract tissue, the ω-oxidising enzymes in this tissue are different from those in the liver, for which E-prostaglandins may act as substrate. In addition, the existence of carboxylated prostaglandin E metabolites in human and rat urine and the intermediate hydroxylated compound in rat urine suggest that an ω-oxidising system, for which prostaglandin E compounds are substrates, exists.

As there are no tetranor 16-hydroxylated or 16-carboxylated prostaglandin E compounds in which the 11-OH group and the 9-10 diene are intact amongst rat urinary metabolites, ω-oxidation seems an unlikely step to follow β-oxidation in the pathway producing metabolites of the prostaglandin B-type. More probably enzymatic dehydration to

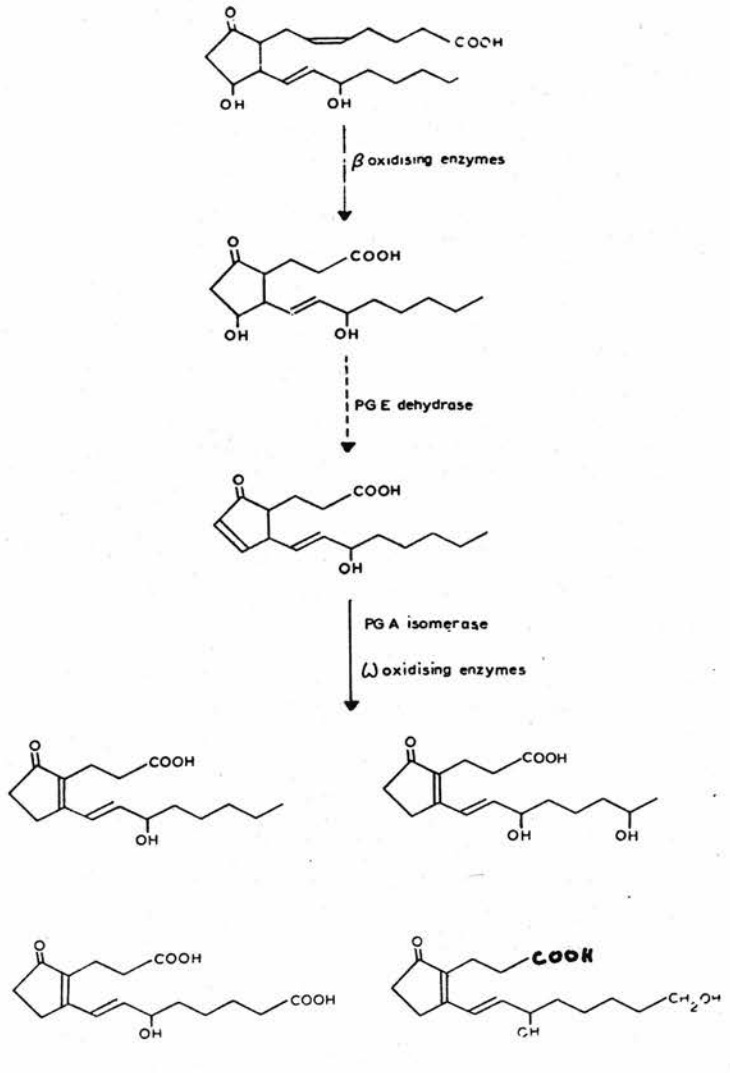
tetranor prostaglandin A occurs. A prostaglandin E dehydrase has not yet, however, been described in the rat. It is at this point in the sequence of reactions that prostaglandin A isomerase could act. If the activity of this enzyme is high, then prostaglandin B compounds might be formed. If not, the products would be of the C-type converted to the corresponding B prostaglandins during extraction and chromatography.

Attempts to prepare dinor and tetranor prostaglandin E_1 from rat liver mitochondria (Hamberg, 1965) and hence the corresponding A prostaglandins for substrate specificity of prostaglandin A isomerase studies were unsuccessful, so it is not known whether β-oxidised prostaglandin A is a substrate for prostaglandin A isomerase. Prostaglandins A_1 and B_1 are equally effective as substrates for guinea pig liver microsomal ω-oxidising enzymes (Israelsson et al., 1969), and 19-OH prostaglandin A_1 is a substrate for prostaglandin A isomerase (Jones, Cammock and Horton, 1972). Thus it is not possible to assess whether isomerisation or ω-oxidation follows dehydration. The pathway by which prostaglandin B-type urinary metabolites might be formed from prostaglandin E_2 in the rat is summarised in Figure 21, page 87.

An alternative pathway is possible if protection from the action of 15-OH prostaglandin dehydrogenase occurred via dehydration of prostaglandin E_2, followed by isomerisation to prostaglandin C_2 or prostaglandin B_2. Little prostaglandin C_2 is converted to 15-oxo prostaglandin C_2 by partially purified 15-hydroxy prostaglandin dehydrogenase from pig kidney, in spite of a rapid initial reaction rate (Jones and Cammock, 1972), whilst prostaglandin B_2 is not a substrate for swine lung dehydrogenase (Änggård and Samuelsson, 1966; Nakano et al., 1969). However it is not known if a prostaglandin E dehydrase

Figure 21

One pathway by which prostaglandin B-type compounds might be formed from prostaglandin E_2 in the rat.



Broken lines denote enzymes which have been postulated but for which evidence has not yet been obtained.

is present in the rat and if so, whether it is as ubiquitous as 15-OH prostaglandin dehydrogenase and the 15-oxo prostaglandin 13,14 reductase (Änggård et al., 1971). There are indications that prostaglandin β-oxidising enzymes are not confined to the liver (Nakano and Morsy, 1971). Thus there is a strong possibility that in the rat β-oxidation can be an initial step in the metabolism of prostaglandin E which protects the 15-OH group and the 13,14 diene from the actions of 15-OH prostaglandin dehydrogenase and 15-oxo prostaglandin, 13,14 reductase. However, no evidence exists to support the suggestion that dehydration and isomerisation can prevent metabolism of prostaglandin E_1 by 15-OH prostaglandin dehydrogenase and 15-oxo prostaglandin, 13,14 reductase in the same way.

Radioimmunoassay of prostaglandins

Prostaglandin A isomerase is significant in the development of radioimmunoassay of prostaglandins. Attempts are being made to raise antibodies specific at least to the 4 major classes of prostaglandin, so that a sensitive specific and more rigid method of estimation of prostaglandins in tissues and body fluids will be available. In most laboratories, antibodies are raised in rabbit sera by immunising with prostaglandin-poly-L-lysine-carbodiimide conjugate (Levine and van Vunakis, 1970; Levine, Gutierrez Cernosek and van Vunakis, 1971; Jaffe, Newton, Parker and Smith, 1971). Whereas immunisation with prostaglandins of the F series produces antibodies with little cross reactivity with E, A and B prostaglandins (Levine et al., 1971; Jaffe et al., 1971), great difficulty is being experienced in obtaining antibodies specific to the immunising prostaglandin when prostaglandins E or A are used (Levine et al., 1971;

Yu and Burke, 1972). Sera containing antibodies raised against prostaglandin E show greater binding capacity for prostaglandin B compounds with less or equal capacity for prostaglandin A ones (Yu and Burke, 1972; Raz, 1973). Heterogeneous sera, containing antibodies specific to E and B prostaglandins, have been detected by an alteration in the apparent specificity of sera specific for prostaglandin B at high and prostaglandin E at low dilutions (Yu and Burke, 1972; Levine, 1973). Most of the antibodies are specific for A and B prostaglandins with a small proportion directed towards prostaglandin E. If prostaglandin E in the poly-L-lysine-carbodiimide conjugate is pure, it is converted to a mixture of prostaglandins before the antibody producing site is challenged. Dehydration to prostaglandin A must occur, followed by isomerisation to prostaglandin B. There is a possibility that the cross reactivity of anti-prostaglandin A antibodies with prostaglandin B is the result of the structural similarity of the two molecules which differ only in the position of the diene structure in the cyclopentane ring. However this alteration induces conformational changes into the molecule at C-8 and C-12 which are recognised by the enzyme 15-OH prostaglandin dehydrogenase. Prostaglandins of the A series but not those of the B series are substrates (Nakano et al., 1969; Vonkeman et al., 1969). Differences in metabolism of the two molecules by the β-oxidation system in rat liver also occur; prostaglandin A is oxidised to a mixture of dinor and tetranor prostaglandin A_1 and conversion is incomplete, whereas prostaglandin B_1 is almost completely converted to dinor prostaglandin B_1 only. Thus recognition of the differences between prostaglandin A and prostaglandin B by enzyme protein makes it likely that antibody protein is also capable of distinguishing these two types of prostaglandin, and that a heterogeneous population of antibodies is the result of challenging

the antibody forming site with a mixture of prostaglandins. The high concentration of prostaglandin isomerase in rabbit plasma makes enzymatic isomerisation of prostaglandin A to prostaglandin B via prostaglandin C a plausable mechanism in the formation of prostaglandin B. More conclusive evidence that prostaglandin A antibodies more specific to prostaglandin B occur from isomerisation rather than lack of recognition by the challenged antibody producing site could be obtained in two ways. One is by raising antibodies to a prostaglandin A, such as 13,14-dihydro A_1, which is not a substrate for the isomerase (Jones et al., 1972). The antibodies produced would therefore be expected to have little binding capacity for 13,14-dihydro B_1. Cross reactivity of prostaglandin B antibodies produced by immunising with prostaglandin A with the prostaglandin A compounds, range from 10% (Levine and van Vunakis, 1970) to 100% (Raz, 1973).

Alternatively, antibodies could be raised in species in which plasma prostaglandin A isomerase has not been detected. Levels of the enzyme in the goat, which is sometimes used to raise antibodies, have not been investigated. It is not clear whether prostaglandin A is formed by <u>in vivo</u> or <u>in vitro</u> dehydration. Improved specificity of antibodies for prostaglandin E when carbodiimide and bovine serum albumin are replaced by poly-L-lysine for the conjugate formation (Raz, 1973), suggests that <u>in vitro</u> dehydration may occur during conjugate formation. Yet estimations of prostaglandin E and prostaglandin A in the prostaglandin reacted with carbodiimide and of prostaglandin E and prostaglandin A in unreacted prostaglandin are not significantly different (Yu and Burke, 1972). Investigations carried out in this laboratory on the stability of prostaglandin E_2 under reaction conditions of carbodiimide conjugate formation have also failed to detect any <u>in vitro</u> dehydration to prostaglandin A_2

(Hensby, personal communication). In vivo conversion therefore seems more likely, though there is as yet no evidence for a prostaglandin E dehydrase in the rabbit.

In vivo and in vitro metabolism of prostaglandins by the lung

There are differences in the in vivo metabolism of prostaglandins of the E series and those of the A series in species in which the plasma prostaglandin A isomerase levels are high. Prostaglandins E_1 and E_2 lose their depressor activity after passing through the pulmonary circulation of the dog (Ferreira and Vane, 1967; McGiff et al., 1969), cat and rabbit (Ferreira and Vane, 1967). Prostaglandins A_1 and A_2 retain their biological activity in the pulmonary circulation of the cat (Horton and Jones, 1969) and the dog (McGiff et al., 1969). In vitro metabolism of prostaglandin E has not been studied in these species but the loss of activity of prostaglandins of the E series has been attributed to metabolism by 15-OH prostaglandin dehydrogenase (Piper et al., 1970; Ångård and Larsson, 1971). This enzyme has been purified from swine lung (Ångård and Samuelsson, 1966) and from bovine lung (Saeed, 1972). Metabolites of prostaglandins E_1 and E_2 incubated with homogenates of guinea pig lung (Ångård and Samuelsson, 1964; 1965) contain the 15-oxo group produced by the action of the enzyme on the 15-hydroxyl group (Ångård and Samuelsson, 1966). This change reduces the biological activity of prostaglandin E_1 on smooth muscle (Ångård, 1966; Pike et al., 1967) and its depressor activity in dogs (Nakano, 1972). The prostaglandin E metabolites in the pulmonary artery of the dog, cat and rabbit were not identified because sensitive specific analytical methods were not available. However the blood bathed organ technique shows that the metabolites are far less biologically active on several smooth muscle preparations than

prostaglandin E_1 (Ferreira and Vane, 1967). Biological inactivation of low concentrations of prostaglandins of the A series cannot be estimated in this way because of the insensitivity of non-vascular smooth muscle preparations to these compounds (Horton and Jones, 1969). Instead the potent depressor activity (Horton and Jones, 1969) is used and the effects of equal doses made at either side of a vascular bed are compared to assess loss of biological activity occurring there. Whereas considerable loss of depressor activity of prostaglandins E_1 and E_2 is thus shown to occur in the lungs, none occurs with prostaglandins A_1, A_2 or 19-OH A_1 (Horton and Jones, 1969). This is evidence that these prostaglandins are not metabolised to biologically less active compounds. If the biologically less active metabolite of prostaglandins of the E series is assumed to be a 15-oxo derivative formed by the actions of 15-OH prostaglandin dehydrogenase, the Km values of the enzyme from swine lung and human placenta for prostaglandins E_1, E_2, A_1 and A_2 are of considerable interest. They are shown in Table 18.

Table 18

Kinetic parameters of swine lung and human placenta 15-OH prostaglandin dehydrogenase for 4 prostaglandins

Enzyme Source		Prostaglandin			
		E_1	E_2	A_1	A_2
Human Placenta (Jarabak, 1972)	Km(μM)	7.7	5.3	8.7	
	Vmax	100	116	94	
Swine lung (Nakano et al.,1969)	Km(μM)	7.7	100	14	25
	Vmax	100	97	86	48
Swine lung (Anggård & Samuelsson, 1966)	relative reaction rate	100	97	45	33

Though the Km values for A prostaglandins are higher and the Vmax lower than for the E prostaglandins, this need not imply that A prostaglandins are not substrates for the enzyme and thereby not susceptible to oxidation and consequential biological inactivation in the lungs. It is possible that the enzymes in cat, dog and rabbit lungs have different substrate specificities than those from swine lung and human placenta. However comparison of Km values of swine and human placental enzyme make marked differences in dehydrogenases from different sources unlikely. Moreover metabolism of prostaglandin A_2 does occur in isolated guinea pig lungs perfused with Krebs solution (Piper, Vane and Wyllie, 1970). 64% of activity on isolated tissues was lost and a less polar metabolite was detected on thin layer chromatography. Thus there is a discrepancy between *in vivo* and *in vitro* metabolism of A prostaglandins. *In vivo* results have been obtained from species in which the plasma prostaglandin A isomerase levels are high. The half life of prostaglandin A in cat blood is less than 30 seconds (Jones, 1972). It is therefore possible that infused prostaglandin A is isomerised to prostaglandin C. Prostaglandin C_1 is a poor substrate for 15-OH prostaglandin dehydrogenase from pig kidney; only 15% conversion is achieved in spite of a rapid initial reaction rate (Jones and Cammock, 1972). However it is 3 times more potent on the cat blood pressure than prostaglandin A_1 (Jones, 1972). Thus isomerisation of prostaglandin A to prostaglandin C might reduce metabolism by 15-OH prostaglandin dehydrogenase in the lung but enhance the biological activity. It is of interest that in the rat, a species in which we found low prostaglandin A isomerase levels, equal loss of depressor activities of prostaglandin E_2 and prostaglandin A_2 occur in the pulmonary circulation (Papanicolaou and Meyer, 1973). Comparison of *in vivo*

and *in vitro* metabolism of prostaglandin A in the lungs correlated with plasma prostaglandin A isomerase levels, would be helpful in assessing whether the actions of this enzyme can explain the difference between *in vivo* and *in vitro* studies which are not carried out on the species.

SECTION II

THE SEARCH FOR A PROSTAGLANDIN E DEHYDRASE

Introduction

There are many reports in the literature of the natural occurrence of prostaglandins of A or B types in animal tissues (Lee, Gougoutas, Takman, Daniels, Crostic, Pike, Hinman and Muirhead, 1966; Lee et al., 1967; Spector, Zusman, Caldwell and Speroff, 1974) and body fluids (Bygdeman and Samuelsson, 1966a; McGiff et al., 1970; Davis and Horton, 1972). The possibility that these may be artefacts of the extraction procedure due to dehydration of the corresponding prostaglandin E must always be considered. However, as discussed earlier in the thesis, evidence at present suggests that although such artefacts can occur, such formation does not account for most of the prostaglandin A and B type compounds so far reported, in human semen at any rate.

In contrast to the route for the biosynthesis of prostaglandins of the E and F series from arachidonic acid or dihomo-γ-linolenic acid (Hamberg and Samuelsson, 1973; Nugteren and Hazelhof, 1973), no pathway has yet been described for the biosynthesis of prostaglandins of the A series. It seems likely that prostaglandins of the B series are formed from the corresponding A by the prostaglandin C intermediate as described at length in Section I. Alternative or additional routes for the biosynthesis of prostaglandin B cannot however be excluded.

The most likely pathways by which prostaglandins of the A series would be synthesised are by dehydration of the corresponding

prostaglandin E or by another pathway from unsaturated fatty acids without prostaglandin E formation. In the present investigation the emphasis has been upon a search for an enzyme which catalyses the dehydration of prostaglandin E.

The most likely source of a prostaglandin E dehydrase would be an organ or tissue known to contain, or to release, a prostaglandin A. One such organ is the kidney. There are reports that rabbit (Lee et al., 1966; 1967) and canine renal tissue contain prostaglandin A_2 and are able to synthesise prostaglandin A_2 from arachidonic acid (Hamberg, 1969; Muirhead, 1972). A prostaglandin A-like compound is also released from kidney into the circulation both spontaneously and in response to nerve stimulation (Davis and Horton, 1972) or ischaemia (McGiff et al., 1969). Moreover, in the rat prostaglandin B-type metabolites of prostaglandin E have been isolated from the urine (Gréen, 1971). For this reason a search for a prostaglandin E dehydrase was made in rat kidney.

Another source of natural prostaglandin A compounds is male reproductive tract tissue. Human semen is a rich source not only of prostaglandin E compounds but also of prostaglandin A and prostaglandin B and their 19 hydroxy analogues (Bygdeman and Samuelsson, 1966a). There is some evidence that these prostaglandins occur in the secretions of the seminal vesicles (Eliasson, 1959), and so a search for the dehydrase was made in human seminal vesicles removed at autopsy. A substance called "Vesiglandin" has been extracted from the seminal vesicles and semen of the monkey "Macacus rhesus" (von Euler, 1936). It differed from 'prostaglandin' by its lack of any significant effect on non-vascular smooth muscle whilst retaining the depressor activity of 'prostaglandin'. On present evidence it is possible that vesiglandin is a prostaglandin A, B or a 19-OH prostaglandin.

Hence a search for a prostaglandin E dehydrase was made in primate seminal vesicles. Finally there has been a more recent report (Christ and van Dorp, 1972) of the presence of a prostaglandin A and B in semen of the domestic fowl. It is not known which organ in the reproductive tract of the cockerel is responsible for the synthesis and secretion of these seminal prostaglandins, and so a search for a dehydrase was made in the entire male reproductive tract of the domestic fowl.

An alternative source of a prostaglandin E dehydrase would be a tissue which metabolises prostaglandin E to prostaglandin A. When ^{3}H-prostaglandin E_1 was added to human plasma, the recovery of biological activity on the rat fundus preparation was low compared with the recovery of radioactivity (Greaves and McDonald-Gibson, 1972). Since the activity of prostaglandin A_1 on this preparation is 300 times less than that of prostaglandin E_1 (Horton and Jones, 1969), this discrepancy may have arisen from formation of prostaglandin A_1 from prostaglandin E_1. Recently (McDonald-Gibson et al., 1972a; McDonald-Gibson, McDonald-Gibson and Greaves, 1972b) conversion by human plasma of ^{3}H-prostaglandin E_1 to material which co-chromatographs with prostaglandin A_1 has been reported. Therefore attempts were made to confirm this result so that the metabolite could be conclusively identified as prostaglandin A_1. This would be strong evidence for the presence of a human plasma prostaglandin E dehydrase.

Materials and Methods

Kidney tissue

Kidneys were obtained from adult Campbell rats of both sexes. Tissue was removed immediately after death and placed in ice cold phosphate buffer before homogenisation or preparation of slices.

Domestic fowl specimens

Specimens from the domestic fowl, Gallus domesticus, were obtained through the kind co-operation of Dr. P.E. Lake, Agricultural Research Council Poultry Research Unit, University of Edinburgh.

Seminal fluid was collected from several fowls and pooled. It was kept chilled in ice until extraction. Seminal plasma was obtained by centrifuging the seminal fluid sample at room temperature to remove sperm. Reproductive tract tissue was removed from fowls immediately after they had been killed with an overdose of sodium pentobarbitone administered intravenously. The tissue was separated into 4 regions described (Lake, 1971), namely the swollen region, epididymal region, vas deferens and testes, and stored on crushed ice. The maximum storage time was three hours.

Human specimens

Autopsy specimens of human seminal vesicles were kindly supplied by the Pathology Department, Royal Infirmary, Edinburgh. The patients had died of heart disease and were in late middle age. None of them suffered from prostatic carcinoma. The interval between death and autopsy varied between 6 and 36 hours. The seminal

vesicles were stored on dry ice during transport to the laboratory, and afterwards at -20°C.

Baboon specimens

Seminal vesicles from the baboon, "Papio cynocephalus", were obtained with the help of Dr. J. Pickard and Mr. E. McKenzie of the Wellcome Surgical Research Institute, University of Glasgow. The animals were used for studies on cerebral blood flow. Anaesthesia was maintained on a nitrous oxide, oxygen mixture (3:1). Doses of phencyclidine (2 mg) and suxamethonium (50 mg) were given every 30 minutes. Seminal vesicles were dissected out at the end of the experiment, whilst the animal was still alive, and placed on dry ice immediately. They were used within 2 weeks.

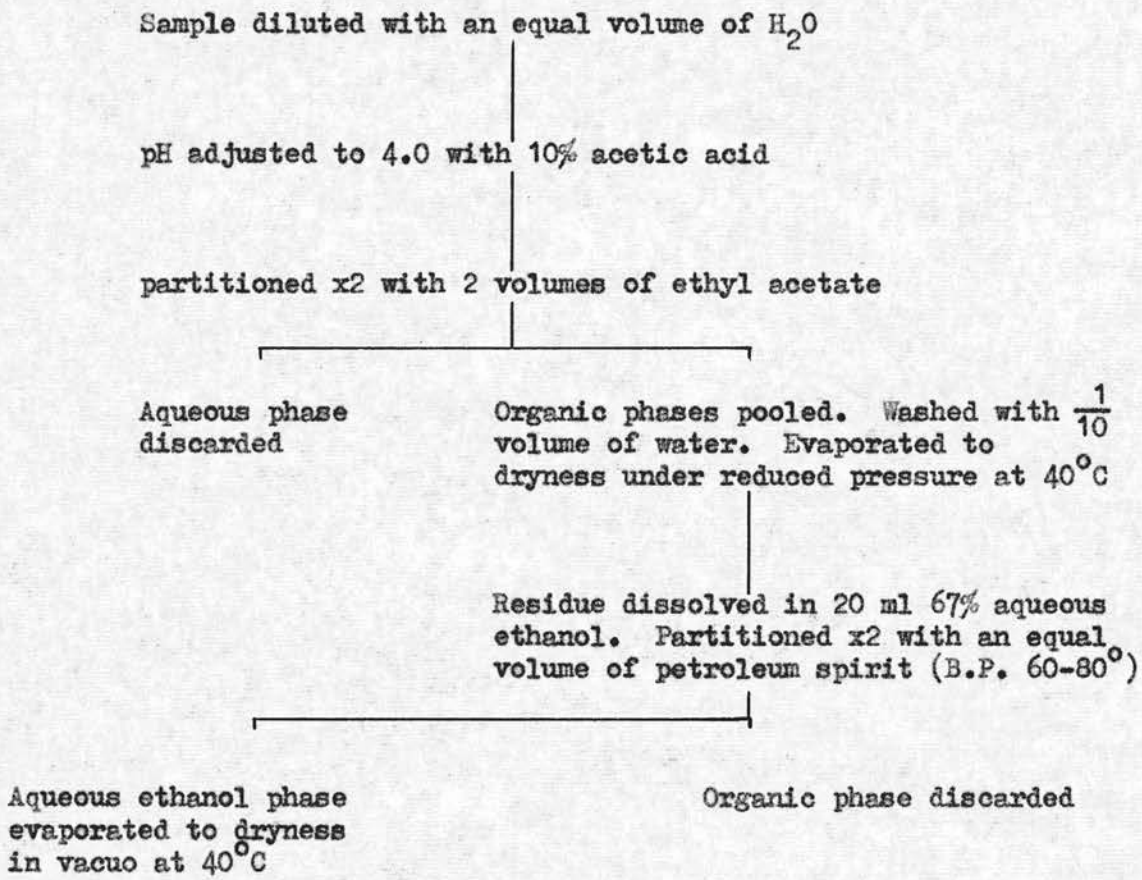
Baboon seminal fluid: A single ejaculate from one animal was obtained by electro-ejaculation. The specimen was collected into 10 ml ice cold ethanol to prevent coagulation which occurs when baboon semen is exposed to air. It was stored in ice until extraction was carried out 3 hours later.

Human Plasma

A 35 ml volume of blood from each of 4 donors aged 22 - 26, 3 of whom were female, was withdrawn by venepuncture and immediately mixed with 5 ml 0.9% NaCl containing 625 I.U. heparin. Blood was then centrifuged at 2,000 g at 4°C for 10 minutes, to separate plasma from cells. The volume of plasma obtained was 10 ml.

Extraction of prostaglandin from avian and baboon seminal fluid and seminal plasma

Prostaglandins were extracted by a modification of the method of Hopkin and Horton (Horton and Jones, 1969), summarised in the following flow diagram :-



Radioactive prostaglandins (0.1 μC 5,6 ^{3}H-$PGF_{2\alpha}$, 5,6 ^{3}H-PGE_1, and 5,6 ^{3}H-PGA_1) were added to the sample immediately before it was diluted to estimate recoveries of these three classes of prostaglandin. The radioactive prostaglandins were also used as markers during thin layer chromatography.

Extraction of prostaglandins from rat kidney and from male reproductive tract tissue

Tissue was weighed and homogenised in 4 volumes of phosphate buffer 0.1 M pH 7.4. The buffer was made up from sodium salts as follows :- NaH_2PO_4 2.7 g per litre, Na_2HPO_4 12.54 g per litre.

The homogenate was incubated at 37°C for 1 hour. All enzymatic activity of the homogenate was stopped with 6 volumes of ice cold ethanol containing radioactive prostaglandins. Proteins were allowed to precipitate for 1 hour at room temperature before the suspension was centrifuged at 1200 g for 10 minutes. The supernatant was decanted off. Aqueous ethanol was removed by evaporation in a partial vacuum at 40°C. The residue was dissolved in 50 ml water and extracted by the modified method of Hopkin and Horton previously described (page 100).

Extraction of prostaglandins from human plasma

Human plasma (10 ml) was incubated with 5,6 ^{3}H-prostaglandin E_1 for 5 minutes. Enzymatic reactions were terminated with 100 ml ice cold ethanol. After 30 minutes, samples were centrifuged at 800 g at room temperature (approximately 20°C) for 10 minutes. Aqueous ethanol was removed by evaporation in a partial vacuum at 40°C. The residue was dissolved in 50 ml water and extracted by the modified method of Hopkin and Horton previously described (page 100).

Group separation of prostaglandins by thin layer chromatography

Preparation of thin layer chromatography plates: Thin layer chromatography (TLC) plates were prepared the day before use. A slurry of Kieselgel G was made by mixing 45 g with 80 ml distilled water in a

flat bottomed, wide necked, stoppered 250 ml flask. Care was taken to avoid the formation of air bubbles. The slurry was then spread uniformly over grooved TLC plates previously well cleaned in acetone and chloroform. A layer of Kieselgel 0.5 mm thick was obtained. The plates were left to dry in air overnight and were activated at 110°C for 30 minutes before use. Ready-made Merck plates 20 x 5 x 0.025 cm were washed in methanol to remove fluorescent indicator.

Solvent systems: Solvent systems used were the A VII (Bygdeman and Samuelsson, 1966), A II and A I (Gréen and Samuelsson, 1964) and a solvent system consisting of ethyl acetate 100 : methanol 10 : acetic acid 1. The composition of the A VII system is glacial acetic acid 20 : water 100 : ethyl acetate 110 : 2,2,4-trimethyl pentane 30, that of the A II is glacial acetic acid 30 : water 100 : methanol 35 : ethyl acetate 110 : 2,4-trimethyl pentane 10, and that of the A I system is glacial acetic acid : 1,4-dioxane 20 : benzene 20. Ethyl acetate was re-distilled once. The other organic solvents were Analar grade. The A VII and A II solvent systems were prepared the day before they were needed and allowed to equilibrate at room temperature for at least 12 hours. Thin layer chromatography tanks were lined with pieces of Whatman No.1 filter paper. Solvent systems were poured down the surface of the filter paper into the tank to a depth of about 1/2", so that the filter paper was completely moistened. This enabled the atmosphere inside the tank to become saturated with solvent vapour. Evaporation of the more volatile components of the solvent was prevented by forming an air-tight seal between the solvent tank and its lid with glycerol. Solvent systems were always freshly prepared and were equilibrated in the tank for 30 minutes.

Thin layer chromatography of extracts: Biological extracts were dissolved in 0.2 ml methanol and loaded on to thin layer chromatography plates approximately 3 centimetres from the bottom end of the plate using a microsyringe. If the extract had been prepared from tissue incubated with radioactive prostaglandin E or radioactive prostaglandin A, standard radioactive prostaglandins were loaded on to another plate. Plates were run in the appropriate solvent until the solvent front was within 2 centimetres from the top of the plate. The plates were then dried at room temperature. The radioactivity on them was located with a Panax thin layer plate scanner. Prostaglandins F, E and A, but not F and 19-OH prostaglandins, are well separated by the A VII solvent system (Figure 22, page 104). Radioactivity due to prostaglandin $F_{2\alpha}$, eluted after TLC in the A VII solvent, was re-run in the A I solvent system to separate prostaglandins of the F series and 19-OH prostaglandins. Separation of F, E, A and 19-OH prostaglandins by TLC is illustrated in the diagram below.

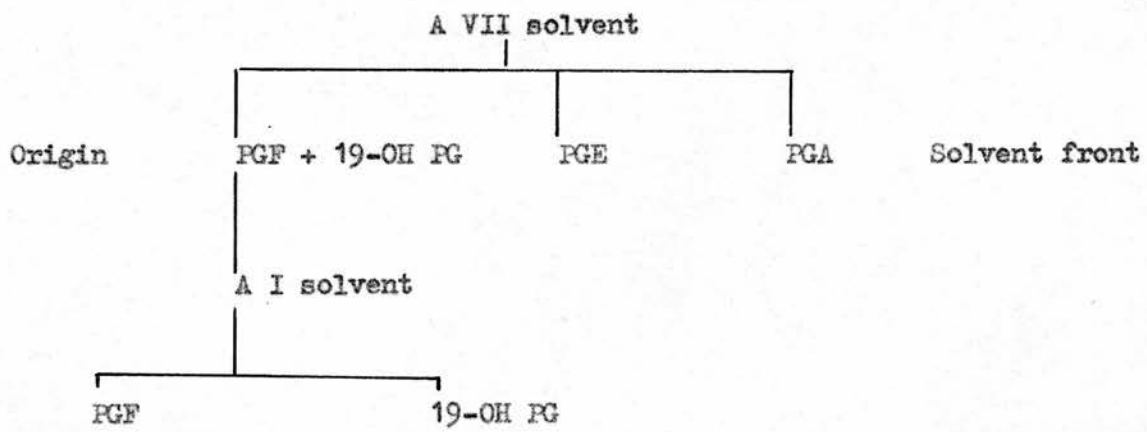
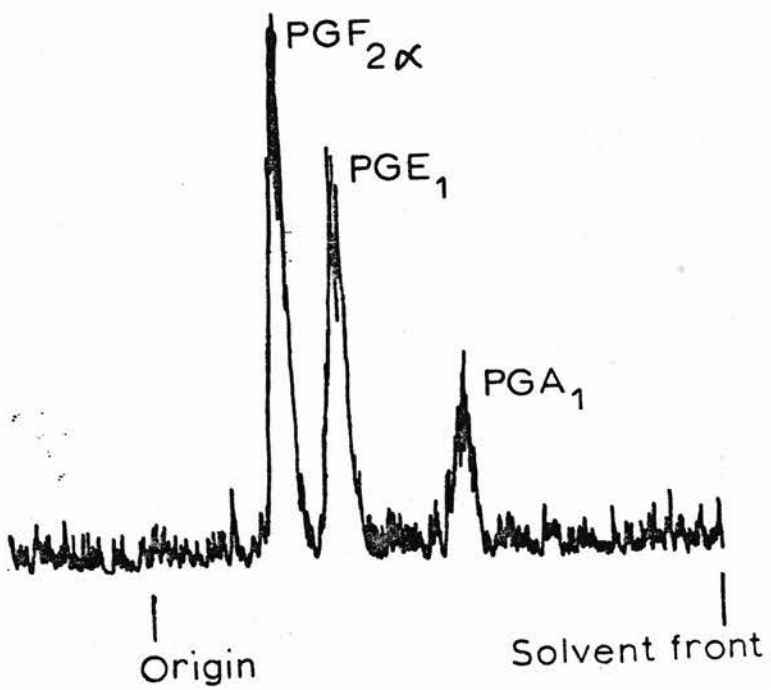


Figure 22

Separation of prostaglandin $F_{2\alpha}$, prostaglandin E_1 and prostaglandin A_1 by TLC in the A VII solvent system.



Elution of prostaglandins from TLC plates: Zones indicated by the peaks of radioactivity due to prostaglandin $F_{2\alpha}$, prostaglandin E_1 and prostaglandin A_1 were scraped off the TLC plate into test tubes to which approximately 10 ml methanol was added. The contents of the tubes were mixed and then centrifuged. The supernatant was carefully poured off and evaporated to dryness. The residue was dissolved in methanol from which a 100 µl portion was removed for quantitative estimation of radioactivity by liquid scintillation counting.

The scintillant (10 ml per sample) was made up as follows :-

toluene 2.5 litres, methanol 62.5 ml, 2,5-diphenyloxazole (PPO) 10.65 g, and 1,4-di-(2-(4-methyl-5-phenyloxazolyl))-benzene (dimethyl POPOP) 0.275 g

Samples were counted on a Nuclear Chicago Mk II liquid scintillation counter. A correction for quenching and an estimate of counting efficiency was obtained by channels ratio or the external standard method. Disintegrations per minute were calculated from recorded counts per minute with an Olivetti P 102 electronic desk-top computer for the channels ratio method or with a PDP8 computer for the external standard method.

Separation of prostaglandins within the E series by TLC: Silver impregnated TLC plates were made by preparing the Kieselgel G slurry with water in which $AgNO_3$ had been dissolved (1.2 g in 60 ml). It was necessary to use the plates immediately they had dried in air as considerable blackening occurred if they were stored overnight. They were activated for 15 minutes at 110°C. This shorter activation time did not prevent separation of standard prostaglandin E_1 and prostaglandin E_2. The solvent used was the A II system of Gréen and Samuelsson (Gréen and Samuelsson, 1964), made up the day before use and equilibrated overnight.

Detection of prostaglandins: Prostaglandins E_1 and E_2 (2.5 µg) were used as standards. After running in a A II solvent system the plate was air dried, sprayed with 10% phosphomolybdic acid in methanol and heated at 110°C for 10 minutes to visualise prostaglandins as blue spots on a yellow-green background (Gréen and Samuelsson, 1964).

Bioassay of prostaglandins

Prostaglandins of the F and E series were assayed on the rat fundus preparation (Vane, 1957). The tissue was suspended in a 10 ml organ bath containing Tyrode solution (5,000 ml H_2O, 40 g NaCl, 5 g glucose, 5 g $NaHCO_3$, 10 ml of 10% solutions of KCl, $CaCl_2$, $MgCl_2$ and 2.5 ml 10% NaH_2PO_4 solution) gassed with 100% O_2. Temperature was maintained at 37°C by a thermostatically controlled water bath. Contractions were recorded isometrically with a Grass transducer FT03 and displayed on a Servoscribe potentiometric recorder. Samples to be assayed were dissolved in distilled water and added to the organ bath from a tuberculin syringe in a volume of 0.2 ml or less. A 6 minute dose cycle was used, comprising a contact time of 90 seconds, a washout of 90 seconds and a rest period of 180 seconds. Tissues were sensitive to 5 ng prostaglandin $F_{2\alpha}$ and prostaglandin E_2, and 10 ng prostaglandin E_1.

Isolated smooth muscle preparations do not respond to prostaglandins of the A series in the same dose range (Horton and Jones, 1969). These prostaglandins were therefore assayed on the kitten blood pressure (Horton and Jones, 1969). Kittens of either sex weighing about 1.5 kg were anaesthetised by an intraperitoneal injection of a 60 mg/ml solution of sodium pentobarbitone (40 mg/kg). Anaesthesia was maintained during the assay by intravenous infusion of a 5 mg/ml solution with a Braun syringe. The rate of infusion

required varied from animal to animal. It was not correlated with the animal's weight and was between 1 mg/kg/hour and 6.5 mg/kg/hour (Horton and Jones, 1969). Blood pressure was recorded with a Statham pressure transducer P23 Db from a common carotid artery. Recordings were displayed on a Grass model 7 polygraph. Samples to be assayed and prostaglandin standards were dissolved in 0.9% NaCl and injected into a femoral vein in a volume not greater than 0.2 ml. Doses of prostaglandin were washed in with 0.2 ml of 0.9% NaCl. A 10 minute dose cycle was used. The threshold dose of prostaglandin A_1 was about 20 ng.

Spectroscopic assay of prostaglandins

Measurement of the 278 nm chromophore can be used to estimate concentrations of prostaglandins of the B series greater than about 1 µg/ml. The ϵ_{278} for prostaglandin B_1 is 27,200 (Andersen, 1969). Prostaglandins of the A series possess a 217 nm chromophore. Measurement of this chromophore is a less sensitive assay for prostaglandins than measurement of the 278 nm chromophore formed by base catalysed isomerisation of prostaglandin A to the corresponding prostaglandin B. Thus 19-OH prostaglandins were assayed spectroscopically by measuring the 278 nm chromophore before addition of KOH which estimated 19-OH B prostaglandins, and after treatment with KOH which estimated 19-OH A and 19-OH B prostaglandins. Prostaglandins of the E series were also estimated by the 278 nm chromophore formed by KOH. A Pye Unicam SP 800 ultraviolet spectrophotometer was used for the spectroscopic assays. A 2 ml volume of the sample in methanol was placed in a 2.5 ml cell, pathlength 10 mm, and a spectrum recorded, against methanol, between 325 nm and 250 nm. A 0.2 ml volume of N KOH in methanol was added

to the sample and spectra recorded at 5 minute intervals until no further increase in extinction at 278 nm occurred. Extinction of 10 µg/ml prostaglandin B is 0.81 OD units.

Radio-gas chromatography

Gas chromatography was performed on a Pye 184 gas chromatograph. The stationary phase of the column was OV1½% supported on Suppelcoport 100-200 mesh. The moving phase was argon and the oven temperature 190°C. Continuous measurement of radioactivity was performed with a Panax radio-gas chromatograph. The methyl ester-trimethylsilyl ether (MeTMS) derivative of the kidney homogenate metabolite of prostaglandin E_1 was prepared by the method of Thompson, Los and Horton (1970). The samples were transferred to Eppendorf polypropylene, capped tubes and evaporated to dryness by a current of dry air. They were methylated by treatment for 15 minutes at room temperature with freshly prepared diazomethane. This was made by reacting 1 g 'Diazald' in 10 ml ether with 2 ml KOH (30 g made up to 50 ml with H_2O) and 9.5 ml 95% ethanol. The vapour was collected into 13.5 ml ether and 1.5 ml methanol chilled in ice. After methylation, diazomethane was evaporated in a current of dry air. Silylation was achieved with 20 µl N, N-bis (trimethylsilyl)-trifluoracetamide (BSTFA). The BSTFA was then evaporated off in a stream of air. The residue was dissolved in 20 µl hexane. A 10 µl portion was injected on to the column.

Combined gas chromatography-mass spectrometry

Combined gas chromatography-mass spectrometry of the methyl ester-trimethysilyl ether derivative of samples with prostaglandin F-like and prostaglandin A-like biological activity, and of the methyl

ester-methoxime-trimethylsilyl ether derivative of those with prostaglandin E-like activity was performed, by the method of Thompson, Los and Horton (Thompson et al., 1970) using an LKB 9000 mass spectrometer. The column was packed with 5% OV1 on 100-200 mesh Supersorb which had been pre-treated with dimethyldichlorosilane. The carrier gas was helium and the oven temperature 196°C or 200°C.

The methyl esters of prostaglandins F, E and A were prepared as previously described. Methoxime derivatives of E prostaglandins were prepared by adding 200 µl of a 10 mg/ml solution of methoxyamine HCl in dry pyridine after methylation, and allowing the reaction to proceed at room temperature overnight. The pyridine was removed before silylation with BSTFA.

Samples were injected on to the column with a 10 µl syringe without removal of BSTFA. Mass spectra were recorded at the appropriate retention time, found by injecting larger amounts of the same derivative of standard prostaglandins. The initial electron energy was 22.5 eV, scanning electron energy 27.5 eV.

Metabolism of prostaglandin E_1 by rat kidney

Incubation of kidney slices with 5,6 ^{3}H-prostaglandin E_1:

Kidney slices approximately 0.5 mm thick were prepared by longitudinal sectioning by hand of kidneys from Campbell rats. After removal from the animal, the kidneys were placed in ice cold phosphate buffer, blotted dry with filter paper and weighed. Approximately 6 g of tissue in 30 ml fresh buffer were incubated with 5,6 ^{3}H-prostaglandin E_1, 15 µg, 0.15 µC, added in 100 µl methanol. The incubation medium was gassed with 100% O_2 or air and incubation at 37°C was continued for 90 minutes. Enzymatic reactions were terminated by the addition of 150 ml ice cold

ethanol. Two types of control experiment were performed. In one, kidney slices were incubated for 90 minutes and prostaglandin E_1 was added immediately before alcohol to estimate non-enzymatic conversion of prostaglandin E_1 to less polar material during extraction and separation of prostaglandins. In the other, kidney slices were incubated in ethanol with prostaglandin E_1 for 90 minutes to estimate non-enzymatic conversion during incubation as well as during extraction and separation of prostaglandins.

Prostaglandins were extracted from the aqueous ethanolic suspension as described on page 100. Separation of prostaglandins by TLC was achieved as described on page 103, using the A VII solvent system. Radioactivity on the plate was located and estimated as described on page 105. Radioactivity in different zones along the plate was expressed as a percentage of the total recovered. Material less polar than prostaglandin E_1 obtained after incubating 5,6 ^{3}H-prostaglandin E_1 with kidney slices was subjected to radio-gas chromatography as described on page 108.

Incubation of kidney homogenates with 5,6 ^{3}H-prostaglandin E_1:

Kidneys were removed from rats immediately after slaughter and placed in ice cold phosphate buffer. They were then cut into small pieces and homogenised in 4 volumes of phosphate buffer with a Polytron homogeniser. The tube was immersed in ice to keep it cool. A 15 ml volume of homogenate was incubated with 5,6 ^{3}H-prostaglandin E_1, 25 µg, 0.63 µC. The incubation mixture was gassed with 100% O_2 and incubation was continued for 90 minutes at 37°C. After this time 5,6 ^{3}H-prostaglandin E_1, 25 µg, 0.63 µC, was added to a second 15 ml portion of homogenate. A 75 ml volume of ice cold ethanol was then added to both portions of homogenate to stop enzymatic reactions. Prostaglandins were extracted and separated on TLC in A VII solvent system.

Estimation of prostaglandins in avian and baboon seminal fluid and avian seminal plasma, and of prostaglandins formed by homogenates of reproductive tract tissue during incubation in phosphate buffer

Tritiated prostaglandin $F_{2\alpha}$, prostaglandin E_2 and prostaglandin A_2 (0.05 μC each) were used as markers for endogenous prostaglandins in seminal fluids and in reproductive tract tissue. They were added to samples of avian and baboon seminal fluid and to avian seminal plasma immediately before extraction of prostaglandins. Homogenates of regions of avian reproductive tract, comprising the whole reproductive tract, and of baboon and human seminal vesicles were incubated in phosphate buffer for 30 minutes, to allow synthesis to occur of prostaglandins from endogenous precursors. Enzymatic reactions were terminated with ethanol containing 0.05 μC each of ^{3}H-prostaglandin $F_{2\alpha}$, prostaglandin E_2 and prostaglandin A_2. Prostaglandins formed during homogenisation of tissues and during incubation were then extracted. Extracts of seminal fluid, seminal plasma and reproductive tract tissue were separated by TLC on Merck plates in A VII solvent system. The zone containing radioactivity due to prostaglandin $F_{2\alpha}$ was run in A I solvent system to separate 19-OH prostaglandins. Radioactivity in zones corresponding to the position of prostaglandin $F_{2\alpha}$, prostaglandin E_2 and prostaglandin A_2 was estimated by liquid scintillation counting and used to calculate recoveries of the 3 classes of prostaglandin. Zones containing radioactive prostaglandin $F_{2\alpha}$ and prostaglandin E_2 were bioassayed on the rat fundus preparation in terms of prostaglandin $F_{2\alpha}$ and prostaglandin E_1 respectively. The zone containing ^{3}H-prostaglandin A_2 and the less polar zone separated from prostaglandin F by TLC in A I solvent system were assayed on the kitten blood pressure in terms of prostaglandin A_1. The potency of

19-OH prostaglandins on this preparation is about 4 times less than that of the corresponding prostaglandin of the A series (Horton and Jones, 1969). Prostaglandin E's and prostaglandin A's in some samples were also assayed spectroscopically.

The prostaglandin E-like material extracted from baboon seminal vesicles was also subjected to argentous TLC in the A II solvent system using prostaglandin E_1 and prostaglandin E_2 as standards. More conclusive identification was provided by combined gas chromatography-mass spectrometry of its methyl ester-methoxime-trimethylsilyl ether derivative.

Samples with prostaglandin F-like and prostaglandin A-like activity extracted from baboon seminal vesicles and those samples with prostaglandin F-like, prostaglandin E-like and prostaglandin A-like activity extracted from human seminal vesicles were also subjected to combined gas chromatography-mass spectrometry.

Incubation of prostaglandin E with male reproductive tract homogenates

Reproductive tract tissue from the domestic fowl, man and baboon was homogenised in 4 volumes of 0.1 M phosphate buffer pH 7.4, and was incubated in 25 ml tared flasks with ^{3}H-prostaglandin E_1 or ^{3}H-prostaglandin E_2. Incubation was for 30 minutes at 37°C in air. All enzymatic reactions were stopped with 10 volumes of ice cold ethanol, and prostaglandins were extracted by the method described for extraction from tissues(see page 109. Boiled homogenate or phosphate buffer was used as controls to estimate the stability of prostaglandin E during incubation, extraction and TLC. Details of the mass of tissue used and the amount of prostaglandin added are shown in Table 19.

Table 19

Composition of incubation mixtures of male reproductive tract tissue homogenates of different species in the study of metabolism of prostaglandin E_1

Species	Tissue	Original mass (g)	Volume of homogenate (ml)	PGE_1 added	Final PGE_1 concentration (per g tissue)
Domestic fowl	Testes	31.65	10 (ex total)	250ng,0.1µC	100ng,0.04µC
	Epididymal region	0.54	3	250ng,0.1µC	463ng,0.19µC
	Vas deferens	1.65	3	250ng,0.1µC	152ng,0.06µC
	Swollen region	0.33	6	250ng,0.1µC	253ng,0.30µC
Human	Seminal vesicles	3.8	10	2.5µg,0.2µC	657ng,0.05µC
Baboon	Seminal vesicles	5.0	25	25µg, 1µC	5 µg, 0.20 µC

In one experiment, 7.3 ml baboon vesicle homogenate equivalent to 0.7 g tissue was incubated with 20 µg, 0.8 µC 5,6 ^{3}H-prostaglandin E_2 (concentration 2.8 µg, 0.10 µC prostaglandin E_2 per g tissue).

Incubation of arachidonic acid with human seminal vesicle homogenates

Human seminal vesicles were homogenised in 4 volumes of phosphate buffer pH 7.4 or 4 volumes of ethanol. 10 ml of homogenate was added to 10 ml of phosphate buffer containing 10 µg/ml hydroquinone as an antioxidant, 100 µg/ml glutathione and 20 µg : 20 µC/ml 5,6,8,9,11,12, 14,15-^{3}H-arachidonic acid. This medium had been prepared immediately before the experiment by dissolving 5 mg hydroquinone in 500ml buffer and adding 50 ml of this solution to 5 mg glutathione. Arachidonic

acid, purified in this laboratory by Mr. A.R. Brash, was dissolved in 10 ml of solution. The incubation mixture was gassed with 100% O_2 during incubation for 60 minutes at 37°C. Enzymatic reactions were stopped with 100 ml ice cold ethanol. Arachidonic acid and prostaglandins were extracted as previously described. Radioactive compounds were separated by TLC in the A VII solvent system.

Estimation of the stability of 5,6 ^{3}H-prostaglandin E_1 during extraction

The stability of 5,6 ^{3}H-prostaglandin E_1 during extraction and TLC was estimated by adding 0.1 µC 5,6 ^{3}H-prostaglandin E_1 tracer in 100 ml ethanol to 10 ml baboon seminal vesicles homogenate in buffer and to 10 ml homogenised in ethanol. Prostaglandins were extracted and subjected to TLC on Merck plates. Zones of radioactivity corresponding to the positions of 0.05 µC prostaglandin E_1 and 0.05 µC prostaglandin A_1 run as markers on a second TLC plate were eluted and counted. Tracer prostaglandin E_1 alone was run on a third plate to estimate any contamination of the tracer with radioactive material with TLC properties of prostaglandin A_1. The percentage of radioactivity in the prostaglandin A zone to that in the prostaglandin E zone was calculated for all three samples.

Incubation of arachidonic acid with baboon seminal vesicle homogenates

Pooled pairs of seminal vesicles were homogenised in 4 volumes of phosphate buffer and incubated with arachidonic acid containing radioactive tracer. The amounts of arachidonic acid used are shown in Table 20.

Table 20

Incubation mixture used in study of prostaglandin biosynthesis from arachidonic acid by baboon seminal vesicles

Volume of homogenate	Mass of tissue	Amount of arachidonic acid	Concentration of arachidonic acid (per g tissue)
12 ml	3 g	100 µg, 10 µC	33 µg, 3.33 µC

Equal volumes of phosphate buffer were incubated with the same amount of arachidonic acid to estimate the stability of radioactive and "cold" arachidonic acid during incubation and extraction. Seminal vesicle homogenate only was incubated to allow prostaglandin formation from endogenous arachidonic acid to be measured. Incubation was in an atmosphere of air at 37°C for 30 minutes. Enzymatic reactions were stopped with 10 volumes of ice cold ethanol. Arachidonic acid and prostaglandins were extracted. Extracts were subjected to TLC in the solvent system ethyl acetate 100 ml : methanol 10 ml : acetic acid 1 ml on prepared Merck plates coated with silica gel (20 x 5 x 0.025)

Zones containing peaks of radioactivity were eluted from the TLC plates and radioactivity estimated. Zones, corresponding to the position of radioactive prostaglandins added as tracers to the homogenate used to measure synthesis of prostaglandin-like material from endogenous arachidonic acid, were bioassayed.

Incubation of ^{3}H-prostaglandin E_1 with human plasma

A 10 ml volume of fresh human plasma was diluted with 10 ml of 0.9% NaCl and boiled to inactivate enzymes. This sample was the control in subsequent incubation experiments being used to estimate non-enzymatic conversion of prostaglandin E_1 during incubation, extraction and separation. Prostaglandin E_1, 500 ng, 0.1 µC in 50 µl methanol was added to 10 ml unboiled plasma and to the diluted boiled plasma. Incubation was at 37°C in an atmosphere of air for 5 minutes. Enzymatic reactions were terminated with 100 ml ice cold ethanol. Prostaglandins were extracted as described on page 100, and then separated by TLC on grooved plates, prepared as described on page 101. The A I solvent system was used. The purity of 5,6 ^{3}H-prostaglandin E_1 incubated with plasma was estimated by TLC of a 250 ng, 0.05 µC amount. Radioactivity on the plates was detected and measured as previously described (page 105). Zones of radioactivity were eluted in methanol (see page 105).

Results

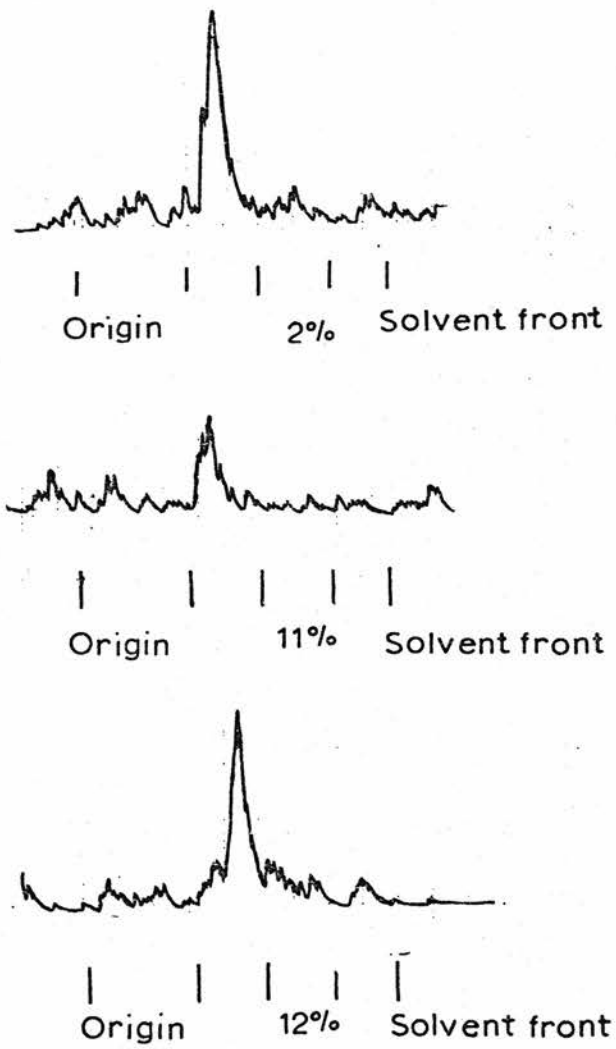
Incubation of kidney slices with 5,6 ^{3}H-prostaglandin E_1

Figure 23, page 118, shows the distribution of radioactivity after TLC of 3 extracts of kidney slices incubated in ethanol with 5,6 ^{3}H-prostaglandin E_1, to estimate non-enzymatic conversion of prostaglandin E_1 to less polar compounds during incubation, extraction and separation of prostaglandins. The proportion of total radioactivity recovered as material less polar than prostaglandin E_1 is 2, 11 and 12. Non-enzymatic conversion during extraction and separation was estimated by adding 5,6 ^{3}H-prostaglandin E_1 to kidney slices immediately before enzymatic reactions were terminated with ethanol. Results in Figure 24, page 119, show that 25% of the total radioactivity was recovered as less polar material after extraction and TLC of this control sample. Possible enzymatic conversion of prostaglandin E_1 to less polar material plus non-enzymatic conversion during incubation, extraction and separation of prostaglandins is shown by the material less polar than prostaglandin E_1 recovered from 5,6 ^{3}H-prostaglandin E_1 incubated with kidney slices in phosphate buffer. The proportion of total radioactivity recovered from this test sample as this material is also shown in Figure 24 and is 30%. This finding suggests that enzymatic conversion of prostaglandin E_1 to less polar compounds may occur in kidney slices.

Figure 25, page 120, shows the peaks of material separated by gas chromatography (upper trace) of the MeTMS derivative 0.75 µg, 0.075 µC of this less polar material in the test sample and of the MeTMS derivative of 2 µg, 0.1 µC prostaglandin A_1. The retention time of the MeTMS derivatives of prostaglandin A_1 and of the less polar

Figure 23

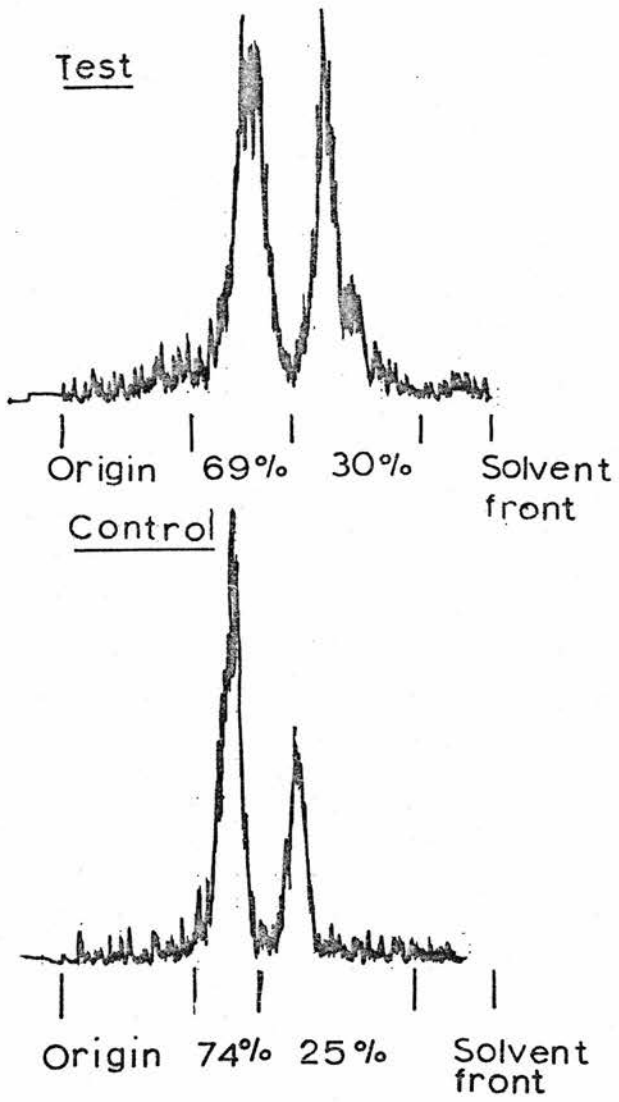
Distribution of radioactivity after TLC of 3 extracts of kidney slices incubated in ethanol with 5,6 ^{3}H-prostaglandin E_1.



Figures show the percentage of total radioactivity recovered which co-chromatographed with prostaglandin A_1.

Figure 24

Distribution of radioactivity after TLC of extracts of kidney slices incubated with 5,6 ^{3}H-prostaglandin E_1.

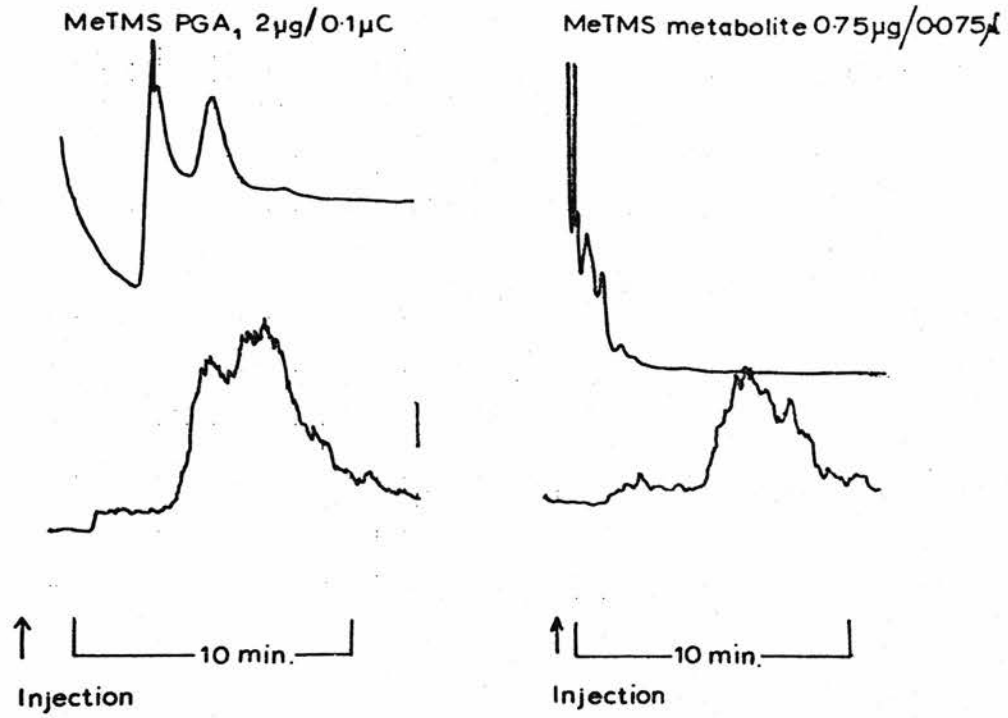


Control: 5,6 ^{3}H-prostaglandin E_1 added immediately before enzymatic reactions were terminated in ethanol.

Test: 5,6 ^{3}H-prostaglandin E_1 incubated for 90 minutes at 37°C. Incubation medium was gassed with 100% O_2.

Figure 25

Radio-gas chromatography of the MeTMS derivatives of prostaglandin A_1 and the rat kidney slices metabolite of prostaglandin E_1.



Upper traces: mass traces

Lower traces: radioactivity traces

metabolite is 4.3 minutes. These results indicate that ^{3}H-prostaglandin A_1 can be extracted from kidney slices after incubation with ^{3}H-prostaglandin E_1. Subsequently, the metabolism of prostaglandin E_1 by kidney homogenates was investigated.

Incubation of 5,6 ^{3}H-prostaglandin E_1 with rat kidney homogenates

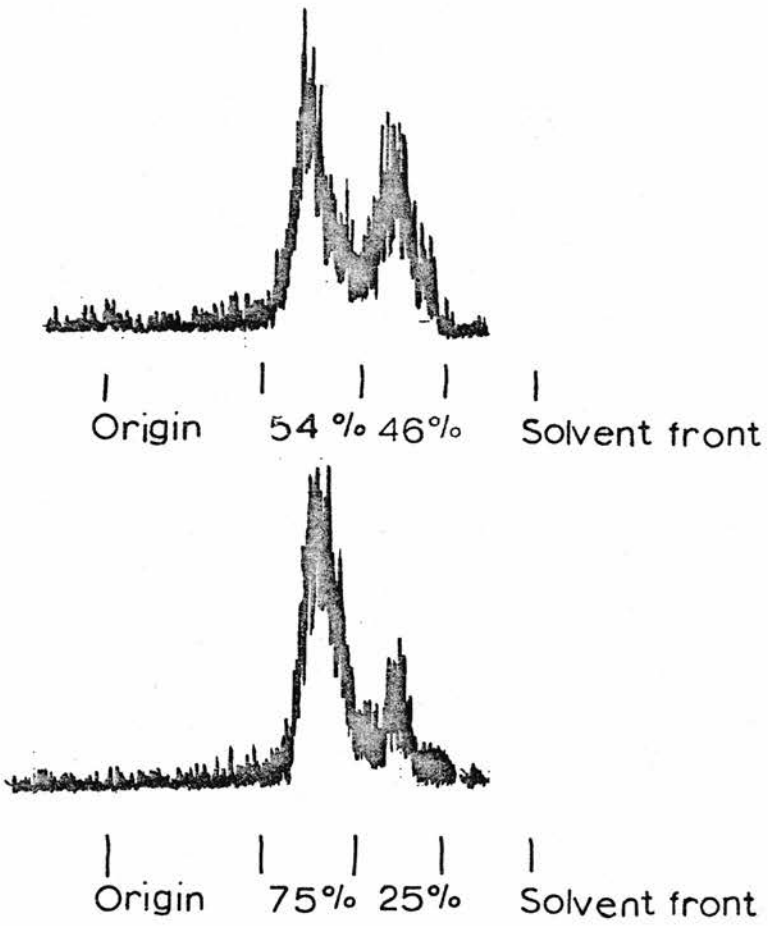
Peaks of radioactivity obtained after TLC of an extract of 5,6 ^{3}H-prostaglandin E_1 incubated with rat whole kidney homogenates in an atmosphere of air at 37°C and in phosphate buffer at 37°C, also in an atmosphere of air, are shown in Figure 26, page 122. Radioactivity detected in the zones indicated is also included. It is expressed as a percentage of the total recovered.

The results show that the incubation of 5,6 ^{3}H-prostaglandin E_1 aerobically resulted in this conversion to at least one less polar compound. Moreover, this conversion was greater than when the prostaglandin E was incubated in phosphate buffer at 37°C. These findings are evidence that kidney homogenates converted prostaglandin E_1 to less polar material and that the conversion may have been enzymatic.

Attempts were made to identify the less polar compound as prostaglandin A_1 by TLC, bioassay on the rat fundus and kitten blood pressure preparations, radio-gas chromatography and combined gas chromatography-mass spectrometry. The results are presented in the following 4 sections.

Figure 26

Distribution of radioactivity after TLC of extracts of 5,6 ^{3}H-prostaglandin E_1 incubated with rat kidney whole homogenates (upper record) and with phosphate buffer (lower record).



Figures indicate the percentage of total radioactivity recovered obtained in each peak.

Thin layer chromatography of the less polar metabolite obtained from rat kidney homogenates

The Rf values of the metabolite and those of prostaglandin A_1, 15-oxo prostaglandin E_1 and 13,14-dihydro prostaglandin E_1 are shown in Table 21. The values are obtained from two samples run in the A VII solvent system.

Table 21

Rf values of the less polar metabolite, prostaglandin A_1, 15-oxo prostaglandin E_1 and 13,14-dihydro prostaglandin E_1 in the A VII solvent system

	Metabolite	PGA_1	15-oxo PGE_1	13,14-dihydro PGE_1
1)	0.56	0.55	0.61	0.44
2)	0.53	0.55	0.56	not run

Results in lines 1) and 2) of Table 21 are from two separate experiments. Although the rat kidney homogenate metabolite was separated from 13,14-dihydro prostaglandin E_1 by TLC on the A VII solvent system, it was not separated from prostaglandin A_1 or from 15-oxo prostaglandin E_1. Therefore on the basis of TLC the metabolite may have been prostaglandin A_1 or 15-oxo prostaglandin E_1 but could not have been 13,14-dihydro prostaglandin E_1.

Bioassay of the less polar metabolite obtained from rat kidney homogenates

The less polar metabolite was bioassayed on the rat fundus and kitten blood pressure preparations in terms of prostaglandin A_1, 15-oxo prostaglandin E_1 and 13,14-dihydro prostaglandin E_1. The doses of

metabolite were calculated from the measurement of radioactivity by liquid scintillation counting. The assumption was made that the specific activity of the radioactive metabolite was the same as that of 5,6 ^{3}H-prostaglandin E_1 incubated with rat kidney homogenates. Figure 27, page 125, shows the log dose response curves obtained on the kitten blood pressure preparation on two occasions. Doses of prostaglandins that were equiactive were calculated from those parts of the log dose response curves that were parallel. The ratios of the activity of metabolite and each of the three standard prostaglandins determined by bioassay on this preparation, on the rat fundus preparation and by liquid scintillation counting are shown in Table 22.

Table 22

Ratios of activities of the metabolite in terms of (a) prostaglandin A_1, (b) 15-oxo prostaglandin E_1 and (c) 13,14-dihydro prostaglandin E_1 measured by bioassay on the rat fundus and kitten blood pressure preparations, and by liquid scintillation counting

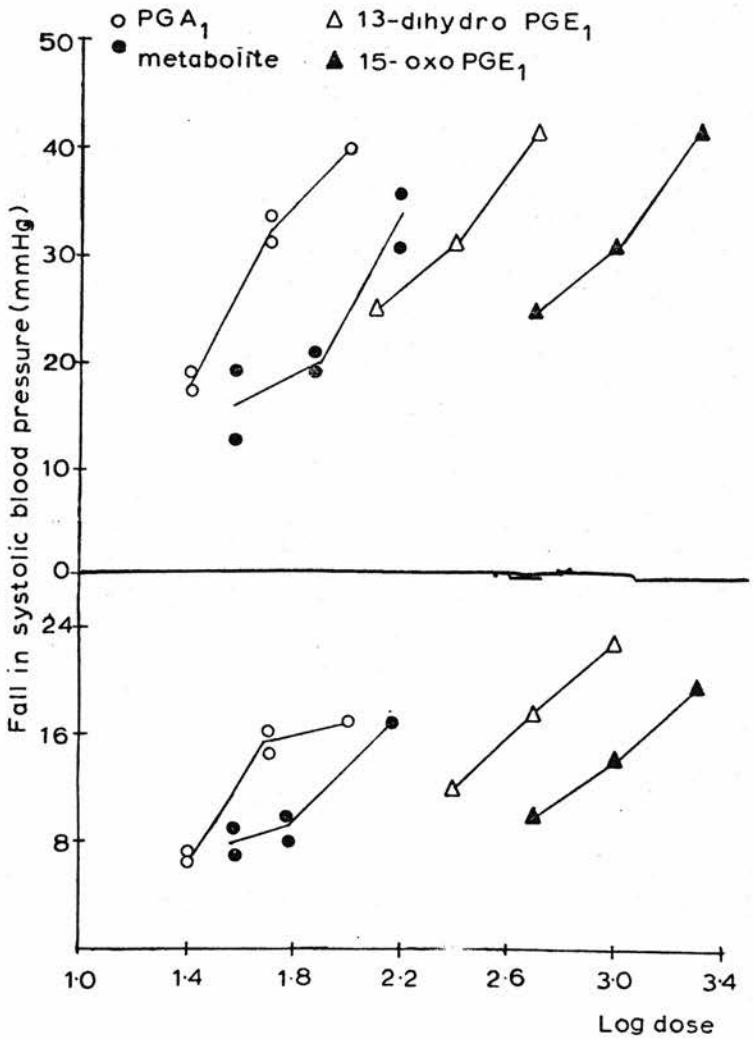
Ratio / Measurement	Metabolite / PGA_1	Metabolite / 15-oxo PGE_1	Metabolite / 13,14-H_2 PGE_1
* Rat fundus preparation	0.69	1.38	14.75
* Kitten blood pressure preparation	2.28	0.06	0.28
Liquid scintillation counting	1.00	1.00	1.00

Equipotent doses of prostaglandin A_1, 15-oxo prostaglandin E_1, 13,14-dihydro prostaglandin E_1, the metabolite and prostaglandin E_1 were 237, 161, 8, 161 and 1 respectively.

* Ratios of activities found by bioassay are the mean of results from 2 samples of the metabolite assayed once on each preparation.

Figure 27

Log dose response curves of prostaglandin A_1, 13-dihydro prostaglandin E_1, 15-oxo prostaglandin E_1 and the rat kidney homogenate metabolite on the kitten blood pressure preparation.



It is apparent that there were discrepancies between the amount of metabolite estimated by liquid scintillation counting and by bioassay on the rat fundus preparation or on the kitten blood pressure in terms of 13,14-dihydro prostaglandin E_1. Thus the metabolite could not have been 13,14-dihydro prostaglandin E_1. The results of bioassay of the metabolite on a single preparation compared with assay by liquid scintillation counting were, however, not able tentatively to identify the metabolite either as prostaglandin A_1 or 15-oxo prostaglandin A_1. Bioassay on the kitten blood pressure preparation is compatible with the metabolite being prostaglandin A_1, because bioassay in terms of prostaglandin A_1 is in agreement with assay by liquid scintillation counting, whereas bioassay in terms of 15-oxo prostaglandin E_1 is not. However, the result of bioassay on the rat fundus preparation in terms of both these prostaglandins is similar to the result obtained from assay by liquid scintillation counting. Thus, on the basis of bioassay on the rat fundus preparation, the metabolite could be either prostaglandin A_1 or 15-oxo prostaglandin E_1. Nevertheless, results of bioassay on this preparation and on the kitten blood pressure preparation can be combined by calculating the index of discrimination between metabolite and prostaglandin A_1, and between metabolite and 15-oxo prostaglandin E_1 on each preparation (Gaddum, 1955).

Indices of discrimination between the metabolite and prostaglandin A_1, the metabolite and 15-oxo prostaglandin E_1 on the rat fundus and kitten blood pressure preparations are shown in Table 23.

Table 23

* Indices of discrimination on the rat fundus and kitten blood pressure preparations between the metabolite and (a) prostaglandin A_1, (b) 15-oxo prostaglandin E_1 and (c) 13,14-dihydro prostaglandin E_1

Metabolite / PGA_1	Metabolite / 15-oxo PGE_1	Metabolite / 13,14-H_2 PGE_1
0.30	21.86	52.12

* Index of discrimination = $\frac{\text{ratio of equipotent dose on rat fundus}}{\text{ratio of equipotent dose on cat blood pressure}}$

A value for the index of discrimination between two substances greater than 10 is evidence that the two substances being compared are not the same (Gaddum, 1955). Therefore the values of indices of discrimination shown in Table 23 suggest that the metabolite was not 13,14-dihydro prostaglandin E_1 or 15-oxo prostaglandin E_1 but do not exclude the possibility that it may be prostaglandin A_1.

Radio-gas chromatography of the metabolite obtained from rat kidney homogenates

The retention time of the MeTMS derivative of 2 µg, 0.1 µC prostaglandin A_1 at 211°C was 4 to 8 minutes. No peak of radioactivity was obtained from radio-gas chromatography of the MeTMS derivative of the metabolite. If the metabolite has retained the 11α-OH group in the cyclopentane ring and also contains a 9-oxo group, the MeTMS derivative would be unstable. Thus the metabolite could not be identified as prostaglandin A_1 on the basis of radio-gas chromatography.

Combined gas chromatography-mass spectrometry of the metabolite obtained from rat kidney homogenates

The retention time of the MeTMS derivative of 2 µg prostaglandin A_1 at 211°C was 12.5 minutes. The mass spectrum taken at this time showed prominent peaks due to ions at $\frac{m}{e}$ values of 422, 407, 391, 351, 332 and 323 (a line diagram of the mass spectrum of the MeTMS derivative of prostaglandin A_2 showing its fragmentation pattern is on page 153). The mass spectrum of the MeTMS derivative of 1.4 µg of the metabolite was taken at 12.5 minutes. It contains many peaks due to compounds other than prostaglandins which masked low intensity prostaglandin peaks. However the peaks at $\frac{m}{e}$ 351 and 323, which are prominent in the mass spectrum of MeTMS prostaglandin A_1, were absent from the mass spectrum of the MeTMS derivative of the metabolite. Therefore combined gas chromatography-mass spectrometry did not identify the metabolite as prostaglandin A_1.

It was decided to examine samples of seminal fluid and seminal plasma of the domestic fowl, Gallus domesticus, to check the levels and identity of prostaglandins previously reported (Christ and van Dorp, 1972).

Levels of prostaglandin-like material in seminal fluid and seminal plasma from the domestic fowl, Gallus domesticus

The levels are shown in Table 24. Material eluted from a zone corresponding to the position of a radioactive prostaglandin was dissolved in 5 ml methanol, of which 1 ml was removed for bioassay and 4 ml retained for spectroscopic assay.

Table 24

Levels of prostaglandin-like material in seminal fluid and seminal plasma from the domestic fowl

Sample	Volume (ml)	TLC zone	% radio-activity recovered	PG in bioassay sample(corrected for recovery) (ng)	PG in total sample (ng)	Level of PG in sample (ng/ml)
Seminal plasma(1)	3.5	PGF	59	42	210	60
		PGE	47	1350	6750	1940
		PGA	37	67	340	90
Seminal plasma(2)	3.0	PGF	80	< 63	< 320	< 80
		PGE	58	< 87	< 450	< 150
		PGA	29	73	370	123
Seminal fluid	2.5	PGF	68	< 29	< 440	< 56
		PGE	45	< 110	< 550	< 220
		PGA	15	135	660	265

Where < is indicated, the total bioassay sample did not elicit a contraction of the rat fundus preparation. Maximum levels of prostaglandin-like activity in seminal plasma and seminal fluid were therefore calculated from the threshold doses of prostaglandin $F_{2\alpha}$ and prostaglandin E_1.

Prostaglandin A-like activity was detected in both samples of seminal plasma and in the sample of seminal fluid. The activity was low compared to the prostaglandin E-like activity found in the first seminal plasma sample, but was of the same order in the second seminal plasma sample and in the sample of seminal fluid. This finding suggests that prostaglandin A-like activity in seminal fluid may be enzymatically derived. A prostaglandin E dehydrase might occur in male reproductive tract tissue from the domestic fowl.

Spectroscopic assay of prostaglandins in seminal plasma from the domestic fowl: The results of a spectroscopic assay for prostaglandin E, prostaglandins A and B and 19-OH prostaglandins A and B in seminal fluid from the domestic fowl are shown in Table 25. The 278 nm chromophore of zones eluted after TLC on the A VII and A I solvent systems were measured before and 30 minutes after the addition of 0.1 N KOH.

Table 25

Absorbance at 278 nm of an extract of avian seminal plasma

TLC zone	Absorbance at 278 nm	
	Before addition of 0.2 ml N KOH	After addition of 0.2 ml N KOH
19-OH PG	0.04	0.04
PGE	0.06	0.06
PGA	0.06	0.06

An increase in absorbance at 278 nm was not produced when N KOH was added to material corresponding to 19-OH prostaglandins, prostaglandin E and prostaglandins A and B. This result shows that the amount of prostaglandin A-like and prostaglandin E-like material is below the threshold of the spectroscopic assay for prostaglandins, and confirms the low levels of prostaglandin E and A-like activities in seminal fluid from the domestic fowl found by bioassay. The levels of prostaglandin A-like activity did not differ between the seminal fluid sample and the sample of seminal plasma. Prostaglandin E-like activity was low in the second seminal plasma sample and in the seminal fluid sample.

The absorbance at 278 nm measured before the addition of N KOH was low. This finding shows that prostaglandin B and 19-OH prostaglandin B, which are not separated from the corresponding prostaglandin A compounds by TLC with the A VII solvent system, are present in amounts below the threshold of the assay, or are absent.

Extraction of prostaglandin-like material from the male reproductive tract of the domestic fowl

The levels of prostaglandin-like material extracted from homogenates of regions of the male reproductive tract from the domestic fowl after 30 minutes incubation in phosphate buffer are shown in Table 26. All the material which co-chromatographed with each of radioactive prostaglandins F, E and A was used for bioassay.

Table 26

Prostaglandin-like material extracted from regions comprising the entire male reproductive tract of the domestic fowl after incubation in phosphate buffer

Region	Zone	% radio-activity recovered	PG in sample (ng)	PG in sample corrected for recovery of radio-active PG	Mass of tissue (g)	PG in tissue (ng/g)
Swollen region	PGF	47	200	425		1420
	PGE	58	240	630	0.3	2100
	PGA	31	-	-	-	-
Ductus deferens + epididymal region	PGF)	incomplete separation on TLC plate. Eluted as one zone 48	25	52		43
	PGE)		300	620	1.2	510
	PGA	42	-	-	-	-
Testes	PGF	34	125	365		12
	PGE	51	420	470	31.5	15
	PGA	8	inactive	-	-	-

This table shows that the levels of prostaglandin F-like and E-like material occurring from regions of male reproductive tract of the domestic fowl after 30 minutes incubation in phosphate buffer are highest in the swollen region, followed by the ductus deferens. In the testes, prostaglandin E and F-like activity is very low. Prostaglandin A-like activity could not be detected. It was regrettable that the samples of prostaglandin A-like activity, extracted from the swollen region and the ductus deferens, were lost as the presence of prostaglandin A-like material indicates that the tissue is able to biosynthesise A prostaglandins and may contain a prostaglandin E dehydrase. However levels of prostaglandin A-like activity assayed in extracts of the swollen region and ductus deferens incubated with 5,6 ^{3}H-prostaglandin E_1 for 60 minutes are shown in Table 27, page 133. They are low and indicate that prostaglandin A-like activity extracted from these tissues after incubation in phosphate buffer is also low.

The results presented in Table 26, page 131, and Table 27, page 133, show that the capacity of male reproductive tract tissue from the domestic fowl to produce prostaglandin A-like material is small compared with the capacity to produce prostaglandin E-like material. The small proportion of prostaglandin A-like material extracted relative to prostaglandin E-like material suggests that most of it may be derived non-enzymatically.

Metabolism of prostaglandin E_1 by the male reproductive tract of the domestic fowl

Radioactive material recovered after incubating 5,6 ^{3}H-prostaglandin E_1 with the epididymal region, the ductus deferens and the swollen region of the reproductive tract of the domestic fowl ran on

TLC in the A VII solvent system as a single peak corresponding to prostaglandin E_1 (Figure 28, page 134). Material recovered after incubation of prostaglandin E_1 with the testes separated into a compound corresponding to prostaglandin E_1, Rf 0.41 and one with an Rf 0.46. This less polar compound did not co-chromatograph with prostaglandin A_1 (Rf 0.59). The amount of radioactivity recovered from the extracts of the 4 regions of the male reproductive tract of the domestic fowl is shown in Table 27, which also shows levels of prostaglandin E and prostaglandin A estimated by radioactivity and by bioassay.

Table 27

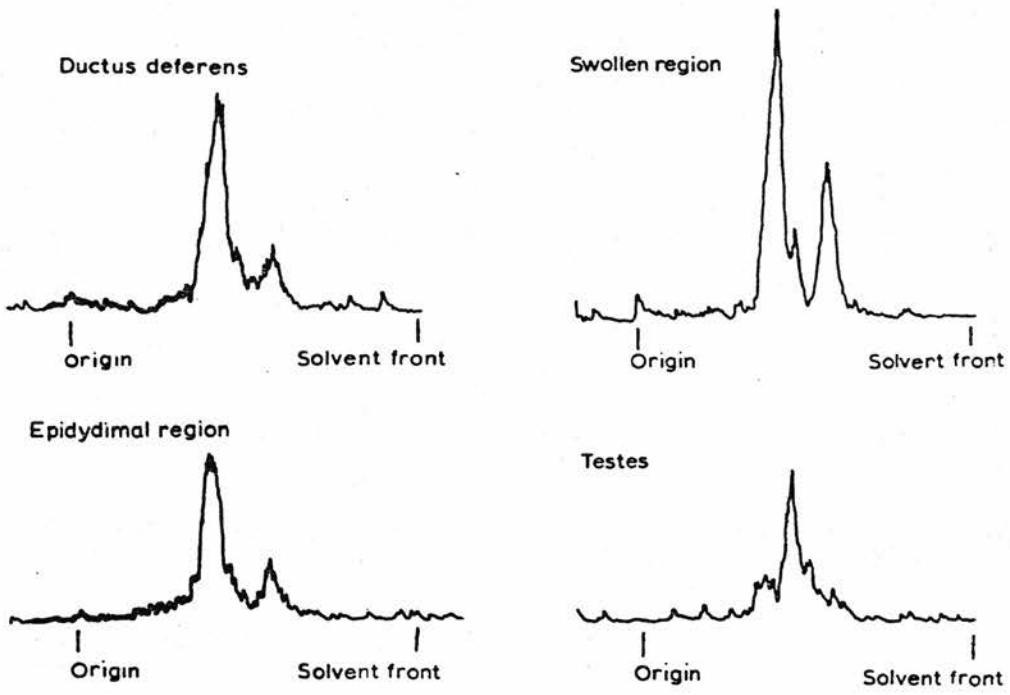
Amounts of prostaglandin E-like and prostaglandin A-like material extracted from the male reproductive tract of the domestic fowl after incubation with prostaglandin E_1 (250 ng, 252,870 dpm)

Region of the reproductive tract	Rf of zone from TLC	dpm recovered	PG estimated from dpm (ng)	PG estimated by bioassay (ng)
Epididymal region	0.32 - 0.50	240,300	335	315
	0.50 - 0.68	52,100	51	25
Ductus deferens	0.33 - 0.51	204,200	197	200
	0.51 - 0.66	47,400	45	25
Swollen region	0.35 - 0.52	225,700	220	750
	0.52 - 0.65	82,100	79	25
Testes	0.33 - 0.40	30,400	29	25
	0.40 - 0.62	95,700	92	50

The Rf of prostaglandin E_1 was 0.41 and that of prostaglandin A_1 was 0.59

Figure 28

Distribution of radioactivity after TLC of extracts of 5,6 ^{3}H-prostaglandin E_1 incubated with homogenates of regions of the reproductive tract of the domestic fowl.



These results show that homogenates of the epididymal region, ductus deferens and the swollen region of the reproductive tract of the domestic fowl do not metabolise prostaglandin E_1. Prostaglandin E_1 metabolism does occur in the testes but the metabolite does not co-chromatograph with prostaglandin A (see Figure 28, page 134). The low capacity of the testes to synthesise prostaglandin E-like material (Table 26, page 131) may therefore have been underestimated owing to metabolism of prostaglandin E_1. There was however no evidence that metabolism of prostaglandin E_1 to prostaglandin A_1 occurred in this tissue.

The low levels of endogenous prostaglandin A-like activity extracted from regions of the reproductive tract of the domestic fowl and the lack of metabolism of prostaglandin E_1 to prostaglandin A_1 indicated that a prostaglandin E dehydrase is not present in this tissue. Consequently, the possibility that human seminal vesicles contain this enzyme was tested by investigating both the biosynthesis of prostaglandins from endogenous and exogenous precursors, and the enzymatic conversion of prostaglandin E to prostaglandin A.

Levels of prostaglandin-like material in human seminal vesicles

The levels of prostaglandin-like material extracted on 2 occasions from autopsy human seminal vesicles and bioassayed on the rat fundus and kitten blood pressure preparations are shown in Table 28. Half the material was homogenised and incubated in ethanol to estimate the prostaglandin-like material present when the vesicles were removed and stored in ice. The remaining material was homogenised in phosphate buffer to assess the capacity of the tissue to synthesise prostaglandins, since prostaglandins can be formed easily during homogenisation and incubation by some tissues (Pace-Asciak, Morawska,

Coceani and Wolfe, 1967). Levels expressed per gram of tissue are corrected for recoveries obtained with radioactive prostaglandins.

Table 28

Prostaglandin-like material extracted from two pairs of autopsy human seminal vesicles

TISSUE HOMOGENISED IN ETHANOL

Sample	Mass of tissue (g)	TLC zone	% radio-activity recovered	PG in sample (uncorrected for recovery)	PG ng per g tissue (corrected for recovery)
1	2.5	PGF	37	500	300
		PGE	29	* 50	38
		PGA	6	< 62	< 27
2	2.5	PGF	35	750	225
		PGE	37	75	35
		PGA	37	124	29

TISSUE HOMOGENISED IN PHOSPHATE BUFFER

Sample	Mass of tissue (g)	TLC zone	% radio-activity recovered	PG in sample (uncorrected for recovery)	PG ng per g tissue (corrected for recovery)
1	2.5	PGF	29	500	400
		PGE	23	75	72
		PGA	23	500	143
2	2.5	PGF	29	300	112
		PGE	23	500	143
		PGA	44	166	48

* An 0.4 ml volume of this sample dissolved in 1 ml 0.9% NaCl was not vasoactive. The threshold dose of prostaglandin A_1 on this preparation was 25 ng.

There is an increase in prostaglandin A-like activity extracted from human seminal vesicles homogenised and incubated in phosphate buffer compared to that extracted from vesicles homogenised and incubated in ethanol. In the first sample, this increase is 5 fold and cannot be accounted for by non-enzymatic dehydration of the increased amount of prostaglandin E-like activity. This is 2 fold higher in phosphate buffer homogenates than in ethanolic ones. However in the second sample, the prostaglandin A-like activity from phosphate buffer homogenates is only 1.8 fold of that obtained from ethanolic homogenates, and may be accounted for by the 4 fold increase in prostaglandin E-like activity detected. The small differences in prostaglandin F and E-like activities between phosphate buffer and ethanolic homogenates of both samples of human seminal vesicles show that the capacity of the vesicles used to synthesise prostaglandin F-like and prostaglandin E-like material was low.

Incubation of human seminal vesicles with arachidonic acid

A single peak of radioactivity (Rf 0.81) was obtained after TLC of an extract of human seminal vesicle homogenate incubated in phosphate buffer. The Rf values of 5,6 ^{3}H-prostaglandin $F_{2\alpha}$, 5,6 ^{3}H-prostaglandin E_1, 5,6 ^{3}H-prostaglandin A_1 and ^{3}H-arachidonic acid are 0.47, 0.53, 0.83 and 0.81 respectively. Thus radioactive arachidonic acid was not converted material which co-chromatographs with F and E prostaglandins by the specimens of human seminal vesicles. The radioactive material that was recovered co-chromatographed with arachidonic acid, but had a similar Rf value to prostaglandin A_2. It was also recovered from an extract of seminal vesicles incubated in ethanol to estimate non-enzymatic formation of prostaglandins.

Therefore it was not due to material formed enzymatically during incubation. Since large amounts (20 μC) of arachidonic acid had been incubated, it is possible that radioactivity due to arachidonic acid remaining after incubation, that may not have partitioned into petroleum spirit during the extraction of prostaglandins, was detectable by the TLC scanner. Alternatively, the radioactive material recovered which co-chromatographs with arachidonic acid may have been due to non-enzymatic formation of a compound other than arachidonic acid but with a similar Rf. The finding that human seminal vesicle homogenates do not synthesise prostaglandins $F_{2\alpha}$, E_2 and A_2 from exogenous arachidonic acid is compatible with the low capacity found of this tissue to biosynthesise prostaglandins from exogenous precursors (see Table 28, page 136). Thus the activity of prostaglandin synthetase in these human seminal vesicle specimens was low. If they do contain a prostaglandin E dehydrase, it may also be inactive. Alternatively, activity may not be detectable because the amount of endogenous prostaglandin E synthesised by prostaglandin synthetase is low.

Metabolism of prostaglandin E by human seminal vesicle homogenates

If the action of prostaglandin E dehydrase ends a sequence of enzymatic reactions started by the action of prostaglandin synthetase, high activity of this enzyme might be required to form sufficient prostaglandin E to act as a substrate for prostaglandin E dehydrase. This may be the reason why little prostaglandin A biosynthesis by human seminal vesicles from endogenous precursors and none from exogenous precursors could be demonstrated. Therefore an attempt was made to demonstrate prostaglandin E dehydrase activity by adding exogenous prostaglandin E.

Incubation of 5,6 ^{3}H-prostaglandin E_1 with human seminal vesicle homogenates and phosphate buffer resulted in one peak of radioactivity after TLC corresponding to prostaglandin E_1 (Figure 29, page 140). Radioactivity recovered in prostaglandin E and prostaglandin A zones of both extracts appears in Table 29.

Table 29

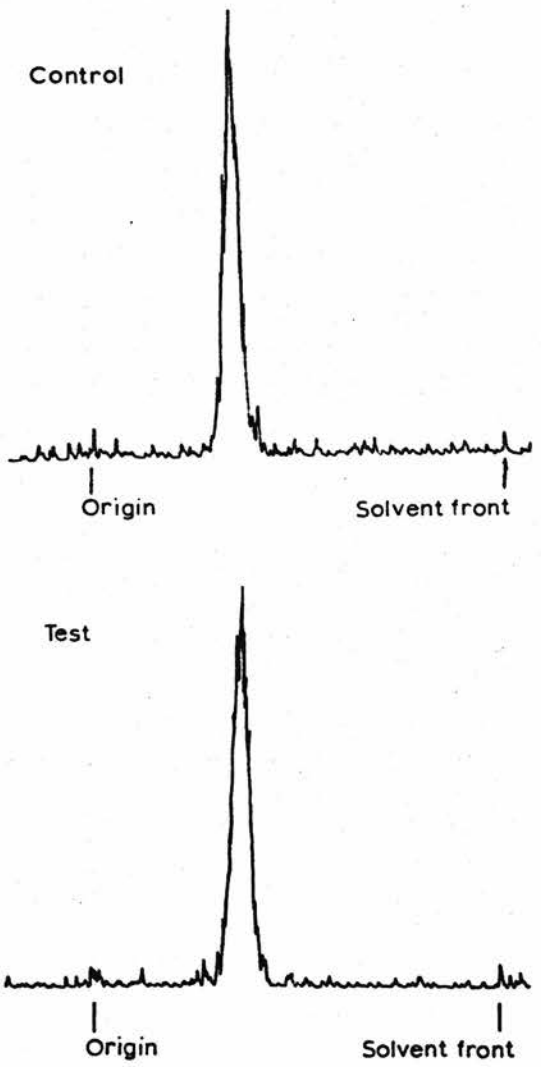
Radioactivity recovered in prostaglandin E and prostaglandin A zones after incubation of 5,6 ^{3}H-prostaglandin E_1 (2.5 µg, 552,530 dpm) with human seminal vesicle homogenates

Sample	TLC zone	dpm	Total dpm	$\frac{\text{PGA} \times 100\%}{\text{Total}}$
PGE_1 + seminal vesicle homogenates	PGE	218,394	219,695	0.6
	PGA	1,301		
PGE_1 + phosphate buffer	PGE	138,130	140,320	1.5
	PGA	2,190		

These results show that the very small amount of radioactive prostaglandin A-like material extracted after human seminal vesicle homogenates have been incubated with 5,6 ^{3}H-prostaglandin E_1 can be accounted for by instability of 5,6 ^{3}H-prostaglandin E_1 during storage, incubation and extraction. Evidence that human seminal vesicles contain a prostaglandin E dehydrase was not obtained by incubating with a possible precursor of prostaglandin A, other than arachidonic acid.

Figure 29

Distribution of radioactivity after TLC of extracts of 5,6 ^{3}H-prostaglandin E_1 incubated with phosphate buffer (control) and with human seminal vesicle homogenates (test).



Extraction of prostaglandins from baboon seminal plasma

The levels of prostaglandin-like activity found by bioassay of TLC zones of an extract of baboon seminal plasma are shown in Table 30. Levels of F, E and A prostaglandins, corrected for recoveries obtained with radioactive compound, are expressed as activity in the original sample, not per unit volume of ejaculate as the exact volume of ejaculate obtained was not measured.

Table 30

Prostaglandin-like activity extracted from baboon seminal plasma and bioassayed on the rat fundus and kitten blood pressure preparations

TLC zone	PG in sample (ng)	Percentage of radioactivity recovered	PG in sample * (corrected for recovery) ng
PGF	< 30	32	< 94
PGE	150	58	500
PGA	< 25	92	< 27
19-OH PG	650	-	-

* The volume of the ejaculate was not known. Therefore levels of prostaglandins could not be expressed per ml of sample.

The prostaglandin F and prostaglandin E zones were assayed in terms of prostaglandin $F_{2\alpha}$ and prostaglandin E_1, the prostaglandin A and 19-OH prostaglandin zones in terms of prostaglandin A_1. The ratio of equiactive doses of 19-OH prostaglandin A_1 to prostaglandin A_1 is about 3.8 (Horton and Jones, 1969). Thus the depressor activity which chromatographs with 19-OH prostaglandins in the A I solvent system is equivalent to 650 x 3.8 ng of 19-OH prostaglandin A_1 (2.47 µg).

The results of a spectroscopic assay of the prostaglandin E and prostaglandin A zones are in Table 31.

Table 31

Spectroscopic assay of prostaglandin E and prostaglandin A TLC zones of an extract of baboon seminal plasma

TLC zone	Volume assayed (ml)	Total sample volume (ml)	Absorbance 278 nm	
			Before addition 0.2 ml N KOH	After addition 0.2 ml N KOH
PGE	2.3	4.9	0.15	0.16
PGA	2.3	4.9	0.28	0.28

There is no change in absorbance at 278 nm when N KOH is added to material in the prostaglandin E and prostaglandin A zones after TLC of an extract of baboon seminal plasma. This finding shows that the amounts of prostaglandin E and prostaglandin A in the extract are below the threshold for the spectroscopic assay. It agrees with the low levels reported in Table 30, page 141. However, the finding that material equivalent to 2.47 µg of 19-OH prostaglandin A_1 could be extracted from baboon semen indicated that baboon seminal vesicles might contain enzymes, including a prostaglandin E dehydrase, that synthesise A prostaglandins. Therefore it was hoped that biosynthesis of A prostaglandins by this tissue would be detected.

Levels of prostaglandin-like material in baboon seminal vesicles

The amounts of prostaglandin-like material extracted on 2 occasions from paired baboon seminal vesicle homogenates after

incubation for 30 minutes in phosphate buffer or ethanol are shown in Table 32. Also shown are the amounts obtained from vesicles which were incubated on 2 occasions in phosphate buffer only. Prostaglandin-like material was assayed in terms of prostaglandin $F_{2\alpha}$ and prostaglandin E_1 on the rat fundus preparation, and in terms of prostaglandin A_1 on the kitten blood pressure. Amounts extracted are expressed per gram of tissue and were corrected for recoveries obtained with radioactive prostaglandins.

Table 32

Prostaglandin-like material extracted from baboon seminal vesicles incubated at 37°C for 30 minutes in air

			Homogenised in ethanol				Homogenised in phosphate buffer			
Sample	Mass of tissue incubated (g)	PG	% radioactivity recovered	PG in sample (µg)	PG in sample corrected for recovery (µg)	PG µg per g tissue	% radioactivity recovered	PG in sample (µg)	PG in sample corrected for recovery (µg)	PG µg per g tissue
1	2.45	F					24	0.20	0.83	0.17
		E					34	13.20	0.04	16.00
		A					22	0.60	2.75	0.55
2	3.0	F	61	0.10	0.15	0.50	33	0.40	1.30	0.43
		E	33	0.20	0.61	0.20	29	5.00	17.30	5.80
		A	35	0.20	0.57	0.19	36	0.40	1.10	0.37
3	1.0	F	66	0.01	0.02	0.02	58	0.10	0.17	0.17
		E	55	0.08	0.15	0.15	56	0.50	0.89	0.89
		A	none added	<0.02			none added	0.05		
4	2.5	F					60	0.33	0.56	0.22
		E					27	8.30	31.00	12.40
		A					23	0.33	1.40	0.57

* An 0.2 ml portion of the sample dissolved in 0.3 ml 0.9% NaCl was not vasoactive. The threshold dose of this preparation was 10 ng of prostaglandin A_1

Results from paired incubations in ethanol and phosphate buffer (samples 2 and 3) show that more prostaglandin-like activity was extracted from homogenates incubated in phosphate buffer than from those incubated in ethanol. An 8 fold increase in ng prostaglandin $F_{2\alpha}$ per gram of seminal vesicle occurs in both samples, and a 25 fold increase in ng prostaglandin E_1 per gram of vesicle in sample 2. The increase in prostaglandin E_1 is only 5 fold in sample 3, but the prostaglandin E-like activity occurring per unit weight of tissue after incubation in phosphate buffer is also lower in this sample than in samples 1, 2 and 4. These results are evidence for the capacity of baboon seminal vesicles to biosynthesise prostaglandins of the F and E series. The increase in ng prostaglandin A_1 per gram of tissue extracted from sample 2 is only 2 fold. This finding, and the low levels of prostaglandin A_1-like activity compared with prostaglandin E_1-like activity extracted from samples 1, 3 and 4 incubated in phosphate buffer, do not suggest that the capacity of baboon seminal vesicle homogenates to synthesise prostaglandins of the A series is high.

Vesiglandin, as already mentioned, may be a prostaglandin of the A, B or 19-OH series and has been extracted from the seminal vesicles of Macacus rhesus (von Euler, 1936). However, the occurrence of prostaglandins in the male reproductive tract tissue of other primate species has not been described previously. Therefore a more positive identification of the large amount of biologically active material extracted from baboon seminal vesicles which co-chromatographed with prostaglandin E_1 was made on the basis of a spectroscopic assay, argentous thin layer chromatography and combined gas chromatography-mass spectrometry. An attempt was also made to identify material which co-chromatographed with prostaglandin $F_{2\alpha}$ and prostaglandin A_1 by combined gas chromatography-mass spectrometry.

Spectroscopic assay of prostaglandin E-like material extracted from baboon seminal vesicles

A 2.8 ml volume of the sample with prostaglandin E-like activity was assayed. Addition of 0.2 ml N KOH turned the sample green and raised the baseline absorbance recorded between 250 nm and 325 nm. Nevertheless it was possible to measure an increase in the 278 nm chromophore.

Prostaglandin E-like material in sample 1, incubated in phosphate buffer, was evaporated to dryness under reduced pressure and was dissolved in 6.0 ml methanol. A 2.8 ml volume was removed for the spectroscopic assay which was performed in a cuvette, pathlength 1 cm, volume 3 ml.

Before the addition of 0.2 ml N KOH A_{278} = 0.11 OD units

Immediately after the addition of 0.2 ml N KOH A_{278} = 0.25 OD units

30 minutes after the addition of 0.2 ml N KOH A_{278} = 0.28 OD units

Therefore the change in A_{278} occurring in 30 minutes = 0.03 OD units

A change in A_{278} of 0.81 OD units is equivalent to the formation of 10 µg/ml prostaglandin B

Therefore a change in A_{278} of 0.03 OD units is equivalent to the formation of $\frac{10 \times 0.03}{0.81}$ µg/ml of prostaglandin B

Volume of fluid assayed = 3 ml

Therefore total prostaglandin B formed = $\frac{10 \times 0.03 \times 3}{0.81}$ µg

= 1.1 µg

Therefore prostaglandin E originally present = 1.1 µg

Therefore prostaglandin E present in 6 ml = $\frac{1.1 \times 6 \text{ µg}}{2.8}$ = 2.3 µg

Prostaglandin E found by bioassay uncorrected for recovery (Table 32, page 143) was equivalent to 13.2 µg prostaglandin E_1. The discrepancy between prostaglandin E found by bioassay and by spectroscopic assay may be due to the high absorbance at 278 nm recorded immediately after addition of N KOH. In addition, the prostaglandin E-like biological activity assayed in terms of prostaglandin E_1 may be accounted for by some prostaglandin E_2, which is about twice as potent as prostaglandin E_1 on the rat fundus preparation (Horton, 1972). Nevertheless the production of a 278 chromophore by treatment of biologically active material, which co-chromatographs with prostaglandin E, is evidence that some of the biological activity is due to a prostaglandin of the E series (Andersen, 1969).

Argentous TLC of prostaglandin E-like material from baboon seminal vesicles

The sample run on $AgNO_3$ impregnated TLC plates in the A II solvent system was obtained from an experiment to study metabolism of prostaglandin E_1 by baboon seminal vesicle homogenates, and contained 6 µg, 0.25µC prostaglandin E_1. Two bands were obtained when the plate was sprayed with phosphomolybdic acid and heated at 110°C. Their Rf values and those of prostaglandin E_1 and prostaglandin E_2 are shown in Table 33.

Table 33

Rf values of material visualised after argentous TLC in A II solvent system of E zone of baboon seminal vesicle extract

Sample	Rf
Baboon vesicle extract (band 1)	0.73
" " " (band 2)	0.68
Prostaglandin E_2	0.75
Prostaglandin E_1	0.69
5,6 ^{3}H-prostaglandin E_1	0.68

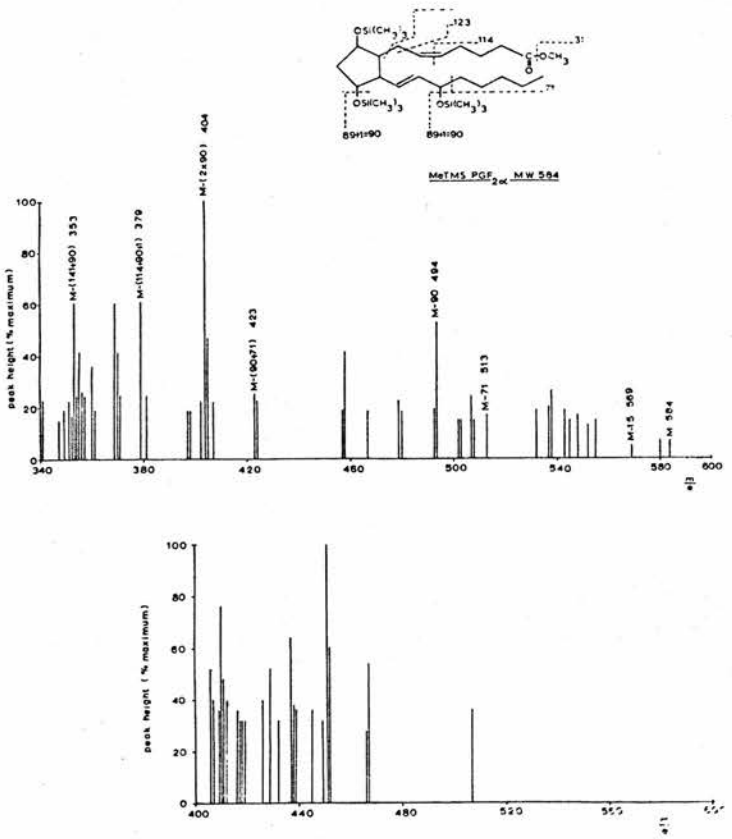
These results show that the extract of baboon seminal vesicles contained material which co-chromatographs with prostaglandin E_1 and prostaglandin E_2. Some of the prostaglandin E_1 is accounted for by 6 µg prostaglandin E_1 added to the homogenate. The prostaglandin-like material extracted from the homogenate is tentatively identified as prostaglandin E_2. It may also comprise some prostaglandin E_1.

Combined gas chromatography-mass spectrometry of prostaglandin-like material extracted from baboon seminal vesicles

A line diagram of the mass spectrum of the methyl ester-trimethylsilyl ether derivative (MeTMS) of 100 ng prostaglandin $F_{2\alpha}$-like material extracted from the 3rd sample of baboon seminal vesicles incubated in buffer (Table 32, page 143) is shown in Figure 30, page 148. Also included is a line diagram of the mass spectrum of the MeTMS derivative of 100 ng of authentic prostaglandin $F_{2\alpha}$ and the fragmentation pattern of the molecule. The part of the spectrum obtained with peaks of

Figure 30

Line diagrams of the mass spectra of the MeTMS derivatives of 100 ng prostaglandin $F_{2\alpha}$ (upper record) and of the prostaglandin F-like material extracted from baboon seminal vesicles (lower record).



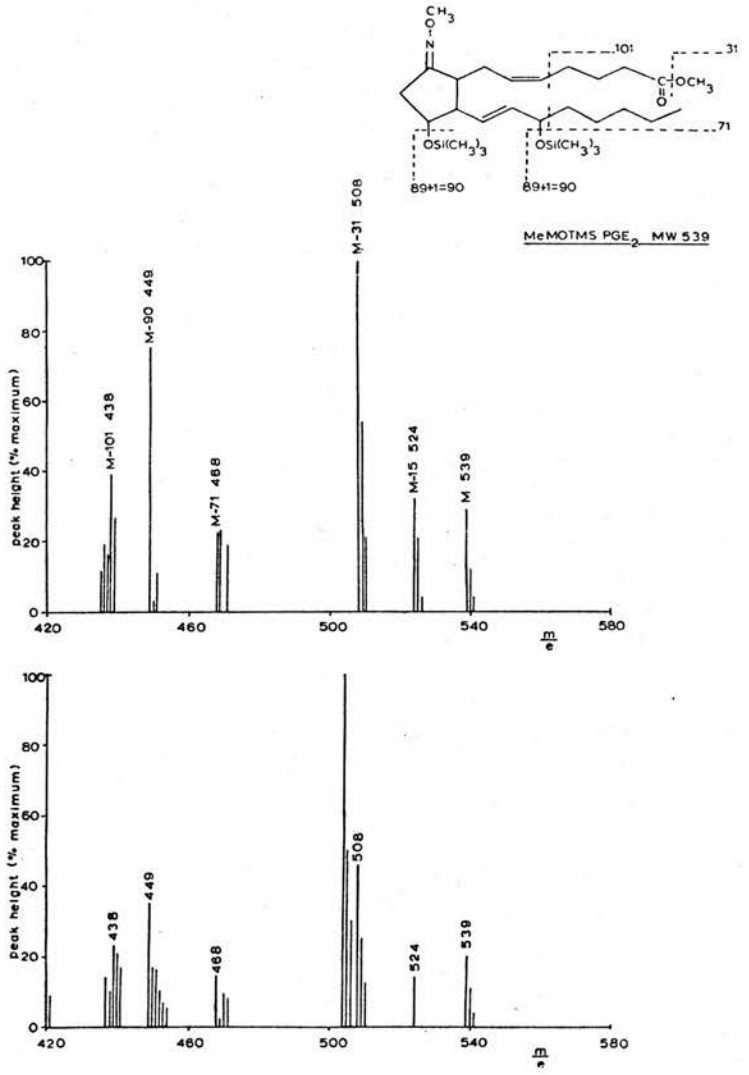
Spectra were taken at the same retention time.

$\frac{m}{e}$ value less than 340 is not included for authentic prostaglandin $F_{2\alpha}$, or below $\frac{m}{e}$ values of 400 for biological extract because peaks at $\frac{m}{e}$ values characteristic of prostaglandin $F_{2\alpha}$ in the extract could not be distinguished from "background peaks" due to other compounds. Ions at $\frac{m}{e}$ values of 584, 569, 513, 494, 423 and 404 obtained from the fragmentation of the MeTMS derivative of prostaglandin $F_{2\alpha}$ did not occur in the mass spectrum of the baboon seminal vesicle extract. Prostaglandin $F_{2\alpha}$ was therefore not identified in the extract.

Line diagrams of the mass spectra obtained from the methyl ester-methoxime-trimethylsilyl ether (MeMOTMS) derivative of 2 µg of authentic prostaglandin E_2 and the MeMOTMS derivative of prostaglandin E-like material extracted from baboon seminal vesicles (sample 1, Table 32, page 143) are shown in Figure 31, page 150. Spectra were taken at the retention time of the second MeMOTMS derivative of prostaglandin E_2 (17.9 minutes). The retention time of the first derivative was 14.5 minutes. Also shown in the line diagram is the fragmentation pattern of MeMOTMS prostaglandin E_2 producing ions of $\frac{m}{e}$ value greater than 400. The intensities of ions below this value are not included in the diagram because of the high intensity of ions due to compounds other than prostaglandin E_2. Ions characteristic of the MeMOTMS prostaglandin E_2 occur in the mass spectrum of the MeMOTMS derivative of the prostaglandin-like material: namely at $\frac{m}{e}$ values of 539, 524, 508, 468, 449 and 438. The ratios of their intensities are of the same order in both spectra. Peaks, characteristic of the MeMOTMS prostaglandin E_1, were not obtained in a spectrum taken at the retention time of MeMOTMS of 2 µg of prostaglandin E_1 (23.9 minutes). Line diagrams of these two spectra and the breakdown pattern of MeMOTMS prostaglandin E_1, producing ions of $\frac{m}{e}$ values greater than 420, are shown in Figure 32, page 151. Thus prostaglandin E_2 but not prostaglandin E_1 was positively identified in baboon seminal vesicle extracts.

Figure 31

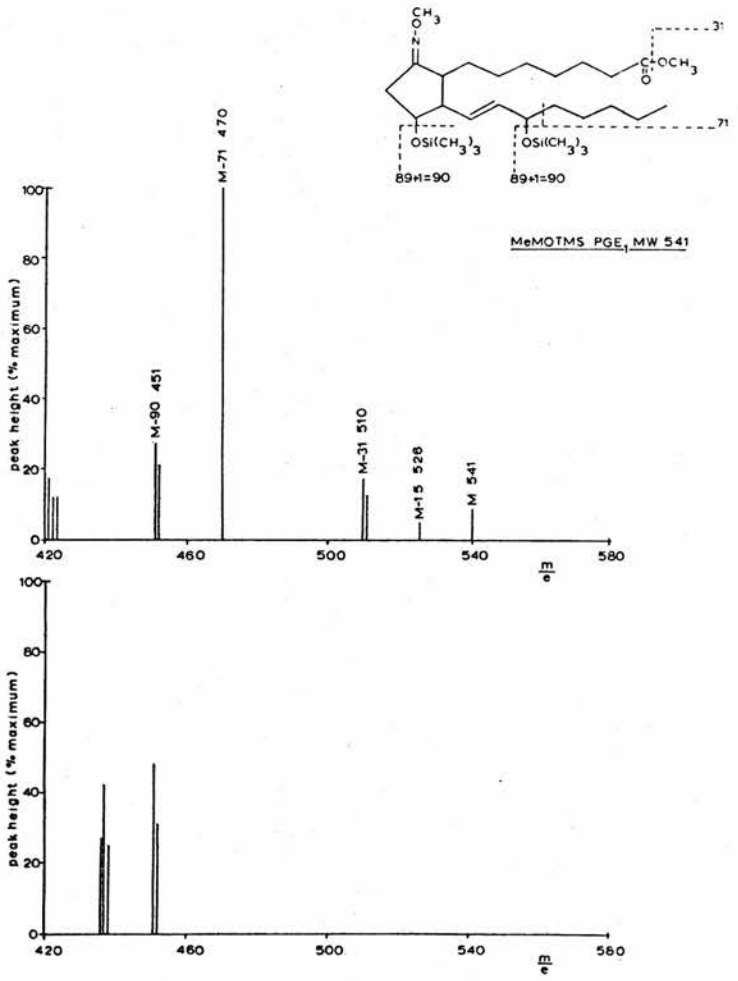
Line diagrams of the mass spectra of the MeMOTMS derivatives of 2 µg prostaglandin E_2 (upper record) and of the prostaglandin E-like material extracted from baboon seminal vesicles (lower record).



Spectra were taken at the same retention time (17.9 minutes).

Figure 32

Line diagrams of the mass spectra of the MeMOTMS derivatives of 2 µg prostaglandin E_1 (upper record) and of the prostaglandin E-like material extracted from baboon seminal vesicles (lower record).



Spectra were taken at the same retention time (23.9 minutes).

Line diagrams of mass spectra of the MeTMS derivative of 500 ng of authentic prostaglandin A_2 and the prostaglandin A-like material extracted from baboon vesicles are shown in Figure 33, page 153. Spectra were taken at the retention time of MeTMS prostaglandin A_2 (9.5 minutes). Ions occurring at $\frac{m}{e}$ values of 420 and 405 in the spectrum of the MeTMS of authentic prostaglandin A_2 did not do so in that of the corresponding derivative of the seminal vesicle extract. Prostaglandin peaks at $\frac{m}{e}$ values of 389, 349, 330 and 321 could not be distinguished from background in the spectra of the extract. Therefore prostaglandin A_2 could not be identified.

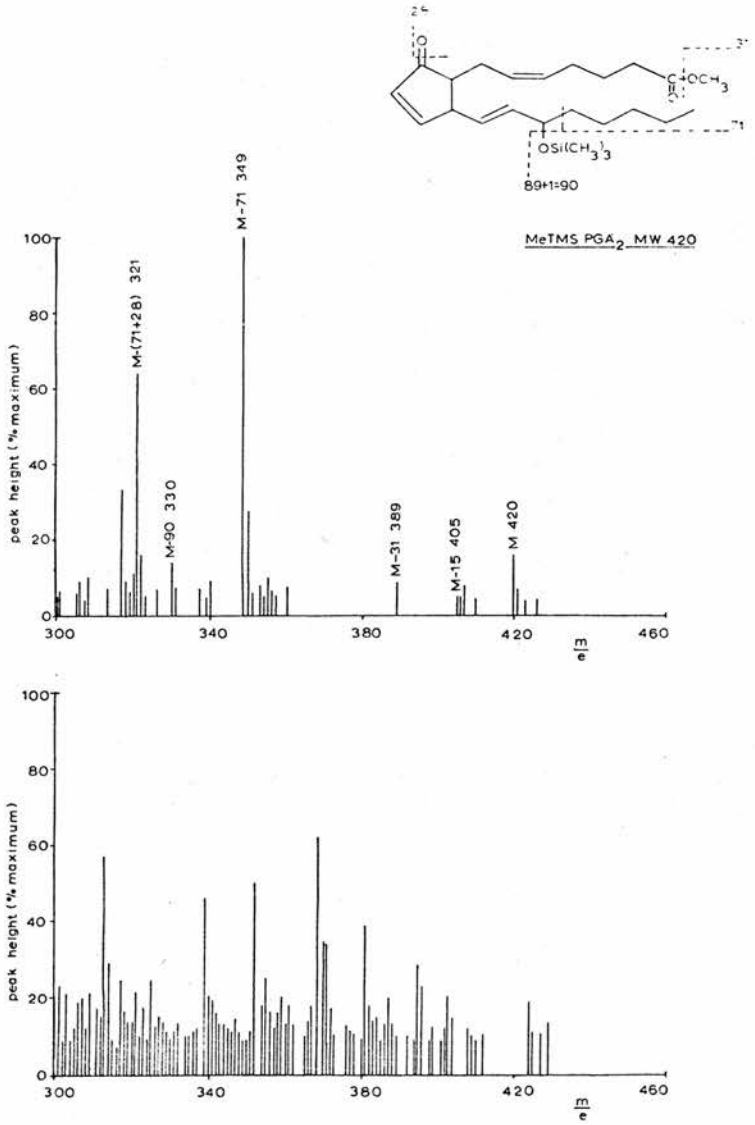
Similarly, the spectrum of the MeTMS derivative of the prostaglandin-like material taken at the retention time (15.4 minutes) of the MeTMS derivative of 500 ng authentic prostaglandin B_2 contained very many ions, whose intensities were greater than those due to breakdown of MeTMS prostaglandin B_2. At $\frac{m}{e}$ values above 360, peaks characteristic of MeTMS prostaglandin B_2 at $\frac{m}{e}$ 389 and 405, were absent (Figure 34, page 154). If the peak at 420 was from the molecular ion of MeTMS prostaglandin B_2, peaks at 405 and 389 should have been detected. Thus prostaglandin B_2 was not identified in an extract of baboon seminal vesicles.

Prostaglandin E-like activity biosynthesised by baboon seminal vesicle homogenates from endogenous precursors was identified as prostaglandin E_2. No prostaglandin E_1 could be identified. Neither could prostaglandin A_2 be identified, although material with prostaglandin A-like biological activity could be extracted from the homogenates.

Subsequently, biosynthesis of prostaglandins from radioactive arachidonic acid was examined to see if the low yield of prostaglandin A-like material obtained from endogenous precursors could be improved by using exogenous ones.

Figure 33

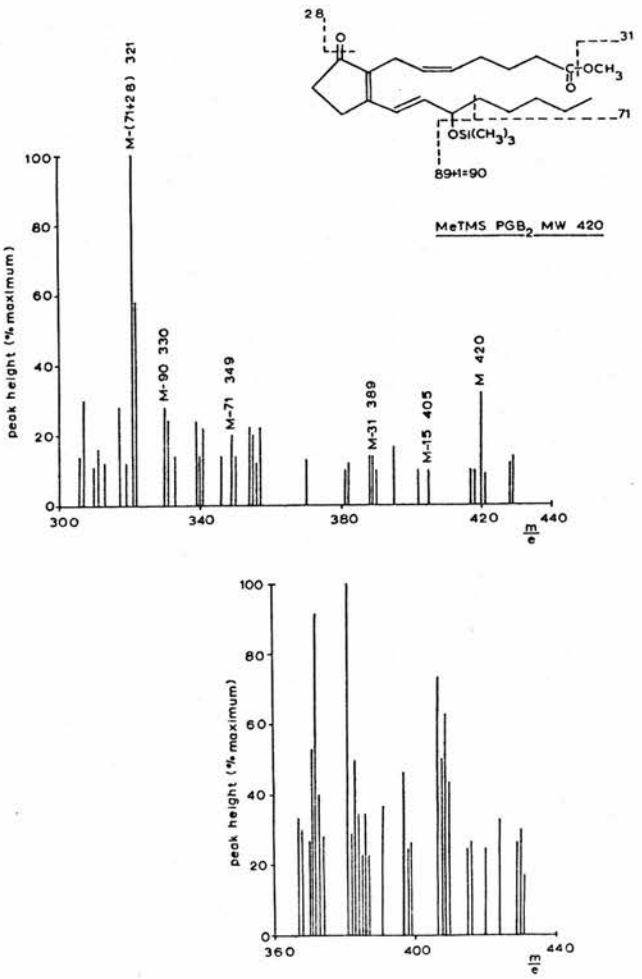
Line diagrams of the mass spectra of the MeTMS derivatives of 500 ng prostaglandin A_2 (upper record) and of the prostaglandin A-like material extracted from baboon seminal vesicles (lower record).



Spectra were taken at the same retention time (9.5 minutes).

Figure 34

Line diagrams of the mass spectra MeTMS derivatives of 500 ng prostaglandin B_2 (upper record) and of material which co-chromatographs with prostaglandin A in the A VII solvent system, extracted from baboon seminal vesicles (lower record).



Spectra were taken at the same retention time (15.4 minutes).

Incubation of baboon seminal vesicles with arachidonic acid

The Rf values of zones eluted after TLC of extracts of 100 µg, 10 µC of arachidonic acid incubated with baboon seminal vesicle homogenates and with phosphate buffer are shown in Table 34. Also shown are the Rf values of ^{3}H-prostaglandins $F_{2\alpha}$, E_1 and A_1.

Table 34

Zones of radioactivity obtained after incubation of ^{3}H-arachidonic acid, 100 µg, 10 µC, with baboon seminal vesicles and with phosphate buffer

Zone	Arachidonic acid incubated with phosphate buffer		Arachidonic acid incubated with seminal vesicle homogenates	
	Rf	dpm	Rf	dpm
1)	)	)	0.25 - 0.37)	)
)	)	)	)	)
2)	0.18 - 0.42)	52,340	0.37 - 0.46)	92,660
3	0.42 - 0.60	105,820	0.46 - 0.64	206,680
4	0.60 - 0.68	148,510	0.64 - 0.70	24,710
5	0.68 - 0.76	138,030	0.70 - 0.80	263,570
6	0.81 - 0.92	4,989,930	0.80 - 0.91	73,380
$PGF_{2\alpha}$	0.24			
PGE_1	0.34			
PGA_1	0.60			

Radioactivity in zones 1 to 5 obtained from the extract of arachidonic acid incubated with phosphate buffer is probably due to trailing of the large amount of material occurring in zone 6. Peaks of radioactivity occurred in extracts of arachidonic acid incubated with seminal vesicle homogenate in zones 1 and 2, 3, 5 and 6. The Rf values

of prostaglandins show that material in zones 1 and 2 co-chromatographed with prostaglandin $F_{2\alpha}$ and prostaglandin E_1, and material in zone 3 with prostaglandin A_1.

Material in zones 1 and 2 was assayed on the rat fundus preparation in terms of prostaglandin E_1. Material in zone 3 was assayed on the kitten blood pressure in terms of prostaglandin A_1. The results are shown in Table 35.

Table 35

Prostaglandin-like activity extracted from baboon seminal vesicles incubated with phosphate buffer and with ^{3}H-arachidonic acid

Sample	Rf	PGE_1 in zone(μg)	Rf	PGA_1 in zone(μg)
Seminal vesicles incubated in EtOH	0.30-0.36	0.2	0.55-0.64	0.2
Seminal vesicles incubated with phosphate buffer	0.31-0.40	5.0	0.56-0.66	0.4
Arachidonic acid incubated with seminal vesicles homogenised in buffer	0.25-0.43	5.0	0.46-0.64	0.4
Arachidonic acid incubated with phosphate buffer	0.18-0.42	0.2	0.42-0.60	< 0.1

The Rf of ^{3}H-prostaglandin E_1 was 0.36, that of prostaglandin A_1 was 0.60

The prostaglandin E-like activity extracted from seminal vesicle homogenates was greater from homogenates incubated in phosphate buffer than in ethanol. This finding is evidence that the homogenates were able to synthesise prostaglandin-like material from endogenous precursors. However the yields of prostaglandin E-like biological

activity in zone 2 and the prostaglandin A-like activity in zone 3 are not increased when arachidonic acid is added to the homogenate. Therefore there is no evidence that the radioactivity in zone 2 (Table 34, page 155) is due to a prostaglandin of the E or F series, or that radioactivity in zone 3 is due to one of the A series. These results show that although baboon seminal vesicle homogenates are able to synthesise prostaglandin E_2 from endogenous precursors, biosynthesis of prostaglandin E_2 or prostaglandin A_2 does not occur from exogenous precursors. Since prostaglandin A biosynthesis from endogenous precursors had also not been demonstrated, the metabolism of ^{3}H-prostaglandin E_1 and ^{3}H-prostaglandin E_2 to prostaglandin A_1 and prostaglandin A_2 was examined in an attempt to demonstrate biosynthesis of prostaglandin A from a possible precursor other than arachidonic acid.

Metabolism of 5,6 ^{3}H-prostaglandin E_1 and 17,18 ^{3}H-prostaglandin E_2 by baboon seminal vesicle homogenates

Table 36 shows the radioactivity recovered after TLC of an extract of ^{3}H-prostaglandin E_2 incubated with baboon seminal vesicle homogenates and in one of ^{3}H-prostaglandin E_2 incubated with phosphate buffer, to estimate the non-enzymatic conversion of prostaglandin E_2 to less polar material during incubation, extraction and separation. The zones correspond to the positions of ^{3}H-prostaglandin E_2 and ^{3}H-prostaglandin A_2.

Table 36

Radioactivity recovered in zones corresponding to prostaglandin E and prostaglandin A after incubation of 17,18 ^{3}H-prostaglandin E_2 with baboon seminal vesicle homogenates

Sample	Zone	dpm	Total dpm	PGA dpm / Total dpm
PGE_2 + homogenate	PGA	60,630	629,490	0.09
2.4 µg, 0.08 µC/ml	PGE	568,860		
PGE_2 + buffer	PGA	113,280	814,560	0.13
2.4 µg, 0.08 µC/ml	PGE	701,280		

The amount of radioactive material which co-chromatographs with prostaglandin A_1 obtained after incubation of ^{3}H-prostaglandin E_2 with baboon seminal vesicle homogenates is less than that after incubation of ^{3}H-prostaglandin E_2 with phosphate buffer. This is evidence that it can be accounted for by non-enzymatic breakdown of ^{3}H-prostaglandin E_2 during incubation with baboon seminal vesicle homogenates, and during extraction and separation.

Results of another incubation experiment in which phosphate buffer and baboon seminal vesicles homogenised in phosphate buffer were incubated with 5,6 ^{3}H-prostaglandin E_1, is shown in Table 37.

Table 37

Radioactivity recovered in zones corresponding to prostaglandin E and prostaglandin A after incubation of 5,6 ^{3}H-prostaglandin E_1 with baboon seminal vesicle homogenates and with phosphate buffer

Sample	Zone	dpm	Total dpm	PGA dpm / Total dpm
PGE_1 + homogenate (incubation 1) (2.5 µg, 0.1 µC)	PGA	46,810	691,780	0.06
	PGE	644,970		
PGE_1 + homogenate (incubation 2)	PGA	18,760	148,720	0.05
	PGE	140,560		
PGE_1 + ethanolic homogenate	PGA	18,730	154,592	0.12
	PGE	135,862		
PGE_1 + phosphate buffer	PGA	8,600	88,810	0.02
	PGE	86,210		

The non-enzymatic conversion of 5,6 ^{3}H-prostaglandin E_1 during incubation and extraction to material which co-chromatographs with prostaglandin A_1 was estimated by incubating 5,6 ^{3}H-prostaglandin E_1 with seminal vesicle homogenates in ethanol and with phosphate buffer. The results in Table 37 show that this conversion of prostaglandin E_1 incubated with seminal vesicles homogenised in ethanol was greater than conversion of prostaglandin E_1 incubated with seminal vesicles homogenised in buffer. Thus incubation of both prostaglandin E_1 and prostaglandin E_2 with baboon seminal vesicle homogenates did not suggest that prostaglandin E is a precursor of prostaglandin A that might be synthesised by this tissue. Consequently, evidence was not obtained for a prostaglandin E dehydrase in baboon seminal vesicles.

Incubation of ^{3}H-prostaglandin E_1 with human plasma

Distribution of radioactivity recovered after TLC of extracts of 5,6 ^{3}H-prostaglandin E_1 incubated with boiled and unboiled plasma is shown in Table 38. Plasma was boiled so that non-enzymatic conversion of prostaglandin E_1 to less polar material during incubation, extraction and separation could be estimated. Also shown is the distribution of radioactivity after TLC of 5,6 ^{3}H-prostaglandin E_1 which had not been incubated with either boiled or unboiled plasma.

Table 38

Radioactivity recovered after TLC of 5,6 ^{3}H-prostaglandin E_1 (220,000 dpm) incubated with unboiled and boiled plasma

Donor	Treatment of plasma	Zone	dpm	Total dpm	Less polar dpm / Total dpm
CH (♂)	Unboiled	Less polar	29,890	100,940	0.29
		PGE	71,050		
	Boiled	Less polar	19,100	73,730	0.25
		PGE	54,620		
MF (♀)	Unboiled	Less polar	13,170	61,920	0.21
		PGE	48,750		
	Boiled	Less polar	28,260	107,480	0.26
		PGE	79,220		
SC (♀)	Unboiled	Less polar	21,510	79,220	0.27
		PGE	57,710		
	Boiled	Less polar	17,200	97,000	0.17
		PGE	79,800		
MD (♀)	Unboiled	Less polar	24,570	116,540	0.21
		PGE	91,970		
	Boiled	Less polar	24,410	100.860	0.24
		PGE	86,450		
PGE_1		Less polar	29,750	93,500	0.31
		PGE	64,750		

Conversion of prostaglandin E_1 incubated with boiled plasma to other compounds is non-enzymatic, whereas conversion of prostaglandin E_1 incubated with unboiled plasma may be both enzymatic and non-enzymatic. Results in Table 38 show that the proportion of total radioactivity recovered as less polar material after incubation of prostaglandin E_1 with boiled plasma is not different from that recovered after incubation with unboiled plasma. This finding is evidence that the 4 samples of human plasma incubated with 5,6 ^{3}H-prostaglandin E_1 did not metabolise prostaglandin E_1 to less polar material. It suggests that these plasma samples do not contain a prostaglandin E dehydrase.

Discussion

The search for a prostaglandin E dehydrase was carried out in 4 tissues, namely rat kidney, male reproductive tract of the domestic fowl, human seminal vesicles and baboon seminal vesicles. In addition, attempts were made to support very tentative evidence (McDonald-Gibson et al., 1972b) that this enzyme occurs in human plasma.

The search for prostaglandin E dehydrase in rat kidney

Kidney slices

Slices of kidney from male Campbell rats metabolised 5,6 ^{3}H-prostaglandin E_1 to at least one less polar compound. Attempts to identify this compound as prostaglandin A_1 by the retention time of its MeTMS derivative on radio-gas chromatography were inconclusive. The MeTMS derivative of the metabolite had the same retention time as that of 5,6 ^{3}H-prostaglandin A_1. However the radioactive peak, due to the MeTMS derivative of 0.075 µC of metabolite, was smaller than expected from the radioactive peak due to the MeTMS derivative of 0.05 µC of prostaglandin A_1. The difference between the expected size of the radioactive peak due to the metabolite and that obtained, has 3 possible causes. Loss of material may have occurred during derivative formation, derivative formation may have been incomplete or radioactivity may have been accounted for by a metabolite other than prostaglandin A_1. This other metabolite may not have been separated from prostaglandin A_1 by TLC in the A VII solvent system. Table 21, page 123, shows that the Rf values of prostaglandin A_1, and 15-oxo prostaglandin E_1 in this system are very similar. Therefore

some of the radioactivity in the less polar material, produced by incubating 5,6 ^{3}H-prostaglandin E_1 with rat kidney slices, may have been accounted for by 15-oxo prostaglandin E_1. Prostaglandin E_1 is oxidised to 15-oxo prostaglandin E_1 by NAD^+ dependent 15-OH prostaglandin dehydrogenase (Änggård and Samuelsson, 1966), which is active in rat kidney (Nissen and Andersen, 1968), swine renal tissue (Änggård and Larsson, 1971) and in rabbit renal medulla (Larsson and Änggård, 1973). The co-enzyme was omitted from the incubation medium to reduce 15-OH prostaglandin dehydrogenase activity but levels of endogenous NAD^+ in kidney slices, in which many cells are intact, may have been high enough for appreciable enzymatic activity to occur. The atmosphere of 100% O_2 may also have enhanced the enzyme's activity. The metabolism of prostaglandin E_1 by kidney whole homogenates in an atmosphere of 100% O_2 and in air was subsequently compared.

Kidney homogenates

The percentage conversion of 5,6 ^{3}H-prostaglandin E_1 to less polar material by rat kidney homogenates incubated in 100% O_2 and in air, was the same. It was concluded that metabolism of prostaglandin E_1 by kidney homogenates did not require an atmosphere of 100% O_2. The metabolite formed after subsequent incubation of 5,6 ^{3}H-prostaglandin E_1 with kidney homogenates in air co-chromatographed with prostaglandin A_1 and 15-oxo prostaglandin E_1 on TLC in the A VII solvent system, and could be distinguished from 13,14-dihydro prostaglandin E_1 and 15-oxo prostaglandin E_1, but not prostaglandin A_1, by parallel bioassay on the rat fundus and kitten blood pressure preparations. However it could not be identified as prostaglandin A_1 from radio-gas chromatography and combined gas chromatography-mass spectrometry of its MeTMS derivative.

The kidney contains 15-OH prostaglandin dehydrogenase (Nissen and Andersen, 1968; Änggård and Larsson, 1971) and 15-oxo prostaglandin 13,14 reductase (Änggård and Larsson, 1971). These enzymes metabolise E prostaglandins to 15-oxo prostaglandin E, 15-oxo 13,14-dihydro prostaglandin E and 13,14-dihydro prostaglandin E (Änggård and Larsson, 1971). It has been shown (Änggård, 1966) that the potencies of 15-oxo prostaglandin E_1 and 15-oxo 13,14-dihydro prostaglandin E_1 on the guinea pig ileum, guinea pig uterus and rabbit duodenum are similar. Both compounds are very much less potent than prostaglandin E_1 on all 3 preparations. In Table 22, page 124, it is shown that likewise 15-oxo prostaglandin E_1 is very much less potent than prostaglandin E_1 on the rat fundus preparation. Therefore 15-oxo 13,14-dihydro prostaglandin E_1 might also be less potent than prostaglandin E_1 and have a similar potency to 15-oxo prostaglandin E_1 on this preparation. It is possible that the activity of the rat kidney homogenate metabolite may be accounted for by 15-oxo 13,14-dihydro prostaglandin E_1. It is more difficult to assess if the depressor activity of an intravenous dose of the metabolite might also be accounted for by 15-oxo 13,14-dihydro prostaglandin E_1. Figure 27, page 125, shows that the potencies of the metabolite, prostaglandin A_1 and 13,14-dihydro prostaglandin E_1 are similar. The ratio of equi-potent dose of intra-arterial 15-oxo 13,14-dihydro prostaglandin E_1 and prostaglandin A_1 on the dog blood pressure is 40 (Nakano, 1972), but intra-arterial 13,14-dihydro prostaglandin E_1 is only 1.5 times less potent than prostaglandin A_1. It is possible that in the kitten the biological activity of intravenously administered 15-oxo 13,14-dihydro prostaglandin E_1 is due to 13,14-dihydro prostaglandin E_1 formed in the pulmonary circulation by the action of 15-oxo prostaglandin 13,14 reductase. This enzyme has been detected in swine lung

(Änggård and Larsson, 1971). The 15-OH dehydrogenase also occurs in lung tissue (Änggård and Samuelsson, 1966; Änggård and Larsson, 1971), and it is thought to be responsible for the biological inactivation of prostaglandins of the E series observed in vivo (Ferreira and Vane, 1967; Horton and Jones, 1969), and in vitro (Piper et al., 1970). These two enzymes do not metabolise 13,14-dihydro prostaglandin E_1 (Änggård and Larsson, 1971). Therefore it is probable that metabolism of 13,14-dihydro prostaglandin E_1 in the pulmonary circulation does not occur. Intravenous and intra-arterial doses would be equiactive. Intra-arterial doses of 13,14-dihydro prostaglandin E_1 and prostaglandin A_1 are almost equiactive in the dog (Nakano, 1972). Intravenous doses of these compounds in the cat are almost equiactive (Figure 27, page 125). Prostaglandin A_1 is not inactivated in the pulmonary circulation (Horton and Jones, 1969). Therefore there is indirect evidence that intravenous and intra-arterial doses of 13,14-dihydro prostaglandin E_1 are equiactive and almost as potent as an intra-arterial or intravenous dose of prostaglandin A_1. Thus the depressor activity of an intravenous dose of the rat kidney homogenate metabolite shown in Figure 27, page 125, could be accounted for by 15-oxo 13,14 dihydro prostaglandin E_1, if this compound is metabolised in cat lungs to 13,14-dihydro prostaglandin E_1.

The rat kidney homogenate metabolite could be tentatively identified as prostaglandin A_1 from its behaviour on TLC in the A VII solvent system and its biological activity on the rat fundus and kitten blood pressure preparations. On this basis, there was evidence for a prostaglandin E dehydrase in rat kidney homogenates. However, there was an alternative possibility that the TLC behaviour and biological activity of the metabolite might be accounted for by 15-oxo 13,14-dihydro prostaglandin E_1. In addition, the metabolite could

not be identified as prostaglandin A_1 from radio-gas chromatography or combined gas chromatography-mass spectrometry of its MeTMS derivative. Therefore enzymatic conversion of prostaglandin E_1 to prostaglandin A_1 by rat kidney could not be detected. Consequently, evidence was not obtained for a prostaglandin E dehydrase in rat kidney.

The search for prostaglandin E dehydrase in male reproductive tract of the domestic fowl, Gallus domesticus

Prostaglandins extracted from seminal plasma and seminal fluid

Prostaglandins in the seminal plasma of the domestic fowl were assayed to check the identity and high levels of prostaglandins reported by Christ and van Dorp (1972). It had been found that cockerel semen contained prostaglandin E_1 and prostaglandin A and that the maximum amounts were 20 µg/ml each. However, the levels of F, E and A prostaglandins found in semen of the domestic fowl were much lower. The maximum amount of E prostaglandins found by bioassay was 1.94 µg/ml and of A prostaglandins 0.27 µg/ml. Spectroscopic assay of an extract of seminal plasma confirmed these low levels. Nevertheless, the finding that domestic fowl semen does contain biologically active material that co-chromatographs with F, E and A prostaglandins was evidence that male reproductive tract tissue might be capable of synthesising prostaglandins. Therefore it was possible that this tissue might contain a prostaglandin E dehydrase.

Biosynthesis of prostaglandins and metabolism of prostaglandin E_1 by male reproductive tract tissue

Prostaglandins released from tissues are newly synthesised and are not found in preformed stores (Shaw and Ramwell, 1966; Davies, Horton and Withrington, 1968; Piper and Vane, 1971). Far greater amounts are released from cat adrenal glands (Ramwell, Shaw, Douglas and Poisner, 1966) and from dog spleen (Davies et al., 1968; Gilmore, Vane and Wyllie, 1968) than can be extracted from the tissue. Prostaglandins can be formed during homogenisation and incubation of some tissue (Pace-Asciak et al., 1967). Thus incubation of regions of the male reproductive tract of the domestic fowl in phosphate buffer would show whether prostaglandin biosynthesis occurs.
In spite of the finding that the swollen region, ductus deferens and the testes were all able to synthesise biologically active material which co-chromatographs with F, E and A prostaglandins, conversion of prostaglandin E_1 to prostaglandin A_1 could not be demonstrated. Therefore evidence that prostaglandin A-like material biosynthesised by these regions of the reproductive tract might be formed by the action of a prostaglandin E dehydrase was not obtained.

The search for prostaglandin E dehydrase in human seminal vesicles

Biosynthesis of prostaglandins by human seminal vesicles

Only a small proportion of the 20 carbon fatty acids, namely all cis 8,11,14-eicosatrienoic acid (dihomo-γ-linolenic acid), all cis 5,8,11,14-eicosatetraenoic acid (arachidonic acid) and all cis 5,8,11,14,17-eicosapentaenoic acid which are precursors of

prostaglandins, are present in tissues as the free fatty acids (Pace-Asciak, 1967; Lands and Samuelsson, 1968; Vonkeman and van Dorp, 1968). Though large amounts are present as phospholipids, it is unlikely that the fatty acids in this form are substrate for prostaglandin synthetase. Very little prostaglandin synthesis occurs from ram vesicular gland prostaglandin synthetase incubated with 2-arachidonyl lecithin (Vonkeman and van Dorp, 1968) or with 1-palmitoyl-2-([2-^{14}C] eicosatrienoyl) glycero-3-phosphoryl choline (Lands and Samuelsson, 1968), unless lecithinase (Vonkeman and van Dorp, 1968) or endogenous acyl hydrolase in the 100,000 *g* supernatant fraction of the sheep vesicular gland preparation (Lands and Samuelsson, 1968) is present. Thus <u>in vitro</u> acyl hydrolases increase the yield of prostaglandin from bound fatty acid, hydrolysing phospholipid molecules into which they are incorporated and presenting the fatty acid in free form as substrates for prostaglandin synthetase. Release of free fatty acids other than prostaglandin precursors also occurs. Of these, oleic acid, linolenic acid and linoleic acid have been found to be competitive, irreversible inhibitors of ram vesicular gland and rat stomach prostaglandin synthetase (Pace-Asciak and Wolfe, 1968).

Very little prostaglandin biosynthesis from endogenous precursors was detected in homogenates of human seminal vesicles (Table 28, page 136). There are 3 possible reasons for this result. One is that human seminal vesicles contain a high proportion of unsaturated fatty acid inhibitors of prostaglandin synthetase. If these acids are released with prostaglandin precursor fatty acids by the action of acyl hydrolases, as just described, prostaglandin synthetase activity would be reduced. It is of interest that ram vesicular glands, in which high levels of prostaglandins are

synthesised from endogenous precursors (Bergström et al., 1962a; 1962b), contain low levels of linolenic acid and trace amounts of linoleic acid (Pace-Asciak and Wolfe, 1968). A second reason is that incubation conditions may not have been those in which prostaglandin synthesis is optimal. Increases in prostaglandin synthesis *in vitro* by rat renal medulla homogenates in response to vasopressin (Kalisker and Dyer, 1972) and by homogenates of rat stomach fundus after addition of noradrenaline, adrenaline and dopamine (Pace-Asciak, 1973) have been reported. Phospholipase A_2, 15-OH prostaglandin dehydrogenase and 15-oxo prostaglandin 13,14 reductase are absent from the rat fundus (Pace-Asciak, 1972). The increased prostaglandin formation is due to activation of prostaglandin synthetase and not to alterations in substrate and inhibitor concentrations produced from effects on phospholipase A_2 or due to changes in metabolism of prostaglandins. Human seminal vesicle prostaglandin synthetase may require the presence of hormones in the incubation medium for prostaglandin synthesis to be detected. A third reason is that human seminal prostaglandins may not originate in the seminal vesicles. Prostaglandin-like activity has been extracted from human prostate and human seminal vesicles (von Euler, 1936). Although Eliasson, using a split ejaculation technique, could correlate seminal prostaglandins with fructose (Eliasson, 1959), which is a marker for secretions of the seminal vesicles, other work correlated them only with acid phosphatase (Bygdeman and Eliasson, 1969), which is secreted by the prostate gland.

Since prostaglandin biosynthesis from endogenous precursors by human seminal vesicle homogenates could not be detected, the failure of the tissue to synthesise ^{3}H-prostaglandin E_2 and ^{3}H-prostaglandin A_2 from ^{3}H-arachidonic acid was not unexpected.

Metabolism of prostaglandin E_1 by human seminal vesicles

The possibility, however, was considered that prostaglandin A biosynthesis could not be detected because prostaglandin synthetase activity in human seminal vesicle homogenates was too low for formation of sufficient prostaglandin E to act as substrate for prostaglandin E dehydrase. However, even though homogenates were incubated with exogenous prostaglandin E_1, biosynthesis of prostaglandin A_1 could not be detected. Therefore evidence was not obtained for a prostaglandin E dehydrase in human seminal vesicles.

The search for prostaglandin E dehydrase in seminal vesicles of the baboon, Papio cyanocephalus

Prostaglandin extracted from seminal plasma of the baboon

The prostaglandin content of baboon semen was examined because vesiglandin had been described in the semen of "Macacus rhesus" by von Euler (1936). Vesiglandin has little smooth muscle stimulating activity so its depressor activity may be due to prostaglandins of the A, B or 19-OH series, which have little action on isolated smooth muscle preparations (Horton and Jones, 1969). In baboon semen, material which co-chromatographed with 19-OH prostaglandins and which was depressor was detected but prostaglandin A-like material was not. However, prostaglandin E-like material was also extracted from baboon semen which, unlike monkey semen, would therefore have smooth muscle stimulating activity. A species difference in the composition of semen from Papio cyanocephalus and Macacus rhesus may account for this finding. Alternatively, vesiglandin may comprise prostaglandin E

compounds which were unstable during storage and extraction of semen. Decrease in prostaglandin E assayed spectroscopically has been reported in human semen stored at room temperature for 1 week (Bygdeman et al., 1969). The β-ketol structure of the cyclopentane ring of prostaglandin E compounds is dehydrated at acid pH to form prostaglandin A (Bergström et al., 1963; Strong et al., 1966), and some conversion of prostaglandin E to prostaglandin A does occur during extraction and separation of prostaglandins (Daniels et al., 1965; Lee et al., 1967). Thus it is possible that the sample of monkey semen was treated in such a way that breakdown of prostaglandin E, perhaps to prostaglandin A, occurred. The consequent loss of smooth muscle stimulating activity would have taken place and this may have accounted for the inactivity of vesiglandin on non-vascular smooth muscle.

Biosynthesis of prostaglandins from endogenous precursors by baboon seminal vesicles

Tables 32 and 35, pages 143 and 156, show that small amounts of biologically active material which co-chromatographs with prostaglandins F and A were biosynthesised by baboon seminal vesicle homogenates. This material could not be more positively identified by combined gas chromatography-mass spectrometry. However, biosynthesis by the homogenates of prostaglandin E-like material in amounts ranging from 0.89 to 16.0 μg prostaglandin E_1/g tissue also occurred. This material was subjected to ultraviolet spectroscopy, which confirmed that it was a prostaglandin of the E series, to argentous thin layer chromatography, which indicated that it was prostaglandin E_2, and to combined gas chromatography-mass spectrometry, which identified it as prostaglandin E_2.

Biosynthesis of prostaglandins of the E and F series by sheep vesicular glands is well documented (Bergström et al., 1962a; 1962b; Samuelsson, 1963; Bergström et al., 1964a; 1964b; van Dorp et al., 1964a; 1964b; Christ and van Dorp, 1972). Not only have the prostaglandins synthesised been identified (Bergström et al., 1962a; 1962b) but some properties of sheep vesicular gland prostaglandin synthetase have been described (van Dorp, 1966; 1967; Ahern and Downing, 1970; Lands, Le Tellier, Rome and Vanderhoek, 1973). Bovine seminal vesicles also are able to biosynthesise prostaglandins from exogenous precursors (Kupieki, 1965; Yoshimoto, Ito and Tomita, 1970). In contrast, attempts to demonstrate prostaglandin biosynthesis by male reproductive tract tissue of some other mammalian species have been unsuccessful. occurrence could not be detected in the seminal vesicles of the rabbit, guinea pig, hamster, rat, mouse (Horton and Thompson, 1964) or of man (van Dorp, 1966). Although "vesiglandin", which has the depressor activity but not the smooth muscle stimulating activity of prostaglandin, has been extracted from the seminal vesicles of Macacus rhesus (von Euler, 1936), its active principle has not yet been identified. Therefore the extraction and conclusive identification of prostaglandin E_2 from baboon seminal vesicles is not only evidence that the baboon is one of the few species in which prostaglandin synthesis by the seminal vesicles has so far been detected, but is also the first reported identification of a prostaglandin from human primate tissue.

Even though baboon seminal vesicles can synthesise prostaglandin E_2 from endogenous arachidonic acid, they failed to form ^{3}H-prostaglandin E_2 from exogenous ^{3}H-arachidonic acid. There is evidence that ^{3}H-arachidonic acid is diluted by endogenous arachidonic acid released during homogenisation and incubation of tissue

(Pace-Asciak et al., 1967). During incubation of rat stomach homogenates, the level of arachidonic acid increases above the level found after homogenisation only. The specific activity of ^{3}H-prostaglandin E_2 produced from ^{3}H-arachidonic acid and the percentage of added label converted, fall as the incubation time is increased (Pace-Asciak et al., 1967). It seems that in rat stomach homogenates endogenous fatty acids are preferred to exogenous fatty acids as substrates for prostaglandin synthetase. The lack of ^{3}H-prostaglandin E_2 biosynthesis from ^{3}H-arachidonic acid by baboon seminal vesicles suggests that prostaglandin synthetase from this tissue also uses endogenous precursors in preference to exogenous ones.

Prostaglandin A_2 biosynthesis could not be detected from either endogenous or exogenous precursors. The ability of baboon seminal vesicle homogenates to synthesise prostaglandin E_2 showed that prostaglandin synthetase in this tissue was active. Thus lack of a substrate for prostaglandin E dehydrase, which might explain why human seminal vesicles are unable to synthesise prostaglandin A, may not be the reason why prostaglandin A biosynthesis from endogenous precursors did not occur in baboon seminal vesicle homogenates.

Metabolism of prostaglandin E by baboon seminal vesicles

Tritiated prostaglandins E_1 and E_2 were incubated with baboon seminal vesicle homogenates to investigate the possibility that this tissue might synthesise prostaglandin A from prostaglandin E, even though endogenous precursors and exogenous arachidonic acid were not converted to prostaglandin A. Metabolism of prostaglandin E was not however detected. Although this finding did not suggest that prostaglandin E dehydrase activity occurs in baboon seminal vesicles,

it does show that the amounts of prostaglandin E_2 extracted from this tissue are not underestimated by conversion of newly synthesised prostaglandin to biologically less active metabolites.

The search for prostaglandin E dehydrase in human plasma

Enzymatic conversion of prostaglandin E_1 to less polar material was not detected when prostaglandin E_1 was incubated with 4 samples of human plasma. The 5,6 ^{3}H-prostaglandin E_1 incubated was contaminated with less polar material. If the equilibrium of the enzymatic reaction by which this material is formed from prostaglandin A_1 did not favour the product of the reaction, the formation of a small amount of less polar material by low enzymatic activity in plasma might not take place. Thus the 4 samples of plasma used in this investigation may contain a prostaglandin E metabolising enzyme which is not very active. Studies on the distribution of the prostaglandin E metabolising enzyme reported in human plasma (McDonald-Gibson et al., 1972b) revealed that whereas some plasmas convert 80% of prostaglandin E_1 to less polar material, others have only a low ability to metabolise prostaglandin E_1. Nevertheless, incubation of 4 human plasma samples with prostaglandin E_1 in the same conditions described by McDonald-Gibson et al., (1972b) could not confirm their report of rapid prostaglandin E_1 metabolism by human plasma to a compound which co-chromatographs with prostaglandin A_1. Tentative evidence for an active prostaglandin E dehydrase in human plasma was therefore not obtained.

The evidence of Greaves et al. (1972) and McDonald-Gibson et al., (1972a; 1972b) is the only report that human plasma metabolises

prostaglandin E_1. Although a discrepancy in recoveries of radioactivity and biological activity of prostaglandin E_1 from dog plasma was reported by Holmes, Horton and Stewart (1968), there was also evidence from this study that the loss in biological activity could be accounted for by binding of prostaglandin E_1 to α_1 and α_2 globulins in plasma. All the prostaglandin E_1 added to dog plasma was bound to these proteins, and biological activity on the rat fundus preparation could be quantitatively recovered from them after starch gel electrophoresis. In addition, recovery of biological activity of prostaglandin E_1 could be improved from 10% to 50% by altering the extraction procedure. Likewise the results of Unger (1972) show that prostaglandin E_1 added to human plasma is bound to plasma proteins but revealed that binding was to the albumin fraction only. None occurred to the α_1 and α_2 globulins. It was also possible to improve the recovery of biological activity of prostaglandin E_1 on the rat fundus preparation by a modified extraction procedure. Proteins, to which prostaglandins are not bound, are precipitated by aqueous ethanol acidified with formic acid before the albumin-prostaglandin complex is dissociated with chloroform. Poor recovery of biological activity of prostaglandin E_1 from human plasma using the unmodified extraction procedure is due to loss of prostaglandin-albumin during precipitation of all the plasma proteins. Another report of binding of prostaglandins to human plasma proteins detects prostaglandin E_2 binding mostly to albumin but also to α_1 and α_2 globulins (Raz, 1972).

These findings, then, imply that the reason for loss of biological activity of prostaglandin E_1 added to human plasma and extracted after incubation, is incomplete recovery of prostaglandin E_1 bound to plasma albumin, α_1 and α_2 globulins and not metabolism to a biologically less active compound which is recovered quantitatively,

as suggested by Greaves et al. (1972). Results of Nakano support this conclusion. He was unable to detect metabolism of ^{3}H-prostaglandin E_1 by human plasma (Nakano, Montague and Darrow, 1971).

Prostaglandin A biosynthesis

Non-enzymatic origin of A prostaglandins

Material which co-chromatographed with prostaglandin A_1 on TLC and which was biologically active on the kitten blood pressure was extracted from male reproductive tract tissue of the domestic fowl, man and baboon. Since prostaglandins can be released from tissues during homogenisation (Pace-Asciak and Wolfe, 1968) as a result of de novo synthesis (Shaw and Ramwell, 1966; Davies et al., 1968; Piper and Vane, 1971), this finding could be evidence that these tissues are able to synthesise prostaglandins of the A series. On the other hand, it should be borne in mind that some of the prostaglandin A-like activity may have been accounted for by prostaglandin A formed non-enzymatically from prostaglandin E, which was also extracted from these tissues. It has already been mentioned (Introduction, page 6) that prostaglandins of the E series are dehydrated in acid conditions to prostaglandin A (Daniels et al., 1965; Strong et al., 1966; Nugteren et al., 1966a). These conditions are drastic: 90% acetic acid at 60°C (Daniels et al., 1965) and pH 1-2 at room temperature for 60 minutes (Strong et al., 1966). Andersen (1969) has studied dehydration of prostaglandin E_1 to prostaglandin A_1 in 4 different conditions, and finds that complete destruction of prostaglandin E_1 occurred in (a) N HCl, in (b) 80% aqueous H_3PO_4 in 100 ml acetone: acetic acid:water 10:4:1 buffer, both at room temperature for 60 hours

and also (c) in the same buffer at room temperature for 9 hours followed by 1 hour at 70°C. When prostaglandin E_1 was kept in buffer at room temperature for 9 hours (d), only 80% destruction took place. He also found that not only did alteration of treatment change the amount of prostaglandin E dehydrated but also the products formed. Treatments (a) and (d) produced only prostaglandin A_1 but additional unidentified products appeared after treatments (b) and (c). Acid conditions do occur during the extraction procedure for prostaglandins at any stage where they are partitioned from aqueous solvents into organic ones. If the organic phase is not washed free of acid, some dehydration of prostaglandin E_1 is possible when the organic solvents are evaporated in a partial vacuum at 40°C to 50°C. There is some evidence that the extraction procedure does dehydrate prostaglandin E_1 to prostaglandin A_1, perhaps due to these acid conditions. The extent to which prostaglandin E extracted from sheep seminal vesicles is dehydrated was altered by modifying the extraction procedure (Schneider et al., 1966; Strong et al., 1966). However, using a different extraction procedure, Lee et al., 1967, could not detect any dehydration of prostaglandin E_2 added to fresh renal medulla before extraction but did recover some prostaglandin A_2 after silicic acid chromatography. There is a difference in the proportion of prostaglandin A to prostaglandin E in tissue extracts prepared in different laboratories. Daniels et al. (1965) obtained a yield of prostaglandin A_1 from dihomo-γ-linolenic acid incubated with sheep seminal vesicles of 9.8% compared with 7.8% prostaglandin E_1. On the other hand, Schneider et al. (1966), using the same procedure on the same tissue, reported a yield of only 0.18% prostaglandin A_1 and 26.1% prostaglandin E . Daniels, Hinman, Leach and Muirhead (1967) obtained 13 mg prostaglandin A_2 and 103 mg prostaglandin E_2 after silicic acid

chromatography of an extract of rabbit renal medulla, but the amounts found by Crowshaw et al. (1970) in an extract of dog renal medulla, again after silicic acid chromatography, were 21 µg prostaglandin A_2 and 40.5 µg prostaglandin E_2. However, Hamberg (1969) could detect only traces of prostaglandin A_2 from rabbit renal medulla, once again after silicic acid column chromatography of the extract. His extraction procedure involved adjustment of pH to 3 only once. These findings would suggest that prostaglandin E is always susceptible to non-enzymatic dehydration during column chromatography and sometimes during some extraction procedures. Thus the amount of artefactual prostaglandin A inevitably varies according to the extraction and separation procedures used. Conclusions about its contribution to prostaglandin A extracted from the kidney cannot be drawn unless an attempt to measure it is made. Only one such attempt has been reported (Lee et al., 1967). It was concluded that most of the prostaglandin A_2 extracted from rabbit renal medulla was artefactual but conclusive evidence was not obtained against a small amount of enzymatically formed prostaglandin A_2. Artefactual and enzymatically formed A prostaglandins extracted from tissues could be distinguished by using a double labelling technique in which ^{14}C-prostaglandin E and ^{3}H-prostaglandin A were added at the start of the extraction and separation procedure. Any radioactivity due to ^{14}C, which co-chromatographed with ^{3}H-prostaglandin A, would have derived from prostaglandin E_2 and would therefore measure the amount of artefactual prostaglandin A.

Prostaglandin E dehydrase in rat kidney

Although there is no conclusive evidence that prostaglandins of the A series are biosynthesised in the kidney, there is also no evidence as yet to suggest that they are not. If a prostaglandin E dehydrase

has been found in rat kidney, the presence of the enzyme would have been evidence in favour of enzymatic formation of A prostaglandins by the kidney.

The presence of prostaglandin B-type compounds amongst rat urinary metabolites of prostaglandin E_2 (Gréen, 1971) suggested that, in the rat, prostaglandin E may be enzymatically dehydrated to prostaglandin A which could then be isomerised to prostaglandin B. A prostaglandin A isomerase was detected in rat plasma (Section I, page 50) but metabolism of prostaglandins of the E series by rat plasma has not been detected (Nakano and Prancan, 1971). Therefore a prostaglandin E dehydrase may be absent from rat plasma but may occur in some other tissue, for instance the kidney.

Metabolism of prostaglandin E_1 by rat kidney did occur. Although evidence from thin layer chromatography and biological activities on the rat fundus and kitten blood pressure preparations was compatible with the metabolite being prostaglandin A_1, the metabolite could not be identified as prostaglandin A_1 on the basis of radio-gas chromatography or combined gas chromatography-mass spectrometry. Thus evidence that a prostaglandin E dehydrase, which may be involved in the metabolism of prostaglandin E to prostaglandin B in the rat, occurs in the kidney was not obtained. Since this part of a pathway by which A prostaglandins may be biosynthesised could not be demonstrated by the rat kidney, evidence was not obtained for non-artefactual A prostaglandins in this tissue.

Non-artefactual A_1, B and 19-OH prostaglandins in human semen

On the other hand, three findings already mentioned (see Section II, Introduction, page95) have been used as evidence that A, B and 19-OH prostaglandins in human semen are enzymatically formed.

However, it is possible that some of these prostaglandins could have been formed from the corresponding E prostaglandins, not during extraction but during storage of the semen samples between collection and extraction of prostaglandins. A decrease in prostaglandin E assayed spectroscopically in human semen stored for 1 week at room temperature has been described (Bygdeman et al., 1969). Recently, the levels of prostaglandins in human semen, which was not stored at room temperature at all but at about 4°C for no longer than 1 hour, have been reported (Horton, Jones and Marr, 1973). Not only were the levels of 19-OH prostaglandins lower than the E prostaglandins, where previously they had been found in amounts in excess of or equal to the E prostaglandins (Bygdeman et al., 1969), but the levels of A or B prostaglandins were only in the order of 1 or 2 μg/ml, although the concentration of prostaglandin E was 50 μg/ml in one subject and 150 μg/ml in another. This finding also contrasts with that of Bygdeman et al. (1969) who found that the total amount of A and B prostaglandins were in the order of 25% of those of total E prostaglandins.

Thus a convincing demonstration of prostaglandin A biosynthesis in human seminal vesicles, which may be the source of seminal prostaglandins (Eliasson, 1959), is required to establish that human seminal A prostaglandins are indeed non-artefactual.

Prostaglandin A biosynthesis in male reproductive tract tissue of the domestic fowl, in human and baboon seminal vesicles

Only a small amount of biologically active prostaglandin A-like material could be detected in human seminal vesicles, baboon seminal vesicles and the male reproductive tract of the domestic fowl. Larger amounts of prostaglandin E-like material were obtained from all 3 tissues.

Non-enzymatic dehydration of this material may have accounted for some of the prostaglandin A-like material obtained, as discussed previously (see page 180). An internal standard had not been used to check the extent of non-enzymatic dehydration of endogenous prostaglandin E during extraction. However material less polar than prostaglandin E_1, recovered from extracts of ^{3}H-prostaglandin E_1 incubated with phosphate buffer, was 2% of the total radioactivity recovered, whereas the same ^{3}H-prostaglandin E_1 extracted after incubation with an ethanolic homogenate in which enzymatic activity could not have been present, was 12%. Therefore enzymatic formation of prostaglandin A would be indicated only if the amount of prostaglandin A detected after this extraction procedure was greater than about 10% of the combined activities of prostaglandins E and A. This level of prostaglandin A-like material was never obtained from homogenates of reproductive tract of the domestic fowl or from baboon seminal vesicle homogenates. Therefore, whilst it was not possible to state that these tissues do synthesise small amounts of prostaglandin A, the possibility cannot be excluded that all this material was formed non-enzymatically from prostaglandin E.

There were 2 occasions when large amounts of prostaglandin A-like material relative to prostaglandin E-like material were extracted from human seminal vesicles (see Table 28, page 136). This material was not further characterised. Though firm evidence that it was prostaglandin A was not obtained, its formulation may be tentative support for biosynthesis of A prostaglandins by human seminal vesicles.

Thus the male reproductive tract of the domestic fowl, human and baboon seminal vesicles may all synthesise small amounts of prostaglandin A, which could be distinguished from artefactual prostaglandin A by using a double labelling technique. A sensitive method, such as quantitative gas chromatography-mass spectrometry, is required to detect these small amounts of enzymatically formed prostaglandin A.

The future search for prostaglandin E dehydrase

Results presented in this thesis do suggest 2 approaches for a future search for prostaglandin E dehydrase. One is to improve the biosynthesis of prostaglandins by human seminal vesicle homogenates. Synthesis of a little prostaglandin E-like material and perhaps some prostaglandin A-like material was obtained by human seminal vesicle homogenates. Some reasons for the lack of enzymatic activity of these specimens have been given (see page 168). One of these was that the specimens were obtained at autopsy. In future, specimens removed immediately after death should be used, if at all possible, to eliminate this potential source of enzymatic inactivity. Another reason was that human seminal vesicle prostaglandin synthetase may require an activator. Rat stomach prostaglandin synthetase is stimulated by noradrenaline (Pace-Asciak, 1973) which can replace hydroquinone as a co-factor for bovine seminal vesicle prostaglandin synthetase (Takeguchi, Kohno and Sih, 1971). A third possible reason for enzymatic inactivity was the liberation from phospholipids of fatty acids, other than prostaglandin precursor fatty acids, which might inhibit prostaglandin synthetase (Pace-Asciak and Wolfe, 1968). This might be tested by estimating the fatty acid composition qualitatively and quantitatively of a total lipid extract of human seminal vesicles by gas chromatography. If such fatty acids are present relative to prostaglandin precursor fatty acids in large amounts, it might be advantageous to separate them from microsomal prostaglandin synthetase by preparing an acetone powder of the enzyme (Wallach, 1965).

An alternative approach in the future search for a prostaglandin E dehydrase would be to investigate biosynthesis of prostaglandins by primate seminal vesicles. One sample of baboon semen was

found to contain material which co-chromatographs with 19-OH prostaglandins. Uncorrected for recovery, it was present in about 10 fold excess of prostaglandin E-like material. The presence of 19-OH prostaglandins should be confirmed in other samples of baboon semen and the material positively identified by combined gas chromatography-mass spectrometry. The capacity of baboon seminal vesicles to synthesise 19-OH prostaglandins should then be examined. In work presented in this thesis, 19-OH prostaglandin-like material extracted from baboon seminal vesicles was not separated from prostaglandin F-like material. Therefore some of the biological activity which chromatographed with ^{3}H-prostaglandin $F_{2\alpha}$ may have been accounted for by 19-OH prostaglandins, even though they have little activity on the rat fundus preparation (Horton and Jones, 1969). Positive identification of 19-OH prostaglandins in baboon semen and baboon seminal vesicles would strengthen the inference from tentative evidence already obtained that, even though baboon semen contains 19-OH prostaglandins which can be synthesised by baboon seminal vesicles, prostaglandin E dehydrase activity cannot be demonstrated in this tissue. However, the identification of prostaglandin E_2 from this source is evidence that prostaglandin synthetase is detectable in baboon seminal vesicles, unlike human seminal vesicles. Thus prostaglandin synthetase may also be active in seminal vesicles of other primate species, including Macacus rhesus from which "vesiglandin" has been obtained (von Euler, 1936). "Vesiglandin" in fresh semen from this species should be identified. If this substance does indeed comprise A, B and 19-OH prostaglandins, which are not non-enzymatically formed during storage of the semen sample, synthesis of these prostaglandins may be detectable in the seminal vesicles of Macacus rhesus. If so, this tissue would be suitable to test the hypothesis that one route by which A prostaglandins are biosynthesised is by enzymatic dehydration of prostaglandins of the E series.

Non-mammalian prostaglandin A biosynthesis

It has not yet been established that renal A prostaglandins are not artefactual, and evidence for a renal prostaglandin E dehydrase to support this statement was not obtained from work presented in this thesis. In addition, evidence that human seminal A, B and 19-OH prostaglandins are enzymatically formed is circumspectual. A prostaglandin E dehydrase could not be detected in homogenates of male reproductive tract tissue from 3 species, to provide additional evidence for the enzymatic origin of these prostaglandins.

However, recently it has been established that the gorgonian Plexaura homomalla contains prostaglandin A_2 and its acetoxy methyl ester greatly in excess of prostaglandin E_2, the A prostaglandins comprising 1.8% of the net dry weight of the organism (Weinheimer and Spraggins, 1969). This finding alone is evidence that prostaglandin A_2 in P. homomalla is unlikely to be an artefact. Moreover, a microsomal preparation of an enzyme, which synthesises prostaglandin A_1 and prostaglandin A_2 from dihomo-γ-linolenic acid and ^{3}H-arachidonic acid, has been obtained from the (S) variety of P. homomalla (Corey, Washburn and Chen, 1973) in which the prostaglandins are in the (S) configuration (Schneider, Hamilton and Rhuland, 1972) of mammalian prostaglandins (Nugteren, van Dorp, Bergström, Hamberg and Samuelsson, 1966b). This is the first conclusive demonstration of prostaglandin A biosynthesis. Nevertheless, prostaglandin A synthetase did not show prostaglandin E dehydrase activity, neither could formation of E prostaglandins be detected when the microsomal preparation was incubated with dihomo-γ-linolenic acid or arachidonic acid. Unlike ovine and bovine prostaglandin synthetase, prostaglandin A synthetase from P. homomalla does not use glutathione as a co-factor. It has

been shown that during prostaglandin E and prostaglandin F biosynthesis from fatty acids via two precursors, namely 15-hydroperoxy prostaglandin R and prostaglandin R, glutathione may be required in the conversion of 15-hydroperoxy prostaglandin R to prostaglandin R, and is an obligatory co-factor in the enzymatic isomerisation of prostaglandin R to prostaglandin E (Nugteren and Hazelhof, 1973). On the basis of these findings, it is possible that prostaglandin A synthetase from P. homomalla does not convert arachidonic acid to prostaglandin E_2 and subsequently to prostaglandin A_2. Instead the enzyme may form prostaglandin A_2 from 15-hydroperoxy prostaglandin R_2 or from prostaglandin R_2. Incubation of these precursors with prostaglandin A synthetase might establish that such a pathway exists, or might suggest that prostaglandins E_2 and A_2 in P.homomalla are biosynthesised from arachidonic acid by two independent routes.

It remains to be seen whether enzymatic dehydration of prostaglandin E, conversion of prostaglandin E precursors or synthesis directly from unsaturated fatty acids is the route by which mammalian A prostaglandins are biosynthesised. Evidence supporting one of these pathways would also favour the notion that these prostaglandins are not artefacts but may have some physiological role.

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SOURCES OF CHEMICALS AND DRUGS

Ammonium sulphate, Analar grade	British Drug Houses Limited
Bovine serum albumin	Sigma Chemical Company
BSTFA	Sigma Chemical Company
Calcium chloride, Analar grade	British Drug Houses Limited
Citric acid, Analar grade	British Drug Houses Limited
DEAE Sephadex	Pharmacia Fine Chemicals A.B.
D-glucose, Analar grade	British Drug Houses Limited
Diazald	Aldridge Chemical Co.
Dimethyl POPOP	Koch-Light Laboratories Limited
Di-sodium hydrogen orthophosphate, Analar grade	British Drug Houses Limited
Hydrochloric acid, Microanalytical grade	British Drug Houses Limited
Magnesium chloride, Analar grade	British Drug Houses Limited
Methoxylamine HCl	Deeside Phase Separations Limited
Phosphomolybdic acid, Analar grade	British Drug Houses Limited
Potassium chloride, Analar grade	British Drug Houses Limited
Potassium hydroxide, Analar grade	British Drug Houses Limited
PPO	Koch-Light Laboratories Limited
Sephadex G-25, G-50, G-200	Pharmacia Fine Chemicals A.B.
Silica	E. Merck A.G.
Silver nitrate, Analar grade	British Drug Houses Limited
Sodium bicarbonate, Analar grade	British Drug Houses Limited
Sodium carbonate	British Drug Houses Limited
Sodium chloride	British Drug Houses Limited
TLC plates, grooved	May and Baker Limited
TLC plates, prepared	E. Merck A.G.
2,4-tri-methylpentane, Analar grade	British Drug Houses Limited

Continued

Tris	British Drug Houses Limited Koch-Light Laboratories Limited
Tri-sodium citrate	British Drug Houses Limited

5,6 ^{3}H-prostaglandins were purchased from New England Nuclear.

Prostaglandins $F_{2\alpha}$, E_1, E_2, A_1 and B_1 were gifts from Dr. J.E. Pike of the Upjohn Company, Kalamazoo, Michigan, U.S.A.

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