STUDIES ON SEMINAL PROSTAGLANDINS

by

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MRC Unit of Reproductive Biology,

To Pat Stevenson, who stuck up for me without letting on...

Contents.

Summary.

The lipid fraction of human semen has been separated by LH 20 Sephadex chromatography and TLC and the resulting fractions analysed by GCMS. Evidence is presented for the existence in semen of several prostaglandins, including 19-OH PG $F_{1\alpha}$, 19-OH PG $F_{2\alpha}$, 8-iso 19-OH PG E_1, 8-iso 19-OH PG E_2, 8-iso PG $F_{1\alpha}$, 8-iso PG $F_{2\alpha}$, 8-iso PG E_1 and 8-iso PG E_2. None of these compounds have previously been described in semen, and the first five have not been described from any source. Evidence is also presented for the existence of a group of prostaglandins isomeric with the dinor prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$.

A method for the preparation of crystalline 19-OH PG E_1 from macaque semen is described. Evidence is presented for the existence of a pair of dihydroxy E prostaglandins, tentatively identified as 18,19 dihydroxy PG E_1 and an isomeric compound in the semen of the stump-tailed macaque.

The effects of 19-OH PG E_1 on non-pregnant and pregnant human isolated myometrial strips have been studied. 19-OH PG E_1, like PG E_1, was found to relax non-pregnant strips, the equipotent molar ratio being E_1:19-OH E_1 = 1:4. 19-OH PG E_1 was also found to relax pregnant strips, unlike PG E_1, and a significant effect was demonstrated at 50 ng/ml.

Examination of homogenates of the seminal vesicle of the stump tailed macaque by GCMS showed them to contain large amounts of 19-OH E prostaglandins, and these levels increased on incubation, an effect which was inhibited by

indomethacin. Incubation of homogenates with radioactively-labelled eicosa-8,11,14-trienoic acid, arachidonic acid, PG E_1, PG E_2, PG $F_{2\alpha}$ or PG $F_{3\alpha}$ produced no significant incorporation of radioactivity into the 19-hydroxy prostaglandin fractions. It is concluded that the biosynthesis of 19-OH PGs takes place via a separate pathway from that leading to the classical prostaglandins.

Some observations on dynamic aspects of seminal prostaglandin production in man are presented, along with the results of a study on the species distribution of the 19-hydroxy E prostaglandins.

Acknowlegements.

A large number of people have contributed directly or indirectly to this work, and to them I owe a debt of gratitude.

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Division of labour.

The work described here was performed as part of a team, and inevitably I have been obliged to include some results obtained by other workers in this thesis. Wherever possible, the contribution of my co-workers has been acknowleged in the body of the text. My own contribution to this work was as follows:

Chapters 1 and 2 are entirely my work, with the exception of the GCMS measurement of prostaglandin D_2, which was performed by Dr. R.W. Kelly, and the measurements quoted in table 1.1 which are attributed by references in the table to their authors.

Chapter 3 describes a project carried out in collaboration with Dr. J.A. Russell of the Department of Physiology, Edinburgh University, as required by the CNAA regulations. Dr. Russell provided the laboratory space, the apparatus and much useful advice and encouragement. I performed the experiments and the presentation of the results is my own.

The experimental animals used in chapter 4 were extremely valuable, and economics dictated that their organs be shared out among several workers. I did not personally perform the dissection, although I was present. The remainder of the work described in this chapter is my own, with the exception of the GCMS measurement of the E prostaglandins (Table 4.5) which was performed by Dr. R.W. Kelly.

Chapter 5 is entirely my work.

The semen samples analysed in chapter 6 were provided

by many different workers, all of whom are listed under "acknowlegements". Concentrations of 19-hydroxy prostaglandins were once more measured by Dr. Kelly, and my part in this work was restricted to the identification of the prostaglandins by GCMS.

Publications in the course of this work.

Taylor P.L. and R.W. Kelly (1975).
"The occurrence of 19-hydroxy F prostaglandins in human semen."
FEBS letters 57, 22.

Kelly R.W. and P.L. Taylor (1976)a
"tert-Butyl dimethyl silyl ethers as derivatives for qualitative analysis of steroids and prostaglandins by gas phase methods."
Analytical chemistry 48, 465.

Kelly R.W. and P.L. Taylor (1976)b
"19-hydroxy prostaglandins in primate semen."
Advances in prostaglandin and thromboxane research 2, 903

Kelly R.W. and P.L. Taylor (1976)c
"Gas chromatography-mass spectrometry of steroids and prostaglandins as t-butyl dimethylsilyl ethers."
Advances in mass spectrometry in biochemistry and medicine, A. Frigerio and N. Castagnoli eds. Spectrum, New York. Vol 1, p. 449.

Kelly R.W., P.L. Taylor, J.P. Hearn, R.V. Short, D.E. Martin and J.H. Marston (1976)
"19-hydroxy prostaglandin E_1 as a major component of the semen of primates."
Nature 260, 544.

Taylor P.L. (1979)
"The 8-isoprostaglandins: evidence for eight compounds in human semen."
Prostaglandins (in press).

Russell J.A., P.L. Taylor and R.W. Kelly (1979)
"Preliminary observations on the effects of 19-hydroxy prostaglandin E_1 on non-pregnant human myometrium"
Journal of reproduction and fertility (in press).

Taylor P.L. and R.W. Kelly (1979)
"Preparation of crystalline 19-hydroxy prostaglandin E_1 from macaque semen."
Preparative biochemistry (submitted for publication).

Introduction.

The prostaglandins are a group of biologically active, hydroxylated fatty acids, originally detected in human semen and subsequently shown to be functionally involved in the operation of most mammalian organs. It is now nearly fifty years since the first observations of the spasmogenic effects of human semen were made, and twenty years since the first prostaglandins extracted from biological sources were crystallised, and in that time the ubiquitous occurrence and wide ranging biological activity of the compounds have led to an exponential growth of publications by workers in the field. Despite this, the role of the seminal prostaglandins in reproduction remains speculative, and this thesis describes a series of studies designed to contribute to an understanding of their function.

Throughout this thesis the individual prostaglandins are referred to by their trivial names, using the semi-systematic nomenclature which has evolved among workers in the field and which was described and codified by Nelson (1974). The systematic names and structural formulae of all the prostaglandins discussed are listed in table 1.1 (page 125). Other abbreviations used are listed on page 215.

Historical background.

Early work.

In the early years of this century, several workers demonstrated autopharmacological effects using extracts of the male reproductive tract. Japelli and Scafa (1906) found that saline extracts of dog prostate, when injected into rabbits produced a depression of the blood pressure and retarded clotting of blood samples taken shortly thereafter. When administered to dogs the same extracts produced a rise in blood pressure, dyspnoea, cardiac arhythmia and arrest. Similar work by Legeu and Gaillardat (1912) using extracts of dog, horse and hypertrophied human prostate showed that the human prostate yielded a much more active and toxic extract than those of the other animals. That this effect was not due to the pathological state of the glands was shown by Battez and Boulet (1913) who made an extract from the prostate of an executed criminal immediately after death, and showed that it produced depression of the blood pressure and contraction of the bladder when injected into decerebrate or curarised dogs. Von Euler (1937) later ascribed some of these effects to adrenaline, which he had demonstrated to be present in the prostate (Von Euler, 1934a), but some may well be due to prostaglandins.

The first observation of an effect which may definitely be attributed to prostaglandins was made by Kurzrok and Lieb (1930), who noted that semen placed in the lumen of

the uterus during artificial insemination could sometimes produce strong uterine contractions. Later, Cockrill et al (1935)demonstrated effects of human semen on isolated human myometrial strips. The majority of semen samples were shown to produce relaxation of the strips, but in some cases contraction resulted.

Meanwhile, Goldblatt (1933, 1935) and Von Euler (1934b) had independantly demonstrated that semen and extracts of male accessory sex glands contained a substance which reduced blood pressure and stimulated contraction of smooth muscles. Von Euler initially suggested that the active principle was basic, but by the following year (Von Euler, 1935a) had identified the substance as a lipid soluble acid and named it 'prostaglandin'. At the same time he demonstrated the presence of a similar substance which he named 'vesiglandin' in the vesicular gland of the rhesus monkey (Von Euler, 1935b). Vesiglandin was shown, like prostaglandin, to have a powerful depressor action but to have little or no effect on isolated smooth muscle preparations, and was also shown to be inactivated more rapidly by extremes of pH (Von Euler, 1937).

In the sheep, the seminal vesicle was the only source from which prostaglandin could be demonstrated when extracts of a number of different organs were assayed for their effect on blood pressure in rabbits and on the isolated rabbit jejunum. It was concluded therefore that prostaglandin must be specifically involved in reproduction since it was

not found other than in the male reproductive tract (Von Euler and Hammarström 1937). Von Euler (1938) and later Bergström (1949) concluded that prostaglandin was a hydroxylated, unsaturated fatty acid on the basis of the chemical and physical properties of preparations purified by precipitation of the barium salt.

Structure identification.

In the late 1950s Bergström separated prostaglandin into two fractions on the basis of their polarity, and named these PG E and PG F, since one could be extracted into ether while the other remained behind in the phosphate buffer (fösfat in Swedish) (Hamberg, 1973). Prostaglandins E and F were obtained in crystalline form by purification of extracts of sheep prostate (Bergström and Sjövall, 1960a, b), and were also shown to be present in ram semen (Bergström et al, 1960), and later in human semen (Bergström and Samuelsson, 1962; Samuelsson, 1963b, c).

Two further prostaglandins were then isolated from the sheep prostate, differing from PG E only in degree of unsaturation. These compounds were named PG E_2 and PG E_3, and the former PG E was renamed PG E_1, the subscript numerals referring to the number of unsaturated groups which the compounds possessed (Bergström et al, 1962a).

The empirical formulae for PG E_1 and PG F_1, $C_{20}H_{34}O_5$ and $C_{20}H_{36}O_5$ respectively were obtained, and it was demontrated that the former could be converted to the latter by borohydride reduction, yielding a pair of compounds now known

as PG $F_{1\alpha}$ and PG $F_{1\beta}$ (Bergström et al, 1962b, c). The naturally occurring PG F compounds belong to the F_{α} series. The structures of the natural prostaglandins were further elucidated by chemical degradation (Bergström et al, 1963a, b; Samuelsson 1963a). The absolute configuration of the C_{11} hydroxyl was not correctly established until 1966 when Nugteren et al (1966a) demonstrated that prostaglandin synthetase enzymes will also convert eicosa-11,14-dienoic acid to 11S-hydroxy 12-trans 14-cis eicosadienoic acid, and determined the configuration of the 11 hydroxyl of this compound by comparison of its optical rotation with that of similar monohydroxylated fatty acids of known configuration. These authors proposed the (now conventional) representation of the prostaglandin molecule with the ring to the left and the use of the Cahn-Ingold-Prelog convention for the notation of the asymmetrical centres in the side chains.

In 1962 the first prostaglandin was identified from a source other than the reproductive system when Bergström et al (1962d) purified and crystallised PG E_2 from 880 lb of swine lung, and two years later PG $F_{3\alpha}$, a prostaglandin which has not been detected in semen, was identified in bovine lung (Samuelsson, 1964). The prostaglandins have since been shown to be almost ubiquitous in mammalian tissues (Karim et al, 1967, 1968a), although levels are generally two to three orders of magnitude lower than in human or sheep semen, and sophisticated techniques are required for their detection and measurement.

The E prostaglandins may be readily dehydrated by exposure to extremes of pH, yielding under acid conditions a compound originally known as PG E_{217} and now called PG A, and under basic conditions PG E_{278} (modern name PG B). The subscripts 217 and 278 refer to the ultraviolet absorption maxima shown by these compounds. A systematic search for compounds having an absorption maximum at 278 nm before or after base treatment produced evidence for eight further prostaglandins in human semen (Hamberg and Samuelsson, 1965, 1966a,b). Extracts of human semen were chromatographed on silicic acid columns along with tritiated PG $F_{3\alpha}$ and aliquots of the fractions obtained were treated with base and their absorption at 278 nm measured. The resulting chromatogram (Hamberg and Samuelsson, 1966a,b) shows four peaks, and further analysis by mass spectrometry, NMR, IR and chemical degradation showed that these contained in order of increasing polarity : I - PGs A_1, A_2, B_1 and B_2; II - PGs E_1, E_2 and E_3; III - PGs 19-hydroxy A_1, 19-hydroxy A_2, 19-hydroxy B_1 and 19-hydroxy B_2. The fourth peak, containing compounds more polar than the 19-hydroxy PGs A and B was not identified.

In order to guard against the possibility that the dehydrated prostaglandins had been formed during storage or extraction the experiment was repeated using semen to which tritiated PG E_1 had been added. It was shown that only 14% of the radioactivity was incorporated into the dehydrated prostaglandins while the unlabelled material

in peak I amounted to 90% of that in peak II. The authors concluded that the dehydrated prostaglandins were of biological origin, and asserted that the 19-hydroxy compounds could not have arisen by dehydration of 19-hydroxy PG Es since these were absent from semen, although they presented no evidence to support this. Initially, the level of the 19-hydroxy compounds was given as about four times that of the E prostaglandins, but later work did not support this, and Bygdeman et al (1970) give a figure roughly equal to that of the E prostaglandins.

The steric configuration of the 19-hydroxyl group was later established by Hamberg (1968) who acetylated 19-hydroxy PG B_1 with tritiated acetic anhydride and degraded the product oxidatively to yield 3-acetoxy butanoic acid. This was then mixed with unlabelled dl 3-acetoxy butanoic acid and the morphine salt of the mixture resolved. The tritium label was found to be incorporated into the D enantiomer, indicating that the original compound had the 19R configuration.

Further indirect support for the conclusion that 19-hydroxy A and B prostaglandins did not arise by dehydration of the analagous 19-hydroxy E compounds was provided by Israelsson et al (1969) who reported that guineapig liver microsomes could hydroxylate PG A_1 at the 19 and 20 positions but not PG E_1 or 13,14 dihydro 15-keto PG E_1. A very recent paper by Kupfer et al (1978) has reported that under slightly different conditions the guineapig liver microsome preparation

can hydroxylate the E prostaglandins, yielding mainly the 19-hydroxy isomer. These authors analysed the products of the reaction by base treatment followed by high pressure liquid chromatography of the resulting 19-hydroxy PG B compounds and therefore did not establish whether the added PG E had dehydrated prior to hydroxylation. It is doubtful whether this work has much relevance to the biochemistry of semen, since liver microsomes can hydroxylate a wide range of compounds in connection with the process of detoxification. Guinea pig semen has not been shown to contain 19-hydroxy prostaglandins and there is no evidence to suggest that 19-hydroxy prostaglandins synthesised in the liver are transported to the seminal vesicles.

Two new prostaglandins were identified in 1968 as a result of work with the prostaglandin synthetase of seminal vesicles. Incubation of seminal vesicle preparations with eicosa-8,11,14-trienoic acid gave rise, in addition to PG E_1 and PG $F_{1\alpha}$, to 8-iso PG E_1 (Daniels *et al*, 1968) and PG D_1 (Granstrom *et al*, 1968). During the course of the work described in this thesis semen was investigated for these and similar compounds and the results are described in chapter 1.

In 1974 we reported the presence of high levels of 19-hydroxy E prostaglandins in human semen (Taylor and Kelly, 1974). The compounds were detected and tentatively identified by GCMS, using an aqueous oximation technique for the first stage of derivatisation which stabilises the

β-ketol system in the ring of the E prostaglandins and eliminates the possibility of subsequent dehydration. The structure of the compounds was confirmed by chemical degradation and identification of the products by comparison of their chromatographic and mass spectral properties with those of authentic compounds. These results were independantly confirmed by Jonsson et al (1975, 1976) who also demonstrated that levels of 19-hydroxy A and A prostaglandins increase in semen samples during incubation, approximately two thirds of the 19-hydroxy PG Es and half of the PG Es being dehydrated after three hours at 37°C.

It would seem probable therefore, that despite the precautions taken by Hamberg and Samuelsson, the high levels of 19-hydroxy A and 19-hydroxy B compounds which they reported were the result of dehydration of 19-hydroxy E prostaglandins. The levels of the 19-hydroxy E prostaglandins which have been reported both in the three papers cited above and in more recent work (Templeton et al, 1978) compare well with levels of 19-hydroxy A and B prostaglandins originally reported by Hamberg and Samuelsson. The Swedish workers used ether extraction at an early stage of their preparation, and the very polar 19-hydroxy E prostaglandins extract only poorly into this solvent. Any dehydrated 19-hydroxy prostaglandins present at this stage would therefore have been concentrated relative to their parent compounds. The subsequent use of silicic acid chromatography introduces an additional risk, as Jouvenaz et al (1970) have pointed out that prolonged

exposure to silicic acid can dehydrate E prostaglandins, and the 19-hydroxy E prostaglandins appear to be rather less stable than the PG Es.

In 1975 we demonstrated the presence of four new compounds in human semen, two of which were identified as 19-hydroxy prostaglandin $F_{1\alpha}$ and 19-hydroxy prostaglandin $F_{2\alpha}$ (Taylor and Kelly, 1975). The remaining compounds were tentatively identified as the 8-epimers of the 19-hydroxy PG Fs. (Evidence for the structure of these compounds forms part of this submission and is described in chapter 1.)

Several other prostaglandins and related compounds have been identified in sources other than semen, and the relevance of these compounds to the problems of semen biochemistry is not known at present; they will be described briefly for the sake of completeness.

Prostaglandin C_1 has been demonstrated as a product of the action of the prostaglandin isomerase enzyme of cat plasma on PG A_1 (Jones, 1972). PG C_1 is unstable in even slightly alkaline conditions, isomerising to PG B_1. Since fresh human semen contains little if any PG A or PG B the presence of C prostaglandins seems unlikely.

Prostaglandins G and H are intermediates in the biosynthesis of the classical prostaglandins, and are also themselves highly active in biological systems. They were first isolated by Hamberg and Samuelsson (1973) and Hamberg et al (1974) as a product of the incubation of sheep seminal vesicle microsomes with arachidonic acid. They will be

discussed in more detail later, as intermediates in the biosynthesis of the seminal prostaglandins. It seems improbable that PG G and PG H occur in semen, since addition of stannous chloride (which converts PG G or PG H to PG F) to fresh semen immediately after ejaculation produced no significant change in the ratio of PG E to PG F (R.W. Kelly, personal communication).

Hamberg et al (1975) have shown that PG G and PG H can be converted by exposure to human platelets into a new group of compounds containing a six-membered oxane ring. The authors named these compounds thromboxanes, and showed that they were extremely active in the aggregation of platelets. The primary product, thromboxane A is extremely unstable, with a half life of about thirty seconds in aqueous solution and decays to thromboxane B which is stable but biologically inactive. Thromboxanes have not been shown to exist in seminal plasma, nor to be produced by the seminal vesicle.

Prostaglandin I_2 (PG X or prostacyclin) has been shown to be produced from PG G_2 or PG H_2 by artery wall microsomes (Gryglewski et al, 1976; Moncada et al, 1976). PG I_2 is a potent inhibitor of platelet aggregation, and is again unstable in aqueous solution, decaying to 6-keto PG $F_{1\alpha}$ (Johnson et al, 1976). The latter compound could not be detected in semen (R.W. Kelly, personal communication) but two groups have reported its detection as a product of the incubation of arachidonic acid with sheep (Cottee et al, 1977) or bull (Chang and Murota, 1977) seminal vesicle

microsomes. Homogenates of seminal vesicle must inevitably include some arterial microsomes since the seminal vesicle is a vascular organ, and these last results may well be due to this.

Origin and biosynthesis.

The origin of the seminal prostaglandins in man was located by Eliasson (1959) using a split ejaculate technique. The various accessory sex glands which contribute to an ejaculate do not discharge their contents simultaneously, and it may be shown that an ejaculate consists of three main fractions. The first fraction consists mainly of prostatic secretion, as evidenced by its high level of acid phosphatase which is secreted solely by the prostate. The second fraction contains the secretions of the epididymes and ampullae and therefore carries the bulk of the spermatozoa. The final fraction consists largely of the secretion of the seminal vesicles, and contains most of the fructose, which is secreted only by the seminal vesicles. The content of prostaglandin in the various fractions of split ejaculates was measured, along with acid phosphatase, sperm count and fructose, and it was shown that prostaglandin activity was positively correlated with fructose concentration, but not with the other markers, indicating that the seminal vesicles were the source of prostaglandin.

Eliasson also examined sheep seminal vesicles, and showed that the amount of prostaglandin which could be

extracted from their homogenates was increased if the homogenate was incubated at 37°C before extraction, and that this process was speeded up if phospholipase A was added to the medium. He concluded that the prostaglandin was stored in an esterified form and that the increase in levels on incubation represented release of prostaglandin from this inactive phospholipid (Eliasson, 1959). More recent work has shown that in other tissues prostaglandins are not stored, but synthesised rapidly in response to demand (Davies et al, 1968; Piper and Vane, 1971) and that the increase in prostaglandin levels observed by Eliasson was probably due to a release of precursor fatty acids which are themselves stored as phospholipid (Kunze and Vogt, 1971).

The first clear demonstration of in vitro biosynthesis of prostaglandins came in 1964 when two groups simultaneously announced their demonstration of synthesis of PG E_2 from arachidonic acid using homogenates of sheep seminal vesicle (van Dorp et al, 1964a; Bergström et al, 1964a). Shortly thereafter the syntheses of PG E_1 from all cis eicosa-8,11,14-trienoic acid (van Dorp et al, 1964b) and of PG E_3 from all cis eicosa-5,8,11,14,17-pentaenoic acid (Bergström et al, 1964b) were announced. Van Dorp et al (1964b) also showed that the prostaglandin synthetase activity of the ram seminal vesicle was located in the microsomal fraction after cell fractionation. Wallach (1965) showed that the prostaglandin synthetase enzymes could be preserved for

storage as an acetone powder, and much of the work on prostaglandin synthesis has since been carried out using acetone powders of seminal vesicle microsomes. The method of preparation is convenient and ensures that endogenous prostaglandins, precursors and cofactors are removed from the enzyme preparation.

That the prostaglandin synthetase was not confined to the seminal vesicles was first demonstrated by Änggård and Samuelsson (1965) who showed that homogenates of guinea pig lung could synthesise PG E_2 and PG $F_{2\alpha}$ from arachidonic acid.

Owing to the difficulty of obtaining fresh human seminal vesicles for experimental purposes, the direct demonstration of prostaglandin synthetase activity in this organ was not made until 1976 when it was shown that incubation of human seminal vesicle homogenate with eicosa-8,11,14-trienoic acid yielded PG E_1 (Hamberg, 1976).

The mechanism of prostaglandin biosynthesis has been the subject of much attention, and has mainly been studied using preparations of sheep or bull seminal vesicle microsomes. Nugteren and van Dorp (1965) and Ryhage and Samuelsson (1965) showed that both the hydroxyl groups of the E prostaglandins were derived from molecular oxygen by performing incubations in the presence of $^{18}O_2$, isolating the products and recording their mass spectra. These experiments gave no information concerning the origin of the 9 carbonyl oxygen since this readily exchanges with water. In the same year Samuelsson,

in a most elegant experiment, showed that not only was the oxygen at C_9 derived from molecular oxygen, but that both the oxygen atoms of the prostaglandin ring were derived from the same oxygen molecule (Samuelsson, 1965). Eicosa--8,11,14-trienoic acid was incubated with the prostaglandin synthetase enzyme in an atmosphere consisting of a mixture of $^{18}O_2$ and $^{16}O_2$. The resulting PG E_1 was reduced to PG F_1 with sodium borohydride to prevent exchange of the 9 oxygen. PG $F_{1\beta}$ was isolated and oxidised to give a C_{13} dicarboxylic acid containing the two oxygens of the ring and the diethyl ester of this compound was subjected to mass spectrometry. The mass spectrum showed that the derivative contained either two ^{16}O atoms or two ^{18}O atoms but not one of each isotope. The experiment was later applied by Foss et al (1971) to PG $F_{1\alpha}$ who showed that the oxygen atoms of the ring of this compound were likewise derived from the same molecule of oxygen.

Synthesis of PG E_1 from eicosa-8,11,14-trienoic acid labelled with ^{14}C at C_3 and ^{3}H at C_8, C_{11} or C_{12} gave a product with unchanged $^{3}H/\ ^{14}C$ ratio, indicating that the reaction did not involve exchange of the hydrogens at C_8, C_{11} or C_{14} (Samuelsson, 1967). Incubation of (13D-^{3}H, 3-^{14}C) and (13L-^{3}H, 3-^{14}C) eicosa-8,11,14-trienoic acid followed by isolation of the resulting PG E_1 and PG $F_{1\alpha}$ showed that the hydrogen removed from C_{13} has the L configuration. 15-hydroperoxy eicosatrienoic acid and 15-hydroxy eicosatrienoic acid were shown not to be substrates

for prostaglandin biosynthesis (Hamberg and Samuelsson, 1967a). These observations led the authors to propose a mechanism involving the initial formation of 11-peroxy eicosatrienoic acid which would then cyclise to the 9,11 endoperoxide. As additional evidence Samuelsson stated that eicosa-11,14-dienoic acid could be converted by the prostaglandin synthetase enzyme into 11-hydroxy-12,14-eicosadienoic acid in high yield (Samuelsson, 1967).

Hamberg and Samuelsson (1967b) showed that incubation of eicosa-8,11,14-trienoic acid with sheep seminal vesicle prostaglandin synthetase preparations produced in addition to the prostaglandins: 11-hydroxy eicosa-8,12,14-trienoic acid, 15-hydroxy eicosa-8,11,13-trienoic acid, 12-hydroxy heptadeca-8,10-dienoic acid and malondialdehyde. It was shown that the last named compound was derived from C_{9-11} and it was suggested that it arose by cleavage of the endoperoxide intermediate at $C_{8/9}$ and $C_{11/12}$, giving rise also to the C_{17} hydroxy acid.

Wlodawer and Samuelsson (1973) used a novel technique to demonstrate that PG $F_{2\alpha}$, PG E_2, PG D_2 and 12-hydroxy heptadecatrienoic acid (HHT) are all formed from the same pool of endoperoxide precursor in the prostaglandin synthetase reaction. They incubated a mixture of unlabelled and 5,6,8,9,11,12,14,15 octadeutero arachidonic acid with PG synthetase in the presence of glutathione and hydroquinone and showed that the D / H ratio for the products PG $F_{2\alpha}$ and HHT increased

with time, whereas that for PG E_2 and PG D_2 did not. Owing to an isotope effect, the formation of PG E_2 and PG D_2 takes place preferentially from the unlabelled endoperoxide, leaving the remaining pool of endoperoxide progressively enriched with deuterium. Since formation of PG $F_{2\alpha}$ and HHT does not involve D-C bond cleavage, the D / H ratio of these compounds reflects that of the pool of endoperoxide precursor at the time of synthesis. Curiously enough, this effect was abolished when adrenaline was used as cofactor in place of glutathione and hydroquinone.

The existence of the postulated endoperoxide precursor was finally proved when two such compounds were isolated from incubations of sheep seminal vesicle microsomes with arachidonic acid (Hamberg and Samuelsson, 1973; Hamberg et al, 1974). The compounds were synthesised during a short incubation in the presence of p-mercuribenzoate (which inhibits the PG E isomerase enzyme which converts the endoperoxide compounds to PG E). The products were separated by silicic acid chromatography, and were shown to act as precursors for the prostaglandins. The two compounds were shown to have the postulated 9,11 endoperoxy structure but to differ in the substituent at C_{15}, one having a 15-hydroperoxy group was named PG G_2, and the other with a 15-hydroxy structure PG H_2. The two compounds were shown to be biologically active, PG G_2 being 50-200 times as active as PG E_2, and PG H_2 100-400 times as active on the superfused

aorta strip. The compounds were unstable in aqueous solution with a half life of about five minutes.

The cofactor requirements of the prostaglandin synthetase system have been extensively studied since Nugteren et al (1966b,c) showed that the biosynthesis of PG E required reduced glutathione, and that antioxidants such as hydroquinone or propylgallate also stimulated the reaction. The quantity of glutathione oxidised was calculated as 2.4 moles per mole of PG E synthesised. Samuelsson (1967) showed that boiled supernatant, boiled rat liver supernatant, reduced glutathione, tetrahydrofolic acid and 6,7 dimethyltetrahydropteridine all stimulated the biosynthesis of PG E by microsomal preparations. NADH, NADPH and ascorbic acid (all at 0.1 mM) were ineffective.

Yoshimoto et al (1970) showed that ascorbate at 10 mM stimulated the reaction and that the microsomes could be rendered inactive by freezing and thawing. Preparations inactivated in this way could be restored to full activity by the addition of haemoglobin, myoglobin, haemin or supernatant. Reduced glutathione was shown to stimulate only if present at the beginning of the reaction and was ineffective if added after four minutes of an eight minute incubation. Omission of reduced glutathione from the reaction mixture drastically reduced the yield of PG E but did not affect the consumption of oxygen, from which it was concluded that the initial oxidation of fatty acid did not require glutathione while the transformation of the endoperoxide intermediate

into PG E did, and that if the glutathione was absent at the beginning of the reaction the endoperoxide was irreversibly transformed into other products and was not subsequently available for the production of PG E. Omission of haem or hydroquinone from the reaction mixture reduced both oxygen consumption and PG E synthesis, showing that these cofactors were involved in the initial, oxidative steps of the reaction.

A wide range of aromatic compounds have been shown to be effective in place of hydroquinone in stimulating the oxidative phase of the prostaglandin synthetase reaction. Sih et al (1970) demonstrated that catecholamines and serotonin were effective, and observed that seminal vesicle homogenates contain high levels of tryptophan, the compound which is now believed to be the natural cofactor for the reaction (Chan et al, 1975).

A major advance came when Vane (1971) demonstrated that non-steroidal anti-inflammatory drugs such as aspirin and indomethacin are potent inhibitors of the prostaglandin synthetase reaction, providing both a physiological explanation for the well known anti-inflammatory, antipyretic and analgesic effects of aspirin and evidence for the involvement of prostaglandin synthesis in inflammation, fever and the perception of pain. These drugs have been shown to act on the oxidative phase of the reaction, and therefore inhibit the formation of the endoperoxides, together with all their

products (Miyamoto, 1976).

Studies of the specificity of the prostaglandin synthetase system towards various polyunsaturated fatty acids have shown that although some acids of differing chain length can act as precursors for prostaglandin-like compounds (Struijk et al, 1966; Van Dorp, 1971), some naturally-occurring acids inhibit the reaction, and it has been pointed out that the rate of the reaction in vivo may depend on the mixture of acids available to the enzyme (Lands et al, 1972).

The production of PG F from the endoperoxide involves a reduction, unlike the production of PG E, and has been shown to be stimulated by Cu^{++} and dithiols such as dithiothreitol and dihydrolipoamide (Lands et al, 1971; Lee et al, 1971). The presence of copper and dithiols stimulates PG F production more than it inhibits PG E production, showing that the effect is due to an increase in the rate of formation of PG F rather than an inhibition of the PG E isomerase system. Chan et al (1975), using a partially purified enzyme system which contained no PG E isomerase activity (as evidenced by the lack of action of reduced glutathione) showed that the pattern of products produced in the absence of the Cu^{++}/ dithiol complex was identical with that produced by the decay of PG H in buffer, and concluded that the production of of PG F was probably therefore non-enzymic. Wlodawer et al (1971) showed that a bicycloheptene analogue of PG H specifically inhibits the PG E isomerase enzyme, and

incubations in the presence of this compound produce low levels of PG E with slightly raised levels of PG F and hydroxy acids.

Several groups have now succeeded in making the enzyme complex soluble, and studies have been published on the separated enzymes (Miyamoto et al, 1974; Chan et al, 1975; Rome and Lands, 1975; van der Ouderaa et al, 1977). Miyamoto (1976) separated the enzymes of bull seminal vesicle microsomes into two fractions and demonstrated that fraction I contained the cyclo-oxygenase activity, and although apparently homogeneous by electrophoresis, catalysed two separate reactions. In the presence of haematin, eicosatrienoic acid was converted to PG G_1, and in the presence of tryptophan and haem PG G_1 was converted to PG H_1. The products of incubations with fraction I were unaffected by the presence of reduced glutathione. Indomethacin and aspirin were shown to block the synthesis of PG G_1, but to have no effect on the conversion of PG G_1 to PG H_1, or on the PG E isomerase which was present in fraction II.

Hemler and Lands (1976) and Lands (1977) have purified the cyclo-oxygenase and shown that it has a molecular weight of 70,000 by SDS electrophoresis but 300,000 by direct measurement, indicating a tetrameric structure. The enzyme was shown to bind labelled aspirin, and in the active form to contain two haem groups and two atoms of non-haem iron per molecule.

The biosynthesis of the 19-hydroxy prostaglandins has so far received little attention. As mentioned previously, two groups have demonstrated 19- and 20-hydroxylation of prostaglandins by guinea pig liver microsomes (Israelsson et al, 1969; Kupfer et al, 1978) but the relevance of these observations to the 19-hydroxy prostaglandins of human semen remains to be established. 19-hydroxy E prostaglandins have been prepared using biosynthetic methods by Sih et al (1969) who demonstrated that the fungus Ophiobolus gramminis could hydroxylate arachidonic acid at the 18 or 19 positions, and incubation of these hydroxy acids with bovine seminal vesicle microsomes gave 18-hydroxy and 19-hydroxy PG E_2, although in low yield. Oxidation of the hydroxyl to a ketone followed by incubation with the microsomal preparation gave 18-oxo and 19-oxo PG E_2 in high yield.

The biosynthesis of seminal prostaglandins in vivo has not yet been examined in detail. Eliasson (1959) measured levels of prostaglandin in ejaculates taken at 24 hour intervals, and showed that these did not differ significantly from those in ejaculates preceded by a longer period of abstinence, concluding that the prostaglandin produced in one ejaculate can be replaced within 24 hours.

The administration of drugs can affect seminal prostaglandin levels, and two groups (Collier and Flower, 1971; Horton et al, 1973) have shown that large doses of aspirin can depress seminal prostaglandin levels in men. Collier

and Flower measured E and F prostaglandins and showed that levels of both groups of compounds were significantly lowered in subjects taking 2.4 g aspirin per day. Levels of E prostaglandins tended to rise after an initial drop while those of the PG Fs remained low for the duration of the aspirin treatment. Horton et al measured 19-hydroxy A and 19-hydroxy B prostaglandins in addition to PG E and PG F, and explored the effect of higher doses of aspirin (up to 7.2 g/day). These authors found all the prostaglandin levels to be depressed by up to 80% at the highest dose rate. The lowering of prostaglandin levels was unaccompanied by changes in seminal volume or fructose levels, and the subjects experienced no difficulty in ejaculating even when receiving the maximum dose. The effect of anti-inflammatory drugs on male fertility has not yet been investigated.

The treatment of hypogonadal men with gonadotrophins (Sturde and Bohm, 1971) or with androgen (Sturde, 1971b; Skakkebaek et al, 1976) has been shown to raise seminal prostaglandin levels. In the case of the latter study the administration of testosterone produced a more pronounced rise in the levels of 19-hydroxy E prostaglandins than in those of the PG Es. The effect followed rapidly upon administration of the drug, and it was later suggested that testosterone may exert a direct effect on prostaglandin synthesis rather than by stimulating proliferation of the tissue responsible for the secretion (Kelly, 1978). Sturde

(1971a) has shown however, that the anti-androgen cyproterone stimulates a steep rise in seminal prostaglandin levels when administered at 200 - 400 mg daily. The effect occurred only after about six weeks of treatment, and the author attributed this result to an inhibition of the feedback effect of testosterone on pituitary gonadotrophin release, leading to increased testicular androgen production.

Levels of prostaglandins in human semen.

The measurement of levels of prostaglandins in human semen is a problem fraught with difficulty. The complex mixture of compounds poses problems of specificity if the levels of individual compounds are to be estimated, and the labile nature of the PG E and 19-hydroxy PG E compounds can lead to erroneous results, especially when measurements are made on frozen semen (Brummer and Gillespie, 1972; Clarke et al, 1974). Other techniques liable to introduce this type of artifact include the use of high temperatures in the extraction procedure (as in Eliasson, 1959) and the use of silicic acid chromatography (many workers, including most work published by Bygdeman et al between 1964 and 1970).

The earliest workers in the field measured the effect of semen or crude extracts thereof on smooth muscle preparations and expressed their results in terms of a standard preparation of 'prostaglandin' or as micrograms of PG E_1 equivalent per millilitre of semen (Asplund, 1947a; Eliasson,

1959; Hawkins and Labrum, 1961; Bergstrom and Samuelsson, 1962; Horton and Thompson, 1964). These studies indicated a figure of around 100 µg PG E_1 equivalent / ml of semen.

Later methods included thin layer chromatography, often preceded by group separation on silicic acid columns and followed by either bioassay, measurement of ultraviolet absorption after base treatment (PG E) or GLC (PG F) (Bygdeman and Samuelsson, 1964, 1966; Bygdeman et al, 1969, 1970, 1973; Bygdeman, 1969; Srivastava and Clausen, 1973; Brummer and Gillespie, 1972; Collier et al, 1975). One group (Clarke et al, 1974) used radioimmunoassay, using an antiserum which did not discriminate between the E and F prostaglandins. Measured levels during this period average about 50 µg / ml for the E prostaglandins and 7 µg / ml for the PG Fs. (For a detailed review of this work see Cenedella, 1975).

Two modern papers give what are currently the most reliable estimates of the prostaglandin content of normal semen. Perry and Desiderio (1977) measured E and 19-hydroxy E prostaglandins in human semen by GCMS using deuterated PG E_2 as an internal standard, and Templeton et al (1978) measured PG Es, PG Fs and their 19-hydroxylated equivalents in the semen of men whose wives had recently given birth, using GLC for the E and 19-hydroxy E prostaglandins and GCMS for the F and 19-hydroxy F prostaglandins. The results of these two studies are given in table 0.1.

Table 0.1

Seminal prostaglandin levels in fertile men.

n.	PG Es	19-OH Es	PG Fs	19-OH Fs	
10	142±102	527±375	-	-	(Perry and Desiderio, 1977)
22	73±72	267±240			(Templeton et al, 1978)
17			2.1±1.8		"
16				18±14	"

(all figures are μg/ml ± σ)

The high levels of E prostaglandins reported in these two studies are not unexpected, since the instability of these compounds must have depressed most previous estimates. The wide range of levels encountered (Templeton et al quote 2 - 272 μg/ml for the PG Es for example) is surprising, since if the seminal prostaglandins have a biological function, the mechanisms involved must be extremely uncritical to operate over such a large dynamic range.

Seminal prostaglandins and fertility.

Several studies have compared the levels of prostaglandins in the semen of fertile and infertile men, and suggested that levels are lower in the infertile group (Hawkins and Labrum, 1961; Bygdeman and Samuelsson, 1966; Hawkins, 1968; Bygdeman, 1969; Bygdeman et al, 1970, 1973; Collier et al, 1975; Perry and Desiderio, 1977). Brummer and Gillespie (1972) could show no significant difference

in levels between the normal and infertile groups, but claimed that the normal group showed a significantly wider scatter. Clarke et al (1974) compared their measured levels in semen from men with suspected infertility with 'normal' levels as defined by Bygdeman (1973) and showed that eleven out of thirty had lower levels. Sturde (1968), however showed that azoospermic men had significantly higher prostaglandin levels as measured by bioassay.

Much of this work is rather unsatisfactory, the statistical analyses used to demonstrate the significance of the observed differences being for the most part perfunctory, and generally based on Student's T test. As long ago as 1961 Hawkins and Labrum pointed out that the statistical distribution of measured seminal prostaglandin levels is extremely skewed, making the T test invalid. These authors corrected for this by taking logarithms of the measured levels and testing these transformed results for significance. The transformed results showed no significant difference between the fertile and infertile groups as originally defined. Much of the work cited above was performed on a number of samples too small to draw conclusions about the nature of the statistical distribution of the results.

Early work on the levels of prostaglandins in human semen tends to show a much narrower spread of results than is revealed by modern methods. The most rigorous investigation of the relationship between seminal prostaglandin levels and fertility was that of Bygdeman et al (1970). These

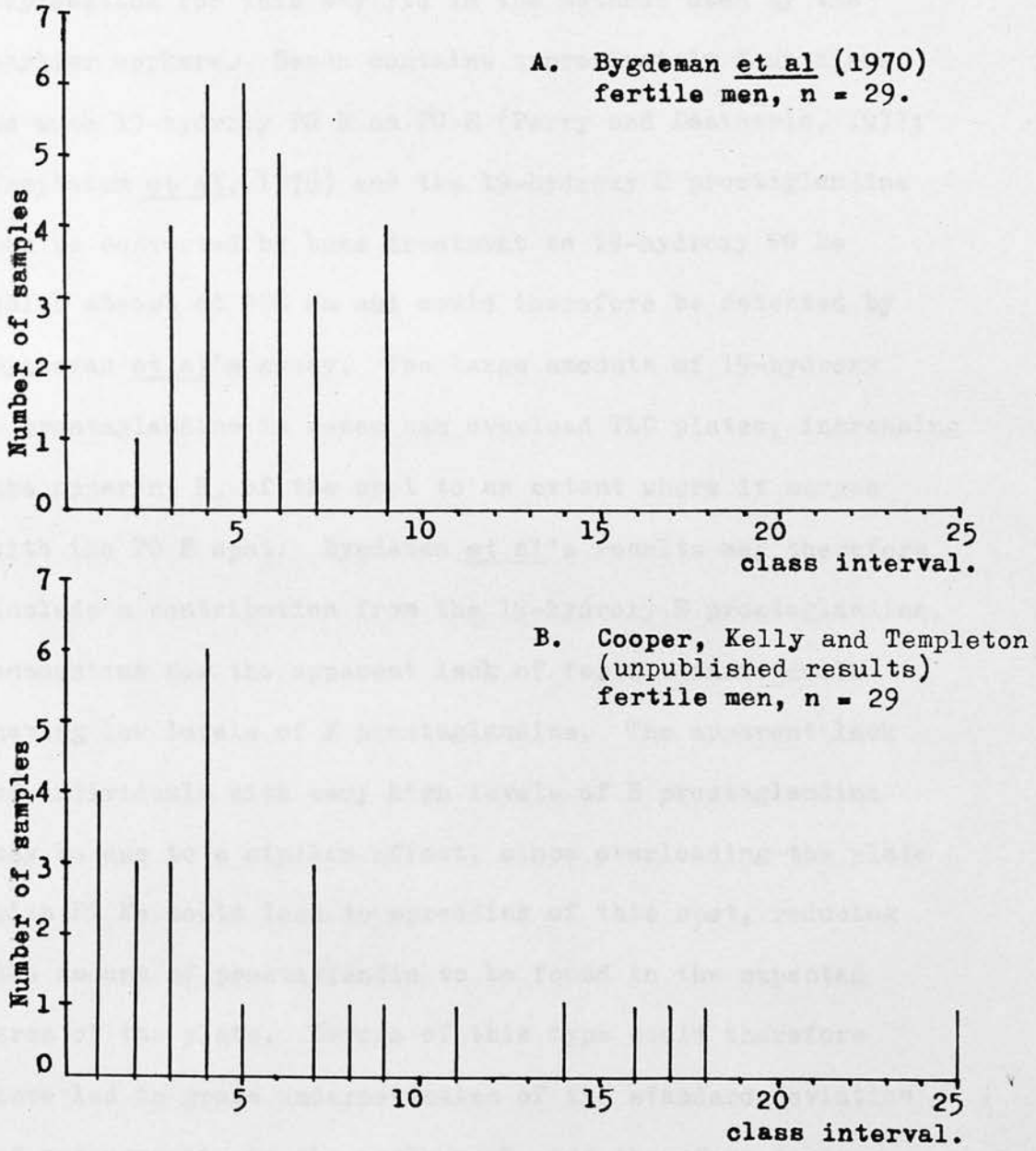
workers measured levels of PG Es, PG A+Bs and 19-hydroxy PG A+Bs in semen samples from three groups. Group A included only men of recently proven fertility (n=29), group B men whose marriages were infertile for reasons which had not been investigated (n=100) and group C men whose marriage remained infertile for unknown reasons after thorough investigation (n=17). The incidence of levels of PG Es below 11 μg/ml (two standard deviations below the mean for the fertile group) in the three groups was 0%, 17% and 41% respectively, a difference which was shown to be highly significant ($P < 0.001$) in the case of groups A and C, and significant ($P < 0.01$) in the case of groups A and B. There were no significant differences in the levels of A and B prostaglandins or 19-hydroxy A and B prostaglandins. Statistical significances were demonstrated by non-parametric tests since the frequency distribution observed in groups B and C appeared anormal. Templeton et al (1978) have suggested that these results may be explained by differing storage times or conditions for the three groups of samples, since it was not known in 1970 that PG E levels decline in frozen semen. However, there is no evidence of a concomitant rise in the measured levels of dehydrated prostaglandins as would be expected if this were the case.

Fig. 0.1 shows the frequency distribution obtained in group A of this study (redrawn after Bygdeman et al, 1970) together with a frequency distribution of levels

of PG Es in the semen of fertile men measured by modern methods (I. Cooper, R.W. Kelly and A. Templeton, unpublished).

Fig. 0.1

Frequency distribution of levels of E prostaglandins in semen of fertile men.



(Class intervals are multiples of 11 μg PG E / ml.)

Comparing the two sets of results in fig. 0.1, it is evident that the spread of measurements is much greater in the modern work. Although the number of measurements is the same in both cases, the modern work shows six cases of levels above 100 μg/ml and four below 11 μg/ml, while the early results all lie between these extremes. The explanation for this may lie in the methods used by the earlier workers. Semen contains approximately four times as much 19-hydroxy PG E as PG E (Perry and Desiderio, 1977; Templeton et al, 1978) and the 19-hydroxy E prostaglandins can be converted by base treatment to 19-hydroxy PG Bs which absorb at 278 nm and would therefore be detected by Bygdeman et al's assay. The large amounts of 19-hydroxy E prostaglandins in semen can overload TLC plates, increasing the apparent R_f of the spot to an extent where it merges with the PG E spot. Bygdeman et al's results may therefore include a contribution from the 19-hydroxy E prostaglandins, accounting for the apparent lack of fertile individuals having low levels of E prostaglandins. The apparent lack of individuals with very high levels of E prostaglandins may be due to a similar effect, since overloading the plate with PG Es could lead to spreading of this spot, reducing the amount of prostaglandin to be found in the expected area of the plate. Errors of this type could therefore have led to gross underestimates of the standard deviation of measurements in the early work, and therefore to over-estimates of the significance of observed differences between

the fertile and infertile groups.

Estimates of the significance of differences in seminal prostaglandin levels between fertile and infertile men using modern methods of assay would require very much larger numbers of measurements (Templeton et al, 1978) and until the results of such work become available the connection between seminal prostaglandin levels and fertility must be considered unproven.

Species distribution.

Von Euler (1934b, 1937) showed prostaglandin to be present in extracts of male accessory sex glands of man and sheep, but could not demonstrate activity in extracts of those of the rabbit, dog, horse, bull or pig. He also demonstrated the presence of vesiglandin, a related substance, in extracts of rhesus monkey seminal vesicles (Von Euler, 1935b). Eliasson (1959) found prostaglandin activity in the semen of sheep and goats but not in horse, bull or pig semen. Extracts of male accessory sex glands showed activity when prepared from the seminal vesicles, ampullae and prostate of the sheep, but not from the organs of other animals, including bull, pig, dog, cat, rabbit and guinea pig.

The distribution of prostaglandins outside the reproductive tract was studied by Karim et al (1967, 1968) who

were able to demonstrate the presence of (or at least the capacity to synthesise) prostaglandins in a wide range of mammalian organs. Prostaglandins have also been detected in non-mammals, perhaps the most striking case being that of the sea whip, Plexaura homomalla, in which Weinheimer and Spraggins (1969) demonstrated the presence of A prostaglandins at a concentration of 1.5% dry weight.

Eliasson (1959) and later Poyser (1974) both suggested that the presence of prostaglandins in the semen may be related to the type of insemination practised by that species; whether intravaginal (man, sheep, cow, rabbit) or intrauterine (horse, pig, dog, rat). Measurements of the seminal prostaglandin levels in various species could not support this hypothesis. Poyser (1974) found that levels of E prostaglandins were less than 5 ng/ml in pig and dog semen, while horse semen contained 24 ng/ml and rabbit 71 ng/ml. Ventura and Freund (1973) found PG E_1 at 1 ng/ml and PG $F_{1\alpha}$ at 0.5 ng/ml in rat semen, but found that rat semen also contains another uterine stimulant which was not extractable into ethyl acetate and was non-dialysable. Voglmayr (1973) measured the level of PG $F_{2\alpha}$ in the semen of dairy bulls and found a level of 0.17 ng/ml, a quantity comparable to that found in venous blood. Estimation of prostaglandin levels in male accessory sex glands of the rat have led to contradictory results. Jouvenaz et al (1970) measured PG E_2 in the prostate and seminal vesicles as 0.25 and

0.16 μg/g respectively. De Cuminsky and Mercuri (1971) measured PG E_1 in the seminal vesicle as 0.5 μg/g. Tan and Privett (1972, 1973), however, claimed that the content of E prostaglandins in the seminal vesicle was as high as 500 μg/g. Bartke and Koener (1974) showed that fluid from the vasa deferentia of rats and mice contained high levels of F prostaglandins.

19-hydroxy prostaglandins were first detected in human semen by Hamberg and Samuelsson (1965, 1966a, b) as 19-hydroxy PG A and 19-hydroxy PG B, but the authors could not detect these compounds in ram semen. Taylor and Kelly (1974) demonstrated the presence of 19-hydroxy E prostaglandins in human semen, and they were later shown to be present in the semen of other primates, including the chimpanzee, gorilla, orang-utan, rhesus monkey and stump-tailed macaque. 19-hydroxy E prostaglandins were not detected in the semen of the horse, bull, sheep, pig, rabbit or chicken (Kelly et al, 1976 and this thesis). In primate species other than man, levels of 19-hydroxy PG E_1 far exceeded those of other prostaglandins detected, and it was suggested that this compound is identical with the vesiglandin extracted by Von Euler from rhesus monkey seminal vesicles. 19-hydroxy F prostaglandins have been demonstrated in the semen and in prostate gland extracts from the brush-tailed possum, Trichosurus vulpecula (Marley et al, 1977).

In summary, high levels of seminal prostaglandins

have been demonstrated only in primates, sheep and goat. Levels of seminal prostaglandins in other species are typically one to ten thousand times lower, and as such may well result from the secretions of organs other than the seminal vesicle. 19-hydroxy prostaglandins have so far been detected only in the semen of primates and one marsupial.

The biological role of the seminal prostaglandins.

The earliest hypothesis on the role of the seminal prostaglandins *in vivo* was that of Goldblatt (1935), who suggested that they might promote vasodilation in the male reproductive organs in the period immediately preceding ejaculation, a proposition which is difficult to test owing to the inaccessibility of most of the male reproductive system.

Several workers have claimed that prostaglandins (Okazaki *et al*, 1978; Webb *et al*, 1974) and human seminal plasma (Stites and Erickson, 1975) possess immunosuppressive properties on the basis of their ability to inhibit phytohaemagglutinin-induced DNA synthesis in cultured lymphocytes. The intravaginal route is adequate for the production of immunity *in vivo*, and Stites and Erickson suggest that the function of the seminal prostaglandins may be to prevent the development of anti-sperm antibodies in the female. The same authors also suggest that the immunosuppressive ability of seminal plasma may be responsible for the poor

immunological response in the male to genital infection by cytomegalovirus or to first infection by Neisseria gonorrhea. A very wide spectrum of compounds has now been shown to inhibit phytohaemagglutinin stimulation of lymphocyte DNA replication, and it is doubtful whether *in vitro* tests of this type are a real guide to the immunosuppressive abilities of compounds *in vivo*. Further work is therefore required before the relevance of this interesting observation can be properly assessed.

The three most probable explanations for the presence of the seminal prostaglandins, however, remain those discussed by Eliasson (1959).

"1. Prostaglandin may act as a chemical stimulator inducing the emptying of the accessory genital glands.

2. Prostaglandin may be of importance for the motility (or other function) of the spermatozoa.

3. Prostaglandin may facilitate the migration of the spermatozoa in the female genital tract"

In order to assess the likelihood of these three explanations, the biological action of the seminal prostaglandins on the male tract, on spermatozoa and on the female tract will now be considered.

The action of the seminal prostaglandins on the male reproductive tract.

Owing to the inaccessibility of the male reproductive tract, most information on the effects of prostaglandins has so far been obtained from experiments performed *in vitro* on isolated organs. In addition, most of this work has been performed on animals whose semen contains little or no prostaglandins, and its relevance to primate reproductive physiology is therefore subject to question.

Holmes *et al* (1963) recorded the pressure in the lumen of the rabbit vas deferens *in vivo*, after cannulating one end of the vas and ligating the other, and showed that PG E_1 inhibited contractions produced by adrenaline. The effect of PG E_1 on the isolated guinea pig vas deferens, studied by Graham and Al-Khatib (1967) was to produce strong contraction (at 0.2 - 0.5 µg/ml). Furthermore, these authors found that after washing out the prostaglandin the action of subsequently-applied noradrenaline was potentiated by a factor of five. Von Euler and Hedqvist (1969) found PG E_1 to be inhibitory on the electrically-stimulated guinea pig vas (at 0.01 - 0.025 µg/ml). PG E_2 was found to be about half as active in this system. Using a similar preparation, Ambache and Zar (1970) found PG E_2 to potentiate the effect of noradrenaline, but inhibit electrically - stimulated contractions. PG $F_{2\alpha}$ was found to have no effect on this preparation. The effect of prostaglandins on isolated

vas deferens preparations from the guinea pig, rabbit, cat and rat were studied by Hedqvist and Von Euler (1972). In all four animals the response to E prostaglandins was found to be biphasic when the tissue was electrically stimulated, doses of PG E_1 or PG E_2 below 0.2 µg/ml inhibiting the contractions, while higher doses enhanced the response. The response of the organs to PG E in the presence of noradrenaline was, however, uniformly one of enhancement. F prostaglandins showed similar effects but were less potent than the PG Es. Bhagat et al (1972) found that PG E_2 increased the response of the isolated guinea pig vas deferens to nerve stimulation or direct electrical stimulation at 10^{-7}M (0.035 µg/ml) but that the effect on the tissue when stimulated simultaneously by nor adrenaline and electrically was once more biphasic.

Similar effects of prostaglandins have been demonstrated on the seminal vesicle. Clegg (1966) showed that PG E_1, $F_{2\alpha}$ and $F_{1\beta}$ all potentiated the response of the isolated guinea pig seminal vesicle to adrenaline, and this effect was shown to last up to 30 minutes after a single dose of prostaglandin. Using a similar preparation, Eliasson and Risley (1966a, b) showed that PG E_1 (0.012 - 0.5 µg/ml) caused a marked potentiation of response to adrenaline, noradrenaline, acetyl choline or serotonin. PG $F_{1\alpha}$ in the same range had no effect. PG E_1 alone provoked contraction at 0.3 µg/ml, while PG $F_{1\alpha}$ had no effect at 1.2 µg/ml.

There was no apparent difference in response between preparations from normal and castrated animals. The effect of PG E_1 on the isolated hamster seminal vesicle was studied by Risley and Hui (1968) who found that contraction of the tissue in response to catecholamines was enhanced at prostaglandin concentrations of 0.01 - 0.1 μg/ml but that the response to acetyl choline was unchanged. Naimzada (1969) found that PGs E_1, E_2, A_1 and $F_{1\alpha}$ all potentiated the response of the isolated guinea pig seminal vesicle to electrical stimulation and sympathetic amines. PG E_1 was effective at 0.1 μg/ml and the ratio of activity was $E_1/E_2/A_1/F_1$ = 100/10/2/1 .

Biphasic response of the isolated guinea pig seminal vesicle to PG E_1 in the presence of nerve stimulation were found by both Sjöstrand and Swedin (1970) and Hedqvist (1972). Both groups found PG E_1 to be inhibitory at low doses (up to 0.06 μg/ml) and stimulatory at high doses (0.1 - 1.0 μg/ml). Hedqvist (1972) proposed that PG E_1 has a dual action, with low doses inhibiting the release of noradrenaline from nerve terminals while high doses potentiate the effect of the neurotransmitter on the receptor, suggesting the presence of an endogenous prostaglandin-mediated regulation of the smooth muscle response to nerve activity.

In the only study to date of the effect of prostaglandins on human seminal vesicles Ito et al (1972) found that PG E_1

at a concentration of 1.3 μg/ml inhibited adrenaline-stimulated contractions *in vitro*. Since human semen contains around 70 μg/ml of PG E and the concentration at the point of secretion must be far higher than this, it would seem that the levels tested by Ito *et al* were far lower than physiological levels.

Stahl (1972) tested the effect of E prostaglandins on the isolated hamster seminal vesicle and vas deferens, and found that PG E_1 inhibited the response to adrenaline of the seminal vesicle at levels below 1 ng/ml and enhanced the response above 100 ng/ml. PG E_2 had no effect at 10 - 100 ng/ml but gave an enhanced response above and below these levels. In the case of the vas deferens, PG E_1 enhanced the response at levels above 100 ng/ml while PG E_2 showed enhancement at all levels from 1 pg/ml to 2 μg/ml. These observations led him to propose that prostaglandin levels control the timing of ejaculation as follows : Initially, low levels of PG E enhance the activity of the vas deferens to bring up spermatozoa while inhibiting the seminal vesicle. As the levels of PG E rise the seminal vesicle is stimulated to contract, expelling its contents behind the mass of spermatozoa.

The low levels at which these effects were observed fits with the low levels of prostaglandins in rodent semen. Ito *et al*'s observation that the human seminal vesicle is inhibited by levels of PG E_1 which are low compared

with those in human semen leads one to speculate that these authors might once more have found a biphasic response had they investigated the effect of levels of the order of hundreds of micrograms per millilitre. Since the prostate gland is the first of the accessory sex glands to discharge in the process of ejaculation, Stahl's hypothesis would predict that sensitivity to stimulation by prostaglandins should be greatest in this organ; however, I can find no reference in the literature to any effect of prostaglandins on the prostate.

Prostaglandins have been shown to have profound effects on the testis, inhibiting contraction of the testis capsule (Hargrove et al, 1971), reducing blood flow and increasing venous pressure at levels which had no effect on central blood pressure (Free and Jaffe, 1972a, b). Studies on the effects of prostaglandins on testosterone secretion have led to conflicting results. Saksena et al (1973) showed that rats injected with PG E_2 (500 µg s/c, twice daily for three days) had lowered plasma testosterone levels and decreased testis weight while plasma LH levels remained unchanged. Similar results were reported by Bartke et al (1973) using mice, however, Eik-Nes (1971) found that levels of testosterone in the spermatic vein of dogs increased when PG E_2 was infused via the spermatic artery at 11 µg/min.

The remote position of the testis relative to the seminal vesicles implies that any physiological effects

of prostaglandins on this organ are probably due to synthesis occurring in the testis itself, and since the testis is a very rich source of prostaglandin 15-dehydrogenase (Nakano and Prancan, 1971) presumably testicular prostaglandins do not contribute to levels in semen.

The epididymis is a contractile organ, and has been shown to respond by contraction to catechol amines and acetyl choline in vivo (Hib, 1976). There is, however, no information so far on the effect of prostaglandins on epididymal smooth muscle, although prostaglandins have been shown to affect sperm maturation (see following section).

Prostaglandin E_1 has been shown to relax the retractor penis muscle of the dog at very low concentration (Luduena and Grigas, 1972). Relaxation of this muscle precedes erection and is mediated via the parasympathetic nervous system, and the authors suggest that PG E_1 is involved in parasympathetic neurotransmission in this muscle.

In summary, prostaglandins may be shown to stimulate contraction of the seminal vesicle and vas deferens, and the biphasic response shown by these organs may indicate the existence of a trigger mechanism operating when a threshold level of prostaglandin is reached. The testes and epididymes are probably too remote from the seminal vesicle to be affected by prostaglandins synthesised there, but the possibility of endogenously synthesised prostaglandins contributing to their discharge cannot be ruled out.

The action of seminal prostaglandins on spermatozoa.

Early work by Horton (1965) showed no effect of added PG E_1 (100 µg/ml) on the motility of ejaculated human spermatozoa, and later Eliasson et al (1968) could show no effect of PG E_1 at physiological levels on the metabolism of washed, ejaculated human spermatozoa. Pento et al (1970) studied the effect of PG E_1 and PG $F_{1\alpha}$ on the carbohydrate metabolism of ejaculated and epididymal ram spermatozoa, and found both prostaglandins to stimulate glycolysis in epididymal spermatozoa but to have no significant effect on ejaculated spermatozoa.

Spermatozoal glycolysis and motility have been shown to be under the control of intracellular cyclic AMP levels (Hoskins and Casillas, 1975), but the mechanisms controlling cyclic AMP levels in the sperm cell are at present unknown. In most tissues other than adipose tissue, E prostaglandins stimulate adenyl cyclase (Horton, 1972). If the seminal prostaglandins could be shown to raise intraspermatozoal cyclic AMP levels and therefore stimulate motility this would provide an explanation for the presence of the seminal prostaglandins. Unfortunately, the only published work to date on this topic showed E prostaglandins to depress intraspermatozoal cyclic AMP levels in washed, ejaculated rhesus monkey spermatozoa (Casillas and Hoskins, 1971). However, the major prostaglandin present in monkey semen is 19-hydroxy PG E_1, the E prostaglandins being present at much lower

levels (Kelly et al, 1976) and prior exposure of ejaculated spermatozoa to prostaglandins may produce irreversible changes which render them insensitive to subsequent prostaglandin treatment.

19-hydroxy E prostaglandins have been shown to depress the respiration of washed, ejaculated human spermatozoa (Kelly, 1977), an effect not seen with E or F prostaglandins. Earlier work had already shown that washing spermatozoa stimulated oxygen uptake by a factor of three (Eliasson, 1971), an effect which is presumably due to the removal of 19-hydroxy E prostaglandins. The aerobic pathway of metabolism is presumably of relatively little importance in ejaculated spermatozoa, since Peterson and Freund (1974) have shown that fructose consumption does not increase under anaerobic conditions (Pasteur effect). Addition of 19-hydroxy E prostaglandins did not affect the anaerobic pathway of metabolism, as evidenced by the unchanged production of lactate, and did not affect the motility of the spermatozoa (Kelly, 1977).

Spermatozoa may be shown to bind prostaglandins, and in the rabbit this binding takes place in the acrosomal region of the sperm plasma membrane (Bartoszewicz et al, 1975). The human spermatozoon has been shown to bind PG E_1 and PG $F_{2\alpha}$ (Mercado et al, 1978) and on the basis of differing binding constants and fluorescence-quenching efficiencies the authors concluded that the two prostaglandins

bind to different receptors.

Exogenous prostaglandins have been shown to affect spermatogenesis and sperm maturation, although the relevance of this information to the seminal prostaglandins is doubtful. Ericsson (1973) showed that twice-daily injection of E prostaglandins in male rats suppressed spermatogenesis as measured by the occurrence of immature germinal cells in the epididymis. This effect may have been due to suppression of testosterone production however, since decreased testicular, epididymal, seminal vesicle and ventral prostate weights were recorded, and other workers have shown that injection of prostaglandins depresses testosterone levels in rats (Bartke et al, 1973; Saksena et al, 1973).

Sorgren and Glass (1972) attempted to demonstrate an effect of PG $F_{2\alpha}$ on the maturation and fertility of rabbit spermatozoa by incubating spermatozoa from the caput and cauda of the epididymis and ejaculated spermatozoa with this compound before artificially inseminating does. They could show no effect on the fertility of ejaculated or caudal spermatozoa, and the caput spermatozoa remained infertile. Injection of male rabbits with PG E_1 or PG $F_{2\alpha}$ was shown to accelerate sperm production in work by Hunt and Nicholson (1972). Rabbits were given a single injection of tritiated thymidine on day zero and were given prostaglandins (1 mg s/c twice daily) thereafter. Radioactive spermatozoa were produced first on day 45 by control rabbits

and two days earlier by the prostaglandin treated animals. Setty and Kar (1972) were unable to show any effect of injected prostaglandins on the residual fertility of castrated male rats maintained on testosterone.

In summary, a conclusive demonstration of the requirement of spermatozoa for seminal prostaglandins has yet to be achieved. Although the work of Kelly (1977) and Pento et al (1970) is especially interesting in this connection, more work with epididymal spermatozoa which have never experienced high levels of prostaglandins will be necessary before the importance of these compounds in relation to sperm motility and metabolism can be assessed.

The effects of seminal prostaglandins on the female reproductive tract.

An extensive literature exists on the effects of prostaglandins on the female, and responses to physiological or pharmacological levels have been demonstrated in the vagina, cervix, uterus and fallopian tubes. The role of prostaglandins in luteolysis and menstruation will not be considered here, as they result from endogenous production in the female and are not relevant to the seminal prostaglandins. As is usual with any aspect of reproductive physiology, marked species differences are evident, and conclusions drawn

from experiments on non-human material must be treated with caution when applied to the human.

Sperm transport in the female tract appears to be extraordinarily rapid, the rate at which spermatozoa are transferred from the vagina to the fallopian tubes far exceeding that which would be expected if the sole means of transport was the motility of the spermatozoa. Settlage et al (1975) showed by artificially inseminating women shortly before surgical removal of the fallopian tubes that sperm can be found in the tubes within five minutes of deposition in the vagina. Work by Bickers (1951) showed a reduced rate of sperm transport in 8/24 women with unexplained infertility. It would seem therefore that the active transport of sperm by the female tract is essential to fertility.

The first barrier faced by the sperm in the female tract is the cervix, and it is not certain at present whether transport of sperm through the cervical canal is an active or a passive process on the part of the female. Bickers (1951) showed that during sexual stimulation uterine activity increases, followed by temporary inhibition after orgasm with a simultaneous increase in vaginal pressure. There exists therefore a pressure gradient between the vagina and the uterus which could facilitate sperm transport by sucking the vaginal contents through the cervical canal. The existence of this pressure gradient has been confirmed

by Fox et al (1970), using direct measurement of the pressures by radio-telemetry.

Early reports by Amersbach (1930) and Trapl (1943) described instances of uptake of carbon particles and carmine dye through the cervix during intercourse. Egli and Newton (1961) placed a suspension of carbon particles in dextran gel in the vagina of anaesthetised women before surgery and simultaneously administered intravenous oxytocin. Carbon particles were found in the fallopian tubes in 2/3 cases 28-34 minutes later. De Boer (1972), however, in a similar investigation studied the transport of carbon particles placed in the vagina, cervical canal or uterus before laparotomy, and later found carbon in the fallopian tubes in 50% (uterus) or 30% (cervical canal) of cases. When the material was placed in the vagina, transport to the fallopian tubes occurred in only one case out of thirty seven. No effect of oxytocin could be detected in this work, and the stage of the patient's menstrual cycle showed no influence on the results.

Masters and Johnson (1966) and Sobrero (1967) both placed radio-opaque material in a cervical cap applied to a woman before masturbation or intercourse, and subsequent radiography showed no radio-opaque material to be drawn into the uterus in either case. Immediate examination of cervical mucus after normal intercourse has shown that spermatozoa reach the internal os of the cervix within 1.5 - 3 minutes of ejaculation (Sobrero and Macleod, 1962). In

three cases where the semen was necrospermic no transport of the immotile sperm occurred, and no vaginal epithelial cells were found in the cervical mucus as might be expected if the semen were drawn in by sucking.

Sperm cells observed in cervical mucus show a higher motility than in the original specimen (Sobrero and Macleod, 1962; Moghissi, 1973) and it would appear that the barrier posed to spermatozoa by the cervical mucus is functional, in that it serves to weed out spermatozoa of reduced motility. In addition, the convoluted folds of the walls of the cervical canal provide a series of crypts which retain sperm and release them for a period of several hours after intercourse (Moghissi, 1973). These functions of the cervix would appear to be incompatible with a mechanism which involves the uterus rapidly sucking the semen through the cervical canal.

Prostaglandins can be shown to affect the motility of the cervix, although the significance of these observations with respect to the transport of spermatozoa through the cervix is at present unknown. The isolated, non-pregnant human cervix was investigated by Hillier and Karim (1970), who found that PG E_2 at levels much lower than those present in semen (0.025 - 0.5 μg/ml) caused a decrease in the spontaneous motility. The response to PG $F_{2\alpha}$ at physiological levels (0.5 - 2.0 μg/ml) was erratic. Intravenous injection of PG E_1 has also been shown to inhibit the motility of the rabbit cervix *in vivo*, along with that of the uterus and oviduct (Horton *et al*, 1965).

Eskin et al (1972, 1973) investigated the effect of added PG $F_{2\alpha}$ on the penetration by spermatozoa of human cervical mucus. Unfortunately these papers are extremely imprecise, almost to the point of being incomprehensible. The authors claim that addition of 250 μμg (sic) of PG $F_{2\alpha}$ to human semen "improves significantly the penetration, motility and drive of sperm into cervical mucus." One may draw conclusions from these papers on the effectiveness of the refereeing process for scientific publications but not, alas, on the effect of prostaglandins on the penetrability of cervical mucus. An earlier (but much better documented) paper by Pomerenke and Viergiver (1947) reported that the ability of spermatozoa to penetrate cervical mucus was not significantly impaired by washing the cells and re-suspending them in Ringer's solution, implying that the endogenous seminal prostaglandins are not essential for this process.

The effects of prostaglandins on sperm transport in the rabbit have been investigated by three groups. Mandl (1972) found that PG E_1 added to rabbit semen at 375 μg/ml greatly increased the rate at which sperm moved from the vagina to the oviduct. Spilman et al (1973) found that intravaginal (100 μg/animal) or subcutaneous (1 mg/kg at 0, 1, 2 and 2.75 hours after insemination) PG $F_{2\alpha}$ increased both the rate of sperm transport and the fertilisation rate. Neither PG E_1 nor PG E_2 could be shown to have any effect.

Precisely the reverse was found by Chang et al (1973) who found intravaginal PG E_1 or PG E_2 (100 µg/ml semen) to increase the fertilisation rate but that PG $F_{2\alpha}$ was ineffective. Levels of prostaglandins used in these studies are enormously higher than those occurring naturally in rabbit semen, where E prostaglandins amount to about 70 ng/ml (Poyser, 1974) and the physiological significance of this work is therefore doubtful.

The effects of prostaglandins on the fallopian tubes have been fairly extensively studied, and the results of different groups agree closely with one another. Preparations of human fallopian tube in vitro were studied by Sandberg et al (1963, 1964, 1965) who showed that PG E_1 stimulates contraction of the proximal (to the uterus) quarter of the tube while relaxing the distal three quarters. PG E_2 has a similar action, while PG E_3 relaxes the entire tube. F prostaglandins were shown to stimulate contraction of the entire length of the tube, and the effects of all of the prostaglandins studied could not be related to the stage of the menstrual cycle.

Horton and Main (1963) showed that all three E prostaglandins decrease the tone and activity of the rabbit oviduct while PG $F_{2\alpha}$ has the opposite effect. Horton et al (1963) showed that intravaginal PG E_1 causes relaxation of the oviduct in rabbits even when the uterine horn is ligated or severed, showing that the prostaglandin must arrive at the organ via the systemic circulation.

Eliasson and Posse (1965) studied the effect of intravaginal application of a crude extract of human seminal prostaglandins (HSF-PG) on the resistance to gas flow of the fallopian tubes in women (Rubin's test), and found a significant increase in resistance in three out of seven cases. A similar technique was employed more recently by Embrey et al (1976), who showed that PG E_2 inhibits and PG $F_{2\alpha}$ stimulates the resting tone, frequency and amplitude of contractions at all stages of the menstrual cycle.

Brundin (1968) recorded the pressure required to force a constant flow of saline through the oviduct of anaesthetised rabbits, and found that PG E_1 antagonised the effect of noradrenaline and electrical stimulation of the hypogastric nerve in stimulating contraction of the oviduct.

Coutinho and Maia (1971) used direct catheterisation to study the effects of prostaglandins on the human fallopian tube in vivo, and once more found PG $F_{2\alpha}$ to stimulate, and PG E_2 to relax the tube. As little as 100 ng of PG $F_{2\alpha}$ introduced into the lumen of the tube produced an increase in motility which was reversible by higher doses of PG E_2. Similar results were obtained by Spilman and Harper (1973) using rabbits in oestrus. These authors observed that the effect was minimal in animals with a low level of spontaneous activity and concluded that the effect of prostaglandins may be related to tubal muscle control mechanisms.

The earliest demonstration of the spasmogenic effects of human semen was on the non-pregnant uterus, and this organ has continued to receive much attention since. Kurzrok and Lieb (1930) observed that semen applied by intracervical injection frequently caused contraction of the uterus, although later work by Cockrill *et al* (1935) showed that semen applied to myometrial strips *in vitro* generally produces relaxation. Extracts of human semen (HSF-PG) were widely used by early workers in the investigation of the effects of prostaglandins on the uterus, until purified preparations of prostaglandins became available in the early 1960s.

The rat uterus was shown by Asplund (1947b) to be stimulated *in vivo* by HSF-PG whether the route of administration was intravenous, intraperitoneal or intravaginal. Karlson (1949) recorded the motility of the human uterus using three separate pickups to measure simultaneously pressure changes in the corpus, isthmus and cervix. At the time of ovulation seminal fluid increased the activity of the corpus but reduced that of the isthmus and cervix, while at other times there was an increase in the activity of all three parts.

Eliasson and Posse (1960) used HSF-PG to show that seminal prostaglandins administered intravaginally produced increased uterine activity around the time of ovulation. When the experiment was repeated in the presence of simultaneous intravenous infusion of oxytocin and vasopressin

the effect was one of relaxation, an interesting observation which does not appear to have been followed up in later work.

PG E_1 was shown to relax human uterine strips *in vitro* by Sandberg *et al* (1963) and the same authors later showed the effect of PG E_2 and PG E_3 to be similar (Sandberg *et al*, 1964), while the F prostaglandins had a stimulatory effect (Sandberg *et al*, 1965). The effect of HSF-PG on human myometrium *in vitro* was shown to be one of relaxation, with the highest sensitivity observed at ovulation time (Bygdeman and Eliasson, 1963). Bygdeman (1964) showed E prostaglandins to inhibit, and F prostaglandins to stimulate the non-pregnant human uterus *in vitro*, and that the effect of HSF-PG was essentially similar to that of PG E. The sensitivity of the tissue was highest at mid-cycle, although addition of progesterone to these preparations reduced their sensitivity towards prostaglandins.

Adamson *et al* (1967) found the rat uterus to show tachyphylaxis to PG E_1 and PG $F_{1\alpha}$ but only slightly to PG E_2. There was no cross-reactivity between the prostaglandins with respect to this effect, and the authors therefore suggested that separate receptors were involved.

The effect of the (then) newly discovered prostaglandins A, B and 19-hydroxy A and B were investigated by Bygdeman and Hamberg (1967). All eight compounds were shown to affect non-pregnant human isolated myometrial strips in

a similar way to PG E, but to be considerably less potent, showing from 1 - 30% of the activity of PG E_1.

The effect of the pure prostaglandins on the non-pregnant human uterus *in vivo* has been studied by a number of authors, and the results obtained for the E prostaglandins confirm those found earlier using semen or HSF-PG. Roth-Brandel *et al* (1970) found that intravenous PG E_1 and PG $F_{2\alpha}$ both invariably caused stimulation of the uterus regardless of the stage of the cycle. Essentially similar results were obtained by Coutinho and Maia (1971) and Karim *et al* (1971). Martin and Bygdeman (1975) found that very small quantities of intrauterine PG E_2 (0.25 μg) stimulated the uterus early or late in the menstrual cycle. At the time of ovulation the sensitivity of the uterus was reduced, while during menses the action of the prostaglandin was reversed, 30 - 40 μg causing inhibition.

The early observations of Kurzrok and Lieb (1930) and recent work by White and Glass (1976) showed that intrauterine instillation of semen frequently results in strong uterine contractions, and the latter authors recommend use of no more than 0.3 ml semen for artificial insemination to avoid this effect. Since this does not happen after normal intercourse, the implication is that the seminal prostaglandins can penetrate the cervix only slowly if at all. Many workers have demonstrated effects of intravaginal prostaglandins on the female tract (Karlson, 1959;

Eliasson and Posse, 1960,1965; Henzl et al, 1972) and the problem arises as to how the seminal prostaglandins reach the uterus and fallopian tubes from the vagina.

Horton (1965) calculated that 5 ml of semen containing 70 μg/ml of PG E would, if deposited in the vagina of a woman with a blood volume of 5 litres and absorbed via the systemic circulation, give rise to a concentration of 70 ng/ml in the blood. In order to act upon the tissues the prostaglandin would need to pass into the interstitial fluid and so further dilution would occur, and in addition the rapid inactivation by the lung would further reduce the available concentration at the presumed target organs. He concluded therefore that systemic absorption of intravaginal prostaglandins could not account for their effects on the female tract.

Sandberg et al (1968) have studied the rate of urinary excretion of radioactivity after vaginal administration of tritium-labelled PG E_1 mixed with human semen. It was found that the rate of appearance of radioactivity in the urine was not affected by the presence of a cervical cap, and the authors therefore concluded that no significant uptake of radioactivity via the cervix occurs. The time taken for the bulk of radioactivity to appear in the urine in this experiment was, however, much longer than the time taken for intravaginal prostaglandins to show their effects, and it may well be that the rate of appearance of prostaglandin

metabolites is determined by processes which are much slower than the rates of uptake via the cervix or vaginal wall. We must therefore conclude that the question of the route of uptake of intravaginal prostaglandins is still to be resolved.

The effects of prostaglandins upon the pregnant uterus have been widely studied, the bulk of the work concerning the utilisation of prostaglandins as abortifacients or in the induction of labour.

One of the earliest demonstrations of an effect which may have been due to prostaglandins was that of Ott and Scott (1909), who reported that a dried extract of prostate gland of unspecified origin strongly stimulated the pregnant cat uterus *in vitro*. The effect of E and F prostaglandins on pregnant myometrium was briefly investigated by Bygdeman (1964), who found that PG $F_{2\alpha}$ strongly stimulated the isolated human myometrium while E prostaglandins caused relaxation, although higher doses were required than those which relaxed non-pregnant strips.

A more thorough investigation by Embrey and Morrison (1968) later showed that the effect of both E and F prostaglandins is to stimulate contraction *in vitro* of myometrial strips taken from the upper segment of human uteri at term. Results obtained with lower segment strips were reported to be erratic, and the effect of the 2 series compounds was found to be more clear-cut than that of the 1 series prostaglandins.

Prostaglandin $F_{2\alpha}$ was shown to stimulate contraction of the pregnant human uterus *in vivo*, and Karim *et al* (1968b) successfully used this compound to induce labour. Embrey (1969) showed that infusion of E prostaglandins into pregnant women induced uterine contractions both at term and (unlike oxytocin) in early pregnancy, and subsequently used this method to induce abortion at 9 - 28 weeks gestation (Embrey, 1970a) and labour at term (Embrey, 1970b).

Roth-Brandel (1971) compared the response of the pregnant uterus *in vivo* to E prostaglandins in mid-pregnancy and at term, showing that the uterus responds at the same dose level in both cases, but that the absolute level of contraction elicited is higher at term.

Brummer (1971, 1972) investigated the interaction between oxytocin and prostaglandins in their action upon isolated pregnant human myometrial strips and showed two types of interaction, potentiation (short term) and enhancement (long term). E and F prostaglandins both potentiated the effect of oxytocin but only the PG Es produced enhancement, and the degree of enhancement was not dose-related. Brummer and Collins (1972) attempted to correlate myometrial and plasma levels of progesterone with the response of isolated pregnant human myometrial strips to prostaglandins, but were unable to demonstrate any correlation. Oestradiol was shown to block the action of PG E_1 on isolated pregnant myometrium in a study by Pinto *et al* (1971). These authors

found a bimodal effect, with low doses of the prostaglandin stimulating contractions while high doses inhibited.

Spilman et al (1977) studied the effect of 19-hydroxy prostaglandins E_2 and $F_{2\alpha}$ on the pregnant rhesus monkey uterus in vivo. Intravenous injection of the methyl esters of these compounds showed that the 19R epimer of 19-hydroxy PG E_2 was as active as PG E_2 in stimulating uterine contractility, while the (unnatural) 19S epimer was about half as active. The methyl ester of 19-hydroxy PG $F_{2\alpha}$ was much less active than PG $F_{2\alpha}$. It was claimed that the methyl esters gave similar results to those obtained with the native compounds, presumably due to hydrolysis by non-specific serum esterases.

Most work on the effects of prostaglandins on the pregnant uterus has been concerned with the pharmacological properties of the compounds, but since copulation continues to occur during pregnancy both in man and in many other primates, the exposure of the pregnant uterus to seminal prostaglandins is also of physiological interest. It has been suggested that coitus during pregnancy may result in premature labour as a result of the vaginal instillation of prostaglandins, and that this may explain the success of hospitalisation in the treatment of this condition as opposed to bed rest at home (Flynn, 1971).

It seems improbable that intercourse during the first and second trimesters of pregnancy could lead to ill effects,

since the quantities of intravaginal prostaglandins required to induce abortion at this time are far larger than those present in normal ejaculates, typical values reported being 50 mg PG $F_{2\alpha}$ or 20 mg PG E_1 (Brenner et al, 1972; Beguin et al, 1972; Pion et al, 1972).

Prostaglandin synthetase inhibitors have been shown to prolong pregnancy in the rat (Aiken, 1972; Chester et al, 1972) and inhibit spontaneous and oxytocin-stimulated contraction of late pregnant isolated rat myometrial strips (Vane and Williams, 1972; Williams et al, 1974). The addition of indomethacin or aspirin to baths containing such strips has been shown to inhibit the release of F prostaglandins into the bathing medium (Aiken, 1972; Williams et al, 1974). It would seem probable in the light of these findings that the action of oxytocin is mediated via increased prostaglandin synthesis. Oxytocin and PG E_2 have been shown to have very similar effects on the distribution of blood flow and uterine contractility in the pregnant rhesus monkey uterus (Novy et al, 1975).

The mechanism of action of prostaglandins on myometrium is unknown at present. On the basis of their action in other tissues, one might expect stimulatory prostaglandins to act by modification of cyclic nucleotide levels. β-adrenergic agents (which relax the uterus) have been shown to raise cAMP levels (Butcher et al, 1965; Triner et al, 1971) and if prostaglandins could be shown to produce the reverse effect, or to prevent β-agonist induced cAMP generation

this would provide strong evidence for a central role of cyclic AMP in mediating the action of these compounds on the uterus.

Adrenaline and E prostaglandins were both shown to stimulate adenyl cyclase in the rat uterus in work by Harbon and Clauser (1971), while oxytocin and PG $F_{2\alpha}$ had no effect on this enzyme. The action of oxytocin and PG $F_{2\alpha}$ in stimulating contraction of the uterus was antagonised by adrenaline and by dibutyryl cAMP but not by E prostaglandins, and the authors concluded that the intracellular cAMP level was not the sole parameter regulating uterine motility. Later, however, it was shown that rat endometrium contains an adenyl cyclase which is stimulated by PG E but not by β-agonists (Bhalla et al, 1972), and the oestrogen-dominated uteri studied by Harbon and Clauser may well have included this enzyme. PG Es and PG $F_{2\alpha}$ have been shown to inhibit the rise in cAMP stimulated by β-agonists in myometrium from oophorectomised rats (Bhalla et al, 1972; Kroeger and Marshal, 1974).

Treatment of oophorectomised rats with oestradiol 17-β has been shown to increase uterine motility, raise levels of PG Fs and raise cGMP levels, however, simultaneous administration of indomethacin did not prevent the rise in cGMP while blocking PG F production (Ham et al, 1975).

PG E_1 has been shown to facilitate the non-energised binding of Ca^{++} to the inner membrane of rat liver mito-

chondria, and to inhibit the mitochondrial swelling produced by energised accumulation of Ca^{++} (Kirtland and Baum, 1972). It was proposed that these effects were due to the prostaglandin acting as an ionophore to facilitate the access of Ca^{++} to binding sites on the membrane, and that calcium-dependant processes elsewhere (such as the contraction of smooth muscle) may likewise be controlled by prostaglandins regulating the passage of this ion across lipoprotein membanes.

Carsten (1974) showed that PG E_2, PG $F_{2\alpha}$ and oxytocin all inhibited the ATP-dependant binding of calcium ions to a subcellular fraction derived from the sarcoplasmic reticulum of bovine myometrium. The author proposed that inhibition of intracellular binding of Ca^{++} increases the concentration of free Ca^{++}, leading to more interaction with the regulatory proteins of the myofibril and subsequent contraction.

The biological effects of 19-hydroxy prostaglandins.

Most work performed on the effects of 19-hydroxy prostaglandins on biological systems has so far remained unpublished, and results which have been made available to us by the authors are quoted here with their permission.

The effects of synthetic (racemic) 19-hydroxy PG E_1, 19-hydroxy PG E_2 and 19-hydroxy PG $F_{2\alpha}$ on the reproductive tract of anaesthetised female rabbits have been investigated by Horton (E.W. Horton, unpublished) who found that they

were similar to, but less potent than the non-hydroxylated parent compounds. The equipotent molar ratios (EPMRs) for the 19-hydroxylated compounds varied from 180 to 550 relative to the parent compounds. Work on the classical bioassay preparations, rat fundus and guinea pig ileum strips (P.S. Agutter, unpublished) and on blood pressure in the sheep and dog (R.L. Jones, unpublished) has shown a similar picture.

The effects of 19-hydroxy PG E_1 on various preparations from one male and one female patas monkey were studied by Vane and Higgs (J.R. Vane and G.A. Higgs, unpublished) who found once more that the action of the hydroxylated compound on vascular and intestinal smooth muscle was similar to, but less potent than that of PG E_1. 19-hydroxy PG E_1 was shown to relax the isolated oviduct at low levels, in contrast to PG E_1, PG E_2 and PG $F_{2\alpha}$ which all produced contraction in this preparation. None of the prostaglandins tested showed any effect on isolated myometrial strips, although the authors point out that since only one animal of each sex was available little weight can be attached to negative results, which may have been due to setting up the tissue in less than optimal conditions.

The work of Spilman et al (1977) showing 19-hydroxy PG E_2 (methyl ester) to stimulate contraction of the pregnant rhesus monkey uterus in vivo has already been described. Similar results were obtained by Novy (M.J. Novy, unpublished) using natural 19-hydroxy PG E_1.

Objectives of the present work.

After the discovery by Hamberg and Samuelsson of the A and B prostaglandins in 1965 little further progress was made in the identification of seminal prostaglandins until 1974, when we reported the presence of 19-hydroxy PG E_1 and 19-hydroxy PG E_2 and showed them to be the major components of the seminal prostaglandins in man. The discovery of these compounds showed that the composition of the mixture of prostaglandins present in semen was still not completely described. In particular, the existence of the 19-hydroxy PG Es made it seem likely that human semen would also contain 19-hydroxy F prostaglandins, and a major objective of the work described in this thesis was the fractionation of the lipid components of semen and identification of any further prostaglandins thus isolated. The results of this work are presented in chapter 1.

Identification of the structures of the isolated compounds was made possible by the technique of combined gas chromatography-mass spectrometry, and a subsidiary aim of the project was to establish a collection of mass spectra of the novel prostaglandins, together with data on their chromatographic properties. A selection of mass spectra are described in chapter 2, along with tables of chromatographic data.

The identification of the 19-hydroxy PG Es as the major components of the human seminal prostaglandins suggested several further projects, including studies on the biological activity, biosynthesis, metabolism and species distribution

of these compounds. The results of a study on the effects of 19-hydroxy PG E_1 on isolated myometrial strips are presented in chapter 3. The investigation of the biosynthesis of the 19-hydroxy PG Es *in vitro* is described in chapter 4, and some observations on the biosynthesis of PG E and 19-hydroxy PG E *in vivo* are presented in chapter 5.

It had been hoped that the work on the biosynthesis of the 19-hydroxy prostaglandins would provide some isotopically-labelled 19-hydroxy PG Es which would be used for studies on the metabolic fate of these compounds. In practice, however, a biological synthesis of labelled 19-hydroxy prostaglandins was not achieved, and this part of the project was therefore postponed until such labelled compounds become available.

The results of the study on the species distribution of seminal 19-hydroxy E prostaglandins are given in chapter 6.

General materials and methods.

Standard prostaglandins.

The following compounds were the gift of the persons listed:-

PGs E_1, E_2, E_3, $F_{1\alpha}$, $F_{2\alpha}$ and D_2. - Dr J.E. Pike (Upjohn Co).

PGs 19-OH E_1, 19-OH E_2, 19-OH $F_{2\alpha}$ and 15R 19-OH E_2 - Dr N. Crossley (ICI).

Dinor PG E_2 - Dr D.A. Van Dorp (Unilever).

8-iso PG E_1, 8-iso PG E_2 - Dr K. Sakai (Sankyo Co).

Prostaglandins dinor $F_{2\alpha}$, 8-iso $F_{1\alpha}$, 8-iso $F_{2\alpha}$ and 9-^{3}H $F_{3\alpha}$ were prepared by borohydride reduction of the appropriate PG E precursor.

Method. Up to 1 mg of the E prostaglandin was treated with potassium borohydride (10 mg/ml in water/methanol 1:2) for one hour at room temperature. The solution was acidified (HCl), diluted with water and extracted with three volumes of ethyl acetate. The extract was dried, and the residue chromatographed on LH 20 Sephadex (1.5M x 5 mm column, chloroform / ethyl acetate / acetic acid 50:50:1) in order to separate the F_α and F_β compounds. PG F_α compounds were identified by their ability to form n-butyl boronate derivatives, and in all cases eluted from the column before the F_β isomer.

In the case of tritium labelled PG $F_{3\alpha}$, PG E_3 (500 µg) was dissolved in 0.5 ml methanol/water (2:1) and injected

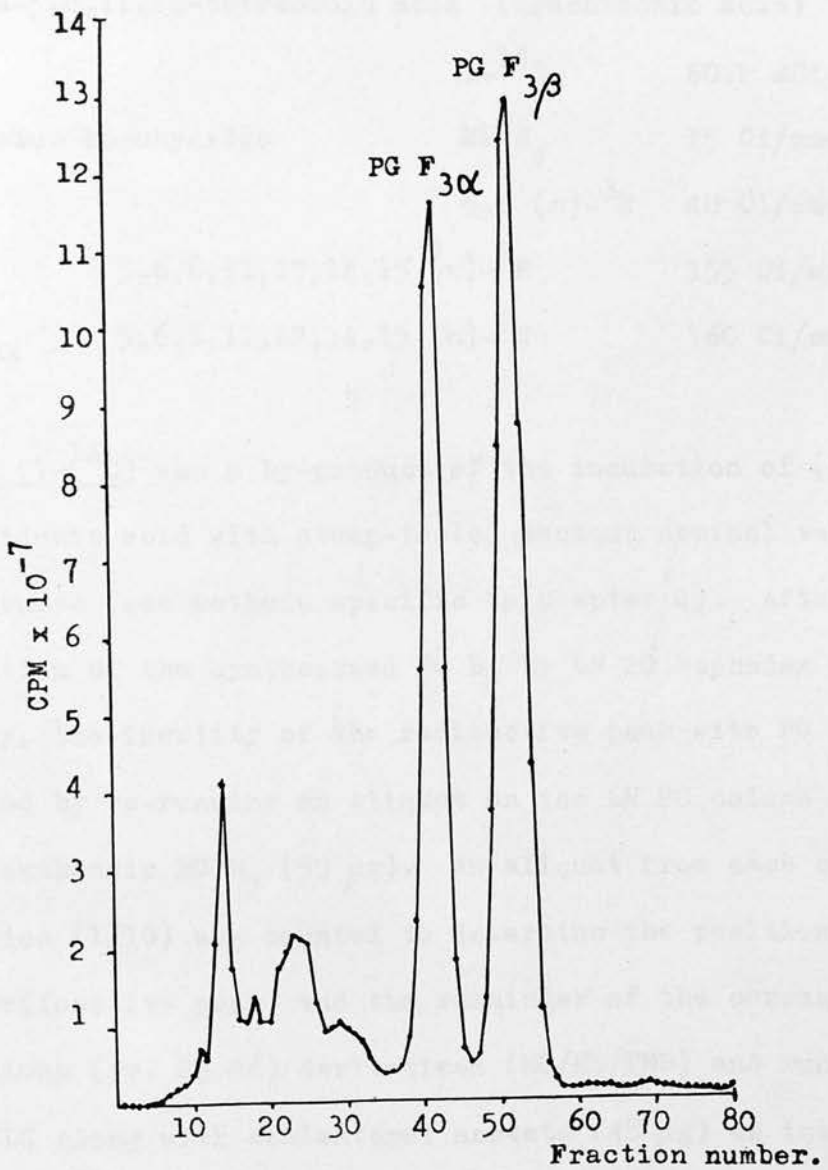
through a rubber seal placed on the neck of a vial containing KB^3H_4 (100 mCi). Excess tritium gas was led away via a syringe needle penetrating the cap, and via a nylon tube to the fume cupboard duct. The tritiated prostaglandins were extracted and separated on Sephadex LH 20 as above, and an aliquot from each fraction counted to determine the position of the radioactive peaks. The reconstructed chromatogram is given in fig. M.1, and shows the typical separation achieved between F_α and F_β compounds in this system. The yield of PG $F_{3\alpha}$ at this stage was 100 μCi.

In most cases PG F compounds prepared by borohydride reduction were intended for reference standards on TLC, GLC and mass spectrometry, and the presence of minor impurities was of no importance. Tritiated PG $F_{3\alpha}$, however, was intended as a substrate for 19-hydroxylation, and since argentation TLC analysis showed the presence of a radioactive impurity corresponding to PG $F_{2\alpha}$ a further stage of purification was undertaken.

An aluminium TLC plate (10 x 20 cm) was dipped in aqueous silver nitrate (5 %) and dried (30 min. at 150°C) in darkness. The sample (half of the previously prepared PG $F_{3\alpha}$) was streaked across 60 mm of the origin, and the plate was developed in methanol / chloroform / acetic acid (40:60:1). Radioactive bands were located using a TLC scanner and that corresponding to PG $F_{3\alpha}$ was eluted with methanol. The methanol eluate was dried and re-run on a newly-packed LH 20 Sephadex column (the effluent from the

original column was too radioactive for further use even after washing for seven days). The final yield of ^{3}H PG $F_{3\alpha}$ was 11 μCi, and the specific activity (calculated from that of the tritiated borohydride starting material) was 3.75 Ci/mmol.

Fig. M.1 Purification of synthesised ^{3}H PG $F_{3\alpha}$.



Isotopically-labelled compounds.

All radioactive compounds used in this work were obtained from the Radiochemical Centre, Amersham, with the exception of ^{14}C eicosatrienoic acid, which was obtained from New England Nuclear Co. The position of the label and specific activities were as follows:-

Eicosa-8,11,14-trienoic acid	1-^{14}C	57.5 mCi/mmol.
Eicosa-5,8,11,14-tetraenoic acid (arachidonic acid)	1-^{14}C	60.2 mCi/mmol.
Potassium borohydride	$KB^{3}H_{4}$	15 Ci/mmol.
PG E_1	5,6 (n)-^{3}H	40 Ci/mmol.
PG E_2	5,6,8,11,12,14,15 (n)-^{3}H	155 Ci/mmol.
PG $F_{2\alpha}$	5,6,8,11,12,14,15 (n)-^{3}H	160 Ci/mmol.

PG D_2 (1-^{14}C) was a by-product of the incubation of (1-^{14}C) arachidonic acid with stump-tailed macaque seminal vesicle homogenate (see methods specific to chapter 4). After isolation of the synthesised PG D_2 by LH 20 Sephadex chromatography, the identity of the radioactive peak with PG D_2 was checked by re-running an aliquot on the LH 20 column along with authentic PG D_2 (50 μg). An aliquot from each column fraction (1/10) was counted to determine the position of the radioactive peak, and the remainder of the corresponding fractions (fr. 23-28) derivatised (MO/ME/TMS) and run on the GLC along with cholesterol acetate (48 μg) as internal standard to determine the position of the peak corresponding

to authentic PG D_2 (fig. M.2).

The remainder of the synthesised (1-^{14}C) PG D_2 was used as a tracer, added to semen to check on the recovery of PG D_2 and to facilitate identification of the corresponding column fractions, and was not further purified before use. It was not possible to estimate the specific activity of this material, since the amount of PG D_2 present was too small to be detected by GCMS.

Commonplace reagents.

All laboratory reagents and solvents used in this work were 'Analar' or equivalent grade, and were not re-distilled or otherwise purified before use.

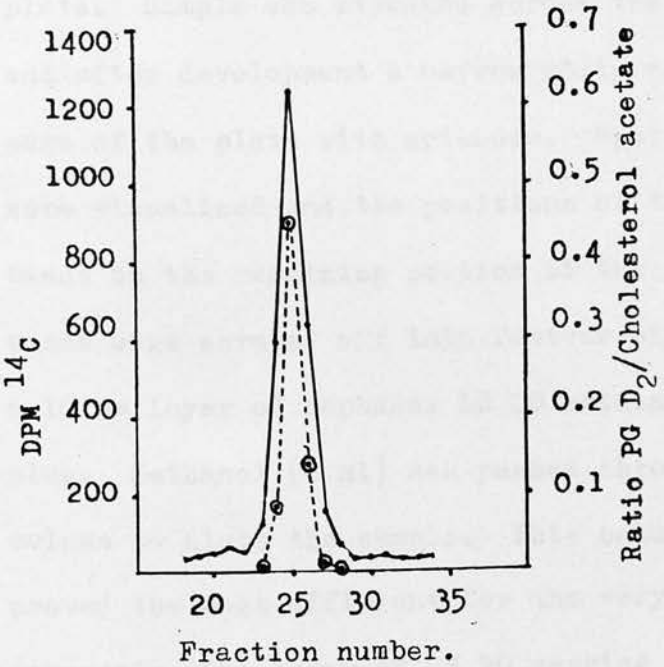


Fig. M.2. Identification of PG D_2.

—•— DPM ^{14}C.

⊙----⊙ Ratio of GLC peak areas.

Thin layer chromatography.

TLC was performed on Merck pre-coated aluminium plates (K 60-F_{254}), the chromatograms being developed in the solvent systems listed below and at 20°C. Prostaglandins were applied to the plate in solution in ethyl acetate and dried at ambient temperature. After development and drying the spots were visualised by sulphuric acid charring.

Three main solvent systems were used:-

(I) Ethyl acetate / acetone / acetic acid (90:10:1)

(II) Chloroform / methanol / acetic acid (80:20:1)

(III) Ethyl acetate / acetic acid (9:1)

System (I) is the F_{VI} system of Anderson (1969). In the case of system (III) the plate was run, dried and re-run to the same mark, and R_f values quoted are for plates developed twice in this way.

Preparative TLC was performed using the same type of plate. Sample was streaked across the width of the plate and after development a narrow strip was cut from each edge of the plate with scissors. Spots on these strips were visualised and the positions of the corresponding bands on the remaining portion of the plate marked. These areas were scraped off into Pasteur pipettes containing a 10 mm layer of Sephadex LH 20 retained by a cotton wool plug. Methanol (4 ml) was passed through this composite column to elute the sample. This column method of elution proved the most efficient for the very polar 19-hydroxy compounds, the layer of LH 20 serving to retain the silicic acid.

Scintillation counting.

Levels of radioactivity were measured by means of a Packard TriCarb liquid scintillation spectrometer. Samples (0.01 - 1 ml in water or ethanol) were placed in 20 ml polypropylene counting vials containing 10 ml scintillant, and were permitted to equilibrate at 4°C for at least 30 minutes before counting, which was performed at this temperature. The composition of the scintillant was as follows:-

2,5 diphenyloxazole (PPO)	10 g.
1,4 di(5-phenyloxazolyl) benzene (POPOP)	0.75 g.
Toluene	2.5 l.
Triton X-100	1.25 l.

Counting was performed using the counting parameters pre-set by the manufacturer for ^{3}H and ^{14}C, and the counting efficiency was monitored continuously by external standard. Vials were counted for 5 minutes or 100,000 counts, and output was via an ASR 33 teletype / tape punch. Paper tape produced, containing data which included the external standardisation ratio and measured counts/minute for each channel was fed to a PDP 8I computer where the counting efficiency for each vial was computed using a program written for the purpose (program listing in appendix). The external standard was calibrated periodically against the standards provided by Packard, and counting efficiencies were typically 35% for tritium and 70% for ^{14}C.

Prostaglandin derivatisation methods.

Prostaglandins are polar, non-volatile, thermolabile lipids, unsuited in their native state to gas liquid chromatography. In order to render these compounds more volatile and less polar it is necessary to derivatise the functional groups of the molecule. Three types of functional group are present in the prostaglandins, requiring up to three stages of derivatisation.

Abbreviations for the various derivatives used throughout this report are given in brackets in the order in which the reactions were performed, thus (MO/ME/TMS) signifies that the compound was first oximated (methyl oxime) then esterified (methyl ester) and finally the hydroxyl groups were derivatised (trimethylsilyl ether).

Carbonyl groups were derivatised as the oxime (O), methyl oxime (MO) or ethyl oxime (EO). Where the (O) derivative was used the free hydroxyl introduced was later derivatised along with the remaining hydroxyl groups. Methods of preparing oximes differed only in the reagent used.

Procedure.

Oximes- Sample material was dissolved in 1 ml pyridinium acetate buffer (1.5 M, pH 5.0) containing the oximating reagent (10 mg methoxyamine HCl (MO), 10 mg ethoxyamine HCl (EO) or 20 mg hydroxylamine HCl (O)) in a 10 ml stoppered test tube. The tube was placed in an ultrasonic bath for 45 minutes. After removal, the contents of the tube were

extracted using 9 ml ether/ethyl acetate (2:1). The organic layer was removed and dried, initially under nitrogen and finally under vacuum at 60°C to ensure removal of all acetic acid.

An alternative procedure was used where the prostaglandins were present in aqueous solution, as in semen or tissue homogenates. This avoided extraction of the labile 19-hydroxy E prostaglandins until after oximation, helping to preserve them from dehydration.-

Semen (0.5 ml) was added to the oximating solution (4.5 ml, composition as above). After treatment in the ultrasonic bath the solution was extracted with ether/ethyl acetate (45 ml), the extract being dried by means of a rotary evaporator.

Carboxyl groups were esterified as methyl- (ME) or ethyl- (EE) esters. If left underivatised at this stage they would be esterified to trimethylsilyl (TMS) or t-butyl dimethylsilyl (tBDMS) esters when the hydroxyl groups were derivatised at a later stage.

Procedure.

Sample was placed in a flat bottomed 4 ml tube and the solvent evaporated. Diazomethane (ME) or diazoethane (EE)

freshly prepared in solution in ether / methanol (9:1) was added (100 µl). The tube was stoppered, mixed and allowed to stand for five minutes at room temperature before removal of the reagent by cautious evaporation under reduced pressure.

Hydroxyl groups were derivatised as various silyl ethers - trimethylsilyl (TMS), dimethylsilyl (DMS) or t-butyl dimethylsilyl (tBDMS) ethers. Alkyl boronates (BB, tBB and MB) are cyclic derivatives which can be formed only by compounds possessing cis 1,2 or 1,3 diols. Of the prostaglandins only the F_{α} compounds form them, and they were used to differentiate between F_{α} and F_{β} compounds. The n-butyl boronate (BB) was most commonly used, but I have also used the t-butyl (tBB) and methyl (MB) boronates.

Procedure.

Sample was placed in a haematocrit tube (2 x 60 mm) and the solvent evaporated in each case.

(TMS) - Bis-trimethylsilyltrifluoroacetamide (up to 25 µl) was added, the tube was sealed and heated at 60°C for 20 minutes. The derivative was injected in the reagent.

(DMS) - Method as for (TMS) except that the reagent was a mixture of equal parts of tetramethyldisilazane and chlorodimethylsilane.

(tBDMS) - t-butyl dimethylsilyl chloride (2 M) and imidazole (2 M) in dimethyl formamide (100 µl) were added, the tube

was sealed and heated at 120°C for one hour. The contents were loaded onto an LH 20 Sephadex column (30 x 5 mm) and eluted with 3 ml heptane / ethyl acetate (3:1). Solvent was evaporated under nitrogen and the residue redissolved in an appropriate volume of toluene for injection.

Alkyl boronates (BB, tBB and MB) - were prepared before the remaining hydroxyls were derivatised. Acetone / benzene (1:1, 40 µl) containing n-butyl boronic acid (BB), t-butyl boronic acid (tBB) or methyl boronic acid (MB) (5 mg/ml) was added and the tube was placed under reduced pressure (100 mm Hg) at 60°C for 20 minutes. Any remaining solvent was removed under vacuum.

Gas liquid chromatography-mass spectrometry of prostaglandins.

Mass spectra of prostaglandin derivatives were produced on an AEI MS 12 mass spectrometer coupled via a Watson-Bieman all glass molecular separator to a Hewlett-Packard 4000 gas liquid chromatograph. A glass U tube column was employed (1.5 M x 3 mm), the stationary phase being Dexsil 300 (1% on Chromosorb G). The flow rate of the helium carrier gas was 40 ml/min and the column oven was operated at 200 - 300°C. On-column injection was employed, with the injection area of the column operated at the same temperature as the remainder. The GLC was coupled to the separator via glass-lined steel tubing, the inlet lines and separator being maintained at 250 - 350°C.

Mass spectra were produced using electron impact ionisation, the electron beam energy being 20 eV and the source block temperature 280°C. The ion accelerating potential was 8 KV and the mass spectrometer was operated at a resolving power of 600-800 (10% valley definition). Detection of the ion beam was by electron multiplier operated at potentials up to 2.5 KV.

Spectra were scanned at 16 seconds per decade and recorded by the PDP 8I computer. Data reduction was accomplished by means of the AEI DS 30 data system and results plotted either in histogram form on an XY plotter or as a numerical listing on the teletype. The instrument was calibrated against perfluorokerosene.

Single ion monitoring GCMS assay of prostaglandin derivatives was performed on a DuPont 490 mass spectrometer coupled via an all-glass single jet molecular separator to a Varian 1400 gas liquid chromatograph. The GLC column was a support-coated open tubular capillary (12.5 M x 0.5 mm, Scientific Glass Engineering Pty.), SE 30 stationary phase. The column was operated isothermally at 270°C and the flow rate of the helium carrier gas was 6 ml/min. The injection temperature was 280°C and the inlet lines and separator were maintained at 250-300°C.

The operating conditions for the mass spectrometer were as follows:- Ionisation energy 20 eV, accelerating potential approx 1.4 KV, electron multiplier potential 2 KV, source block temperature 230°C, resolution 600.

Multiple ion detection apparatus was available, enabling up to six channels to be recorded simultaneously, however, in the present work only two channels were used for recording, a third channel remaining tuned to a known mass in the spectrum of perfluorokerosene. Perfluorokerosene was introduced into the source via the batch inlet system and the mass spectrometer was tuned to m/e 792.9506 using the 'tuning' channel of the MID. The high tension reference voltage was then measured by means of a digital voltmeter, and the voltage required to tune in the sample ions calculated. The 'sample' channels of the MID were then set to the calculated voltages and the perfluorokerosene pumped from the source before injection of the sample. Tuning of the mass spectrometer was checked

at intervals by readmitting perfluorokerosene, switching to the 'tuning' channel and optimising the magnet current for maximum detector response.

Assay of prostaglandins.

Assay of E and 19-hydroxy E prostaglandins by GLC.

The measurement of E and 19-hydroxy E prostaglandins in semen using modern methods poses no serious problems since the compounds are present in relatively large amounts, and the method of aqueous oximation described previously has enabled the tendency of the 9-ketoprostaglandins to dehydration to be overcome. The method described here is a variation on that of Cooper and Kelly (1975).

Method

Sample (0.5 ml) was derivatised (MO/ME/TMS) using the techniques described under "derivatisation methods". After methylation, but before formation of the TMS derivative, an accurately measured quantity of cholesterol acetate (ca. 25 µg) was added as an internal standard. (If the internal standard is added at the beginning of the procedure a certain amount of hydrolysis to free cholesterol takes place during oximation.) The final volume of the silanising agent was 10-25 µl.

An aliquot (up to 10 µl) was injected into the Hewlett-Packard / AEI MS 12 GCMS combination using the operating conditions specified previously. The column temperature was programmed from 200° to 300°C at 2°/min. Peaks were detected by the total ion current monitor of the mass spectrometer and quantified by computer integration using a program developed for the purpose (program listed in appendix).

A standard curve for 19-hydroxy PG E_2 is reproduced in fig. M.3. The detection limit of the system depends critically on the age and past history of the column, but lies in the range 1-5 μg per injection of standard compounds. (The detection limit is here defined as the minimum quantity of standard required to give a peak which exceeds system noise by 3σ). The ability of the system to differentiate between the 1 and 2 series compounds depends on the relative proportions of the two species present. In human semen, where the two series of prostaglandins are present in similar amounts they can be measured independently with reasonable accuracy. In the semen of non-human primates the peak corresponding to 19-hydroxy PG E_2 is reduced to a shoulder on the side of the 19-hydroxy PG E_1 peak, causing the integrator to lump the results together, and estimates of the relative proportions of the two compounds must be made manually from the chart record.

Precision of the assay has been tested by running six replicate samples from pooled semen. Results indicated mean levels of $182 \pm 10\%$ (σ) μg for 19-hydroxy PG Es and $49 \pm 12\%$ (σ) μg for PG Es (per ml of semen in both cases).

Assay of 19-hydroxy E prostaglandins in tissue homogenates.

The method described above was modified to increase the sensitivity towards 19-hydroxy PG Es at the expense of the E prostaglandins. Sample (1 ml) was precipitated by the addition of ethanol (10 ml) and the precipitate was

centrifuged down. The precipitate was washed with ethanol (10 ml) and the ethanol extracts combined and evaporated. The residue from this evaporation was redissolved in potassium phosphate buffer (10 ml, 1.0 M, pH 5.0) and extracted with ether (10 ml) and ethyl acetate (3 x 30 ml). The ether layer was discarded and the combined ethyl acetate extracts were dried and derivatised (MO/ME/TMS) as in the previous method. Gas liquid chromatography was performed isothermally at 270°C.

The differential extraction procedure removes many compounds less polar than the 19-hydroxy PG Es, while losing only 5% of the 19-hydroxy prostaglandins present. The cleaner sample provided by this method allowed isothermal operation of the GLC column at the optimum temperature for detection of the 19-hydroxy E prostaglandins. The detection limit for standards using this method was about 0.1 µg, however, in practice the limit was set by the presence of interfering compounds in the sample, and an arbitrary figure of 1 µg has been adopted for the purposes of this report.

Precision of the assay was tested by running six replicate standards (19-hydroxy PG E_1, 5.2 µg each). Results indicated a standard deviation of 21% at this level.

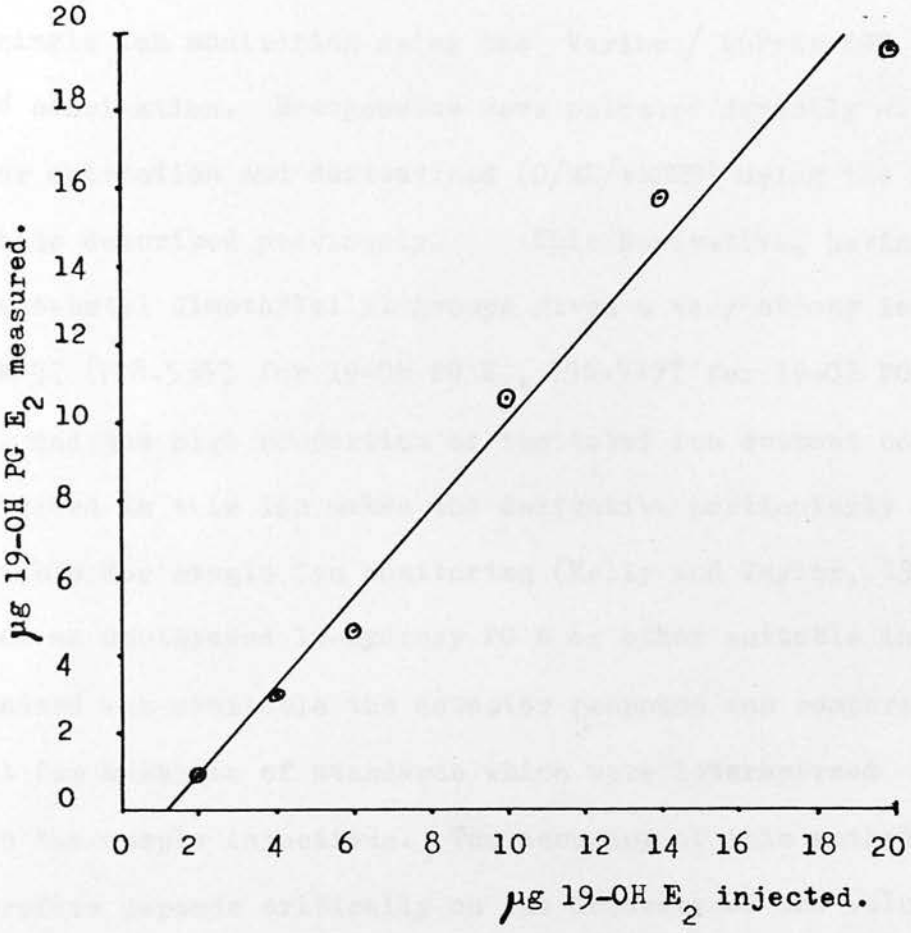


Fig. M.3.

Standard curve for 19-hydroxy PG E_2 (GLC assay).

Results are calculated as follows:-

If a is the measured area of the unknown peak,
b is the measured area of the internal standard peak,
c is the weight of internal standard added,

$$\text{weight of unknown} = \frac{b \times a \times 368}{c \times 627}$$

(368 is the molecular weight of 19-OH PG E_2, 627 is the molecular weight of 19-OH PG E_2 (MO/ME/TMS)).

GCMS measurement of low levels of 19-hydroxy E prostaglandins.

Measurement of low levels of 19-hydroxy E prostaglandins in tissue homogenates and incubations was accomplished by single ion monitoring using the Varian / DuPont 490 GCMS combination. Homogenates were oximated directly without prior extraction and derivatised (O/ME/tBDMS) using the methods described previously. This derivative, having four t-butyl dimethylsilyl groups gives a very strong ion at M-57 (798.5353 for 19-OH PG E_1, 796.5197 for 19-OH PG E_2), and the high proportion of the total ion current concentrated in this ion makes the derivative particularly suitable for single ion monitoring (Kelly and Taylor, 1976a). Since no deuterated 19-hydroxy PG E or other suitable internal standard was available the detector response was compared with that for a series of standards which were interspersed with the sample injections. The accuracy of this method therefore depends critically on the accuracy of the volume of each injection and the stability of the detector response. In order to minimise these sources of inaccuracy, six replicate injections were made of each sample and standard, and the results quoted are therefore the mean of six estimates.

Samples and standards were run isothermally (oven temperature 270°C) and the detector response was quantitated by measuring the peaks on the chart trace by hand. Measurement of peak height rather than area gives a more reproducible measurement provided that the oven temperature (and therefore the peak width) does not change. Either of the two oxime

isomers may be used for measurement, but the first isomer to appear gives a more linear calibration curve at low levels, and was therefore used exclusively in these measurements.

A standard curve for 19-hydroxy PG E_1 is reproduced in fig. M.4. The method is extremely sensitive, since the absence of an internal standard eliminates the crossover between channels which is normally a problem when monitoring more than one ion. As little as 50 pg 19-hydroxy E_1 or 19-hydroxy E_2 injected gives a visible peak, however, all measurements quoted here were made using injections in the range 1-10 ng.

Precision of the assay was checked by running six replicate samples of human semen (2.0 µl each). The derivatised samples were dissolved in toluene (100 µl) and six replicate injections of 1.0 µl were made from each. Results indicated a level of 2.6 ng/injection (130 µg/ml semen) of 19-hydroxy PG E_1, and the standard deviation between replicate samples was 19%. It should be noted that this figure was not different from that for replicate injections from the same derivatised sample (ranging from 9% - 52% in the present case) and the variance contributed to the results by sampling errors and losses during derivatisation is therefore small compared with that introduced by the mass spectrometer. Advantage has been taken of this in the presentation of results on the time course of synthesis of 19-hydroxy PG E_1 in seminal vesicle homogenate (fig. 4.3), where the standard

deviations quoted refer to replicate injections rather than replicate samples. Such standard deviations may not be used to infer statistical significance, but do convey some useful information since they indicate the magnitude of the major source of error.

The precision between assays performed on different occasions has not been checked for any of the three assay systems described, and absolute levels of compounds quoted should therefore be treated with caution. In all cases samples derived from the same experiment were run together in the same assay, and relative concentrations quoted are therefore subject only to the errors imposed by the inter--sample precision of each assay.

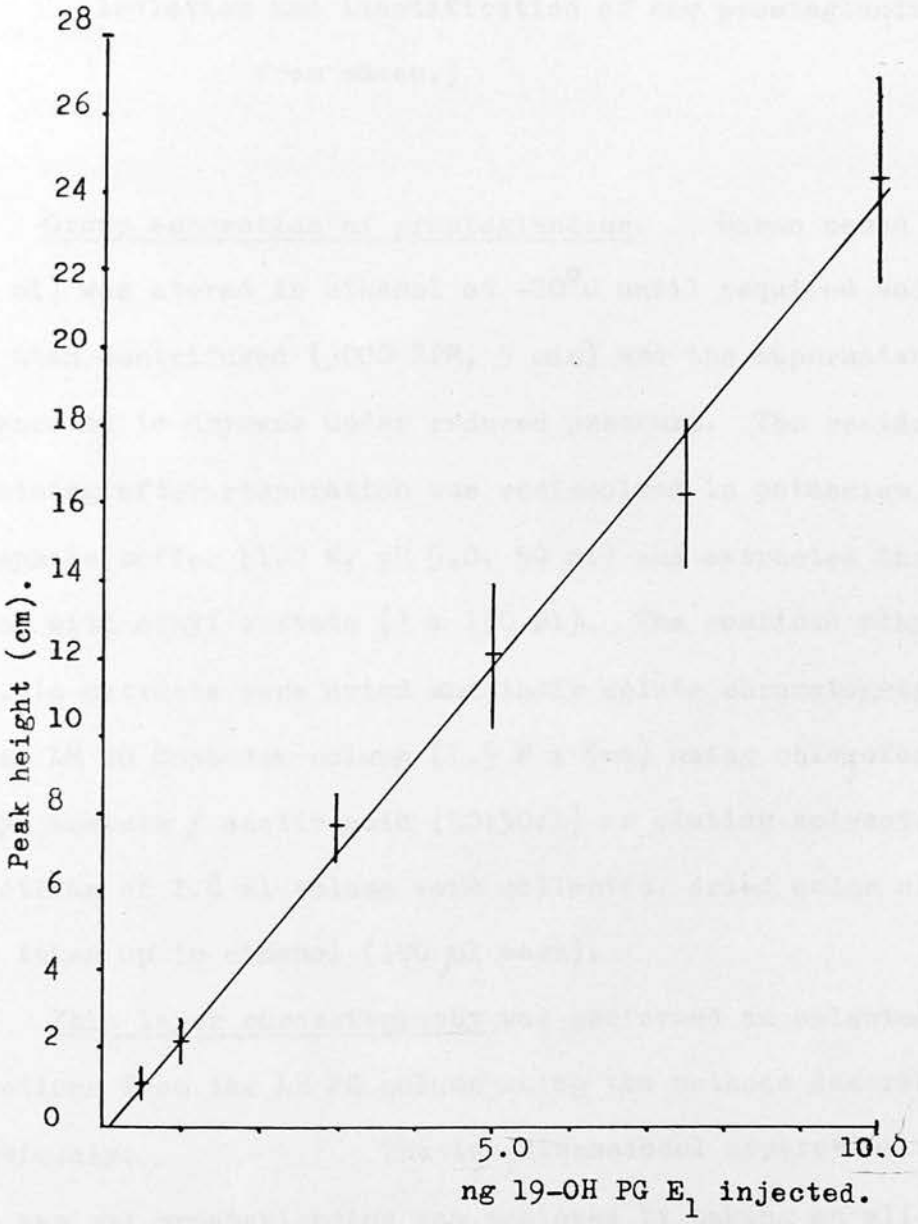


Fig. M.4.

Standard curve for 19-hydroxy PG E_1 assayed by single ion monitoring. Bars represent standard deviation of replicate injections from the same derivatised standard. (n = 6)

Methods specific to chapter 1.

(Isolation and identification of new prostaglandins from semen.)

Group separation of prostaglandins. Human semen (68 ml) was stored in ethanol at -20°C until required and was then centrifuged (3000 RPM, 5 min) and the supernatant evaporated to dryness under reduced pressure. The residue remaining after evaporation was redissolved in potassium phosphate buffer (1.0 M, pH 5.0, 50 ml) and extracted three times with ethyl acetate (3 x 150 ml). The combined ethyl acetate extracts were dried and their solute chromatographed on an LH 20 Sephadex column (1.5 M x 5mm) using chloroform/ ethyl acetate / acetic acid (50:50:1) as eluting solvent. Fractions of 1.8 ml volume were collected, dried under nitrogen and taken up in ethanol (100 µl each).

Thin layer chromatography was performed on selected fractions from the LH 20 column using the methods described previously. The two-dimensional separation of the seminal prostaglandins was achieved by taking an aliquot (5 µl) from each fraction (fractions 1-30), from alternate fractions (31-100) or from every fourth fraction (101-160) and loading onto a 20 x 20 cm TLC plate, which was then developed using solvent system III. The plate was inspected under ultraviolet light, and absorbing or fluorescing spots were ringed. The remaining spots were made visible with sulphuric

acid. Spots were identified by their retention volumes and R_f values, and also by colour (E prostaglandins produce a brown spot, F prostaglandins a pink spot).

Borohydride reduction of 9-ketoprostaglandins was performed by treatment with potassium borohydride (10 mg/ml in methanol / water 2:1) for one hour at room temperature. The solution was acidified (HCl), diluted with water and extracted three times with three volumes of ethyl acetate. The ethyl acetate extracts were combined, dried and the residue chromatographed on the LH 20 Sephadex column described above, in order to separate the resulting F_α and F_β compounds. The F_α compounds were identified by their ability to form n-butyl boronate derivatives, and in all cases eluted from the column before the F_β isomers.

Dehydration of 9-ketoprostaglandins. 9-ketoprostaglandins were dehydrated to the equivalent PG Bs by treatment with strong base (1 N NaOH) for one hour at room temperature, followed by acidification and extraction. 19-hydroxy E prostaglandins may be dehydrated and simultaneously derivatised by making the (ME/TMS) derivative without prior oximation, this process yielding the equivalent A prostaglandin derivatives.

Isomerisation of 9-ketoprostaglandins was performed using the method of Daniels et al (1968). The compounds were treated with potassium acetate (2% in ethanol) for seven days at room temperature followed by dilution with water, acidification and extraction into ethyl acetate. This process yields an equilibrium mixture of E and 8-iso E prostaglandins

containing about 10% of the 8-isoprostaglandin.

Incubation of semen with labelled prostaglandins.

To a single fresh ejaculate (2.8 ml volume) was added (5,6 (n) ^{3}H) PG E_1 (1.0 μCi) and (5,6,8,11,12,14,15 (n) ^{3}H PG $F_{2\alpha}$ (1.0 μCi), each dissolved in ethanol (5 μl). The semen was allowed to stand for four hours at room temperature, and was then precipitated with four volumes of ethanol and stored at - 20°C for seven days. The prostaglandins were then extracted and subjected to chromatography on LH 20 Sephadex using the method described above. 1/10th of each fraction was removed and counted to determine the position of the PG E and PG F peaks and the relative quantities of labelled PG $F_{2\alpha}$ and 8-iso PG $F_{2\alpha}$. The fractions corresponding to the E prostaglandins were pooled and rechromatographed on TLC (system I). The plate was examined using a radioactive TLC plate scanner, and the areas corresponding to PG E_1 and 8-iso PG E_1 were scraped off into scintillation vials and counted to determine the relative proportions of labelled PG E_1 and 8-iso PG E_1.

Prostaglandin D_2. The presence of this prostaglandin in semen was separately investigated using the following method.

Human semen (67.5 ml) was stored in acetone at - 20°C until required. ^{14}C PG D_2 (58,000 DPM) was added and the precipitate centrifuged down. The supernatant was evaporated to dryness under reduced pressure and the residue from this evaporation taken up directly in 0.5 ml chloroform / ethyl acetate / acetic acid (50:50:1) before running on the 1.5 M x

5 mm LH 20 Sephadex column. Fractions of 1.8 ml were collected, dried and taken up in ethanol (1.0 ml). An aliquot of 50 µl was taken from each fraction for scintillation counting, and after location of the radioactive peak, half of each of the corresponding fractions were derivatised (MO/ME/TMS) for GCMS (scanning) and the remaining halves as (O/ME/tBDMS) for single ion monitoring GCMS analysis.

Preparation of crystalline 19-hydroxy PG E_1 from the semen of Macaca arctoides.

Seminal coagulum was collected from the cage floors of two stump-tailed macaques that masturbated frequently. Thirty two specimens were stored in ethanol at - 20°C until required. The original weight of coagulum was not recorded since the samples were inevitably contaminated with faeces and other cage floor detritus.

The coagulum was crushed in a mortar and homogenised in the ethanol used for storage. Solid materials were removed by centrifugation and the supernatant evaporated to dryness under reduced pressure at 40°C. The solid residue from this evaporation was redissolved in potassium phosphate buffer (100 ml, 0.5 M, pH 5.0). This was then extracted first with ether (100 ml) and then three times with ethyl acetate (3 x 300 ml). The ether extract was discarded, and the ethyl acetate extracts combined and dried. The residue was redissolved in 0.5 ml ethyl acetate / chloroform / acetic acid (50/50/1) and loaded onto a 400 x 5 mm LH 20 Sephadex column which was then eluted with the same solvent. 3.2 ml fractions

were collected, the fractions being immediately evaporated under nitrogen and redissolved in ethanol (100 µl). An aliquot (5 µl) was taken from each fraction for GCMS analysis.

Fractions 13 and 14, containing 19-hydroxy PG E_1, were retained, along with fractions 15 - 22 which contained dihydroxy E prostaglandins. Fractions 10 - 12, which contained 19-hydroxy E_1 along with some 19-hydroxy E_2 were pooled and rechromatographed (run 2). Fractions 13 and 14 from run 2 were pooled with fractions 13 and 14 from run 1 and subjected to a third stage of chromatography (run 3). Fractions 13 and 14 from run 3 contained material which partially crystallised on evaporation, and this material was further purified by recrystallisation.

The partially crystalline 19-hydroxy PG E_1 was dissolved in acetone (2 ml) and n-heptane added until a faint precipitate appeared. The solution was then left to evaporate from an open tube at room temperature until the volume was reduced by about half.

<u>18,19-dihydroxy PG E_1.</u> The presence of dihydroxy E prostaglandins in human semen was investigated separately. Semen (30 ml) was precipitated with acetone and separated using the method applied to stump-tailed macaque semen above. The fractions corresponding to the putative dihydroxy E compounds were pooled and derivatised (MO/ME/TMS) for GCMS analysis.

Methods specific to chapter 2.

(Physical properties of the seminal prostaglandins)

Measurement of GLC retention times.

Retention time data for the prostaglandins was produced using the Hewlett-Packard / AEI GCMS combination described previously, and results are quoted as equivalent carbon values relative to the series of n-paraffins. Compounds were derivatised and injected into the GLC along with a mixture of paraffins of known chain length. Peaks were detected by the total ion current monitor of the mass spectrometer, the chromatogram was digitised and the retention times of all peaks recorded by computer using a program written for the purpose. Given the equivalent carbon value of any four peaks, the computer then calculated the slope and intercept of the graph of log (retention time) against carbon value, and using this data calculated the carbon value of all the peaks detected, including those of the reference compounds. The re-calculation of the carbon values of the reference compounds provided a built-in check on the accuracy of the system. Provided that the unknown compounds emerge between the reference compounds the accuracy of the measurement is $\pm$ 0.1 CV.

Measurement of the equivalent carbon values was performed at a column temperature of 260°C for the 19-hydroxy prostaglandins and at 240°C for the remaining compounds. A program listing is given in the appendix.

Methods specific to chapter 3.

(The effect of 19-hydroxy prostaglandin E_1 on isolated myometrial strips.)

Natural 19-hydroxy PG E_1 was prepared from the semen of the stump-tailed macaque using the method described in chapter 1. The effects of this compound were compared with those of PG E_1 and synthetic 19-hydroxy PG E_1 (racemic, and with mixed epimers at C_{19}).

Non-pregnant human tissue was obtained from patients undergoing hysterectomy, and pregnant material was obtained at caesarian section. In all cases the pregnancy was at term, and the tissue was removed from the lower uterine segment. Pregnant marmoset tissue was removed from the upper uterine segment during hysterotomy performed at various stages of gestation.

Human and marmoset tissue was transported in physiological saline and the experiment was commenced within one hour of operation. The tissue was cut into strips approx. 30 x 2 mm (human) or 10 x 1 mm (marmoset) before suspension in the organ bath. Organ baths of 20 ml capacity were used containing Tyrode's solution (pH 7.4), the baths being continuously aerated with oxygen containing 5% carbon dioxide, and maintained at 37°C by an external water jacket. Isometric contractions of the tissue were monitored by means of Washington strain gauges and recorders.

The tissue was allowed to equilibrate in the baths

with occasional washing with fresh solution until a steady series of contractions was obtained. In some cases it proved necessary to stimulate the tissue by the addition of oxytocin (up to 0.75 mU/ml) or prostaglandin E_1 (50 ng/ml). The minimum concentration of oxytocin necessary to sustain activity was used in each case. Prostaglandins were added in solution in up to 5 µl of ethanol, this concentration of ethanol having been shown to have no effect on the activity of the tissue. The effect of addition of prostaglandin was recorded, and the tissue was then washed with two changes of fresh medium. The activity of the tissue was then once more recorded before making the next injection.

Activities were calculated from the chart recordings by measuring the area under an integral number of contractions (the record was photocopied and the areas for measurement cut out and weighed) and dividing this by the corresponding time. This process gives an arbitrary measure of the power output of the muscle strip, taking into account both frequency and amplitude of contraction. The effect of added prostaglandin was expressed as a percentage inhibition, compared to the mean of the activity before injection and the activity after washing with fresh medium.

If the initial activity = a

Activity after injection = b

Activity after washing = c

$$\text{Effect of added prostaglandin} = \frac{((a+c)/2-b) \times 100}{(a+c)/2}$$

This rather cumbersome procedure was necessitated by the fact that the observed activity of the muscle strips varied with respect to time even in the absence of added prostaglandin, and the activity after washing the prostaglandin out was often quite different from the initial activity.

Methods specific to chapter 4.

(*In vitro* biosynthesis of 19-hydroxy prostaglandins.)

Work described in this chapter has been directed toward elucidation of the route of biosynthesis of the 19-hydroxy prostaglandins in the seminal vesicle of the stump-tailed macaque (Macaca arctoides). Seminal vesicles were obtained on two occasions, giving rise to two series of experiments, and since the experimental techniques employed differed, the two series are described separately below.

Series 1.

Stump-tailed macaque reproductive organs were obtained immediately after the animal was killed by exsanguination under ketamine anaesthesia. The seminal vesicles, testis, epididimes, prostate and ampullae were removed, weighed and homogenised in sodium phosphate buffer (50 mM, pH 7.4). An aliquot (1 ml) was taken from each homogenate and extracted and derivatised (MO/ME/TMS) for GCMS analysis. Four further 1 ml aliquots from each homogenate were incubated with either no substrate, eicosa-11,14,17-trienoic acid, arachidonic acid or eicosa-5,8,11,14,17-pentaenoic acid (100 µg each). Incubations were carried out under an atmosphere of oxygen / 5% carbon dioxide at room temperature. After one hour the incubations were stopped by the addition of 5 ml of a solution of methoxyamine hydrochloride (10 mg/ml) in pyridinium acetate buffer (1.5 M, pH 5.0). The material was derivatised (MO/ME/TMS) for GCMS analysis of the synthesised prostaglandins.

The remaining homogenate was stored at -20°C and used for the following experiments in this series.

When stored at -20°C both the activity of the prostaglandin synthetase enzymes and the amount of endogenous 19-hydroxy PG E present decline, and results obtained with the frozen homogenate must be interpreted with this in mind. The following experiments are therefore presented in chronological order, and the time of storage (in days) is indicated in each case.

Time course of 19-hydroxy PG E synthesis. (29 days storage)

Seminal vesicle homogenate (10 ml) was thawed and placed in a 250 ml conical flask containing arachidonic acid (1 mg). The flask was gassed with oxygen / 5% CO_2 and incubated at 37°C. Samples (1 ml) were withdrawn at intervals of zero, 15 min., 30 min., 1, 2, 4 and 8.5 hours. After each withdrawal the flask was gassed, shaken and returned to the incubator. The samples were precipitated with ethanol (10 ml) and stored at -20°C until the following day. The precipitate was removed by centrifugation and the supernatant dried under vacuum. The residue after drying was redissolved in potassium phosphate buffer (10 ml, 1.0 M, pH 5.0) and extracted with ether (10 ml) and ethyl acetate (3 x 30 ml). The combined ethyl acetate extracts were dried and derivatised (MO/ME/TMS) for GLC analysis of 19-hydroxy PG Es. The use of the differential extraction technique provides a cleaner sample for GLC, allowing 19-hydroxy PG Es to be measured down to 1 µg.

Incubation with PG E_1. (43 days storage)

Four incubations were set up in 100 ml conical flasks with thawed seminal vesicle homogenate (2 ml) in each. Two of the flasks contained PG E_1 (70 μg) and the remainder had no additions. The flasks were gassed, incubated (1½ hours) and the contents precipitated, dried, differentially extracted and assayed as before.

Incubation with PG E_2 and PG $F_{2\alpha}$. (46 days storage)

Six incubations were set up as in the previous experiment. To two of the flasks was added PG E_2 (60 μg), while two had PG $F_{2\alpha}$ (57 μg) and the remaining two had no addition.

Series 2.

Reproductive organs were dissected from the animal under Saffan anaesthesia, and transported to the laboratory on ice. The organs were homogenised in ice-cold sodium phosphate buffer (50 mM, pH 7.4) and an aliquot removed from each for prostaglandin assay, which was performed in this case using single ion monitoring (O/ME/tBDMS derivative). Prostaglandin synthesis was investigated only in the case of the seminal vesicle homogenate. Labelled substrates and cofactors were placed in 50 ml conical flasks and dried under vacuum. Seminal vesicle homogenate (1 ml) was added and the flasks were gassed with oxygen, stoppered and incubated on a shaking waterbath (1 hour, 37°C, 140 strokes per minute). Incubations

were stopped by the addition of acetone (50 ml).

Two methods were used for the extraction of prostaglandins prior to chromatography. Initially, the acetone extracts were simply dried under vacuum and taken up in the eluting solvent for transfer to the LH 20 column. This method gave variable and sometimes low recovery of radioactivity, and was later replaced by the following method which gave better results:- After drying, the acetone extracts were taken up in potassium phosphate buffer (50 ml, 1.0 M, pH 5.0) and extracted three times with ethyl acetate (3 x 150 ml). The ethyl acetate extracts were pooled, dried and the residue transferred to the LH 20 column in the eluting solvent.

Chromatography was performed on a 1.5M x 5 mm LH 20 Sephadex column which was eluted with chloroform / ethyl acetate / acetic acid (50:50:1). Fractions of 1.8 ml were collected, dried under nitrogen and taken up in ethanol (100 µl), from which an aliquot (10 µl) was removed for scintillation counting. Products were identified by their retention volumes on the LH 20 column, compared with those of standards.

The remaining seminal vesicle homogenate was stored under liquid nitrogen, and although there was a slight decline in prostaglandin synthetase activity on freezing, no further loss of activity occurred over one year's storage. Incubations with the stored homogenate were performed in the same way as those with the fresh homogenate, only sufficient material being thawed at any one time for the immediate purpose, the main bulk of homogenate remaining at liquid nitrogen temperature

throughout. At the end of the series of experiments described here the capacity of the stored homogenate to synthesise 19-hydroxy PG E_1 from endogenous precursors was checked by means of the following experiment:-

Frozen seminal vesicle homogenate (0.5 g) was thawed in sodium phosphate buffer (5 ml, 50 mMolar, pH 7.4) at 4°C. 2.5 ml of this suspension was then placed in each of two flasks, both of which contained tryptophan and reduced glutathione (both 1 mMolar), and one of which contained indomethacin (20 μMolar). An aliquot (250 μl) was removed immediately from each, and the flasks were then gassed with oxygen and placed on the shaking waterbath. Further samples were removed at 5, 10, 20 and 40 minutes. Samples were immediately oximated and derivatised (O/ME/tBDMS) for single ion monitoring GCMS assay of 19-hydroxy PG E_1.

Methods specific to chapter 5.

(Dynamic aspects of seminal prostaglandin production in man.)

Semen samples were provided by masturbation by a fertile volunteer who had not ejaculated for at least 36 hours before each experiment. Sample volumes were measured after the semen had liquefied (15 minutes at room temperature) and an aliquot (0.5 ml) removed and derivatised (MO/ME/TMS) for GLC analysis of PG Es, 19-hydroxy PG Es and cholesterol.

Serial ejaculates. The subject provided three samples at half hour intervals. The experiment was performed on two occasions, and the results are presented separately in fig. 5.1 A and 5.1 B.

Effect of aspirin. The subject, who had fasted for 14 hours previously, took a single oral dose of 2.1 g soluble aspirin 30 minutes before the experiment, then provided three ejaculates as in the previous experiment.

Effect of prior sexual arousal. The subject masturbated three times at half hour intervals, as in the previous experiments, but on the first two occasions stopped as close as possible to the point of orgasm without ejaculating. The ejaculate produced on the third occasion was compared with normal ejaculates produced on the previous day and on the following day.

Methods specific to chapter 6.

(The species distribution of the 19-hydroxy E prostaglandins.)

Semen samples were obtained by various methods, including voluntary masturbation, electroejaculation, drug-induced ejaculation, artificial vagina and recovery of semen from the vagina after coitus. Semen samples were transported in 4-10 volumes of acetone or ethanol, and stored in these solvents at - 20°C until required. The semen of most species gels after ejaculation, and where necessary these gelled samples were homogenised in the solvent in which they arrived. The homogenates were centrifuged and the supernatants evaporated to dryness before derivatisation (MO/ME/TMS) and GCMS analysis. In all cases where identification of 19-hydroxy prostaglandins is claimed mass spectra were obtained.

Chapter 1.

The isolation and identification of new prostaglandins from semen.

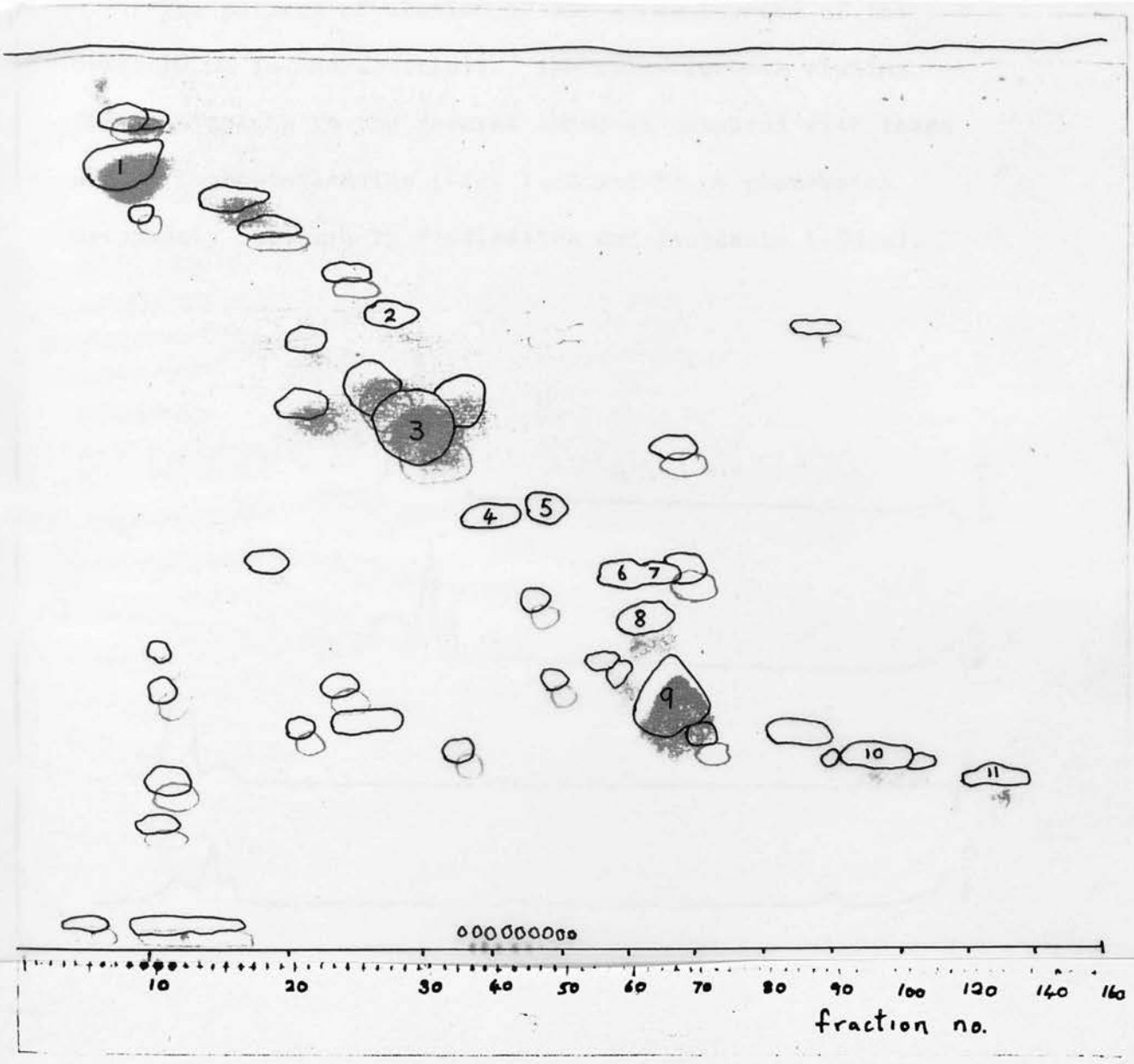
Two dimensional chromatography.

The results of the two dimensional separation of the human seminal prostaglandins are given in fig. 1.1. The diagram was produced by making a Xerox copy of the plate, and a key to the spots identified is given on the overlay.

GCMS analysis of the fractions obtained from the group separation showed the E prostaglandins to be located in fractions 29 - 34, PG Fs in fractions 45 - 55, 8-iso PG Fs in fractions 57 - 64, 19-hydroxy PG Es in fractions 61 - 72, 19-hydroxy PG Fs in fractions 88 - 101 and 8-iso 19-hydroxy PG Fs in fractions 121 - 137. The column did not separate the 8-iso E prostaglandins from the PG Es, nor the 8-iso 19-hydroxy PG Es from the 19-hydroxy PG Es, and these separations were subsequently made by preparative TLC.

8-iso PG Es. Fractions 29 - 34 were pooled, and an aliquot run on TLC system I along with standard PG E_1 and 8-iso PG E_1 showed two spots corresponding to the standard compounds. The remaining material was therefore separated by preparative TLC (system I) into two fractions containing PG Es and 8-iso PG Es respectively. The identity of the 8-iso PG Es was confirmed by comparison of their chromatographic properties with those of the standard compounds. The compounds were shown to have identical retention volumes on the LH 20 column, and mixtures of the biological material with the standards were shown to co-chromatograph on all three TLC systems, and

Fig. 1.1 A two dimensional separation of the seminal prostaglandins.

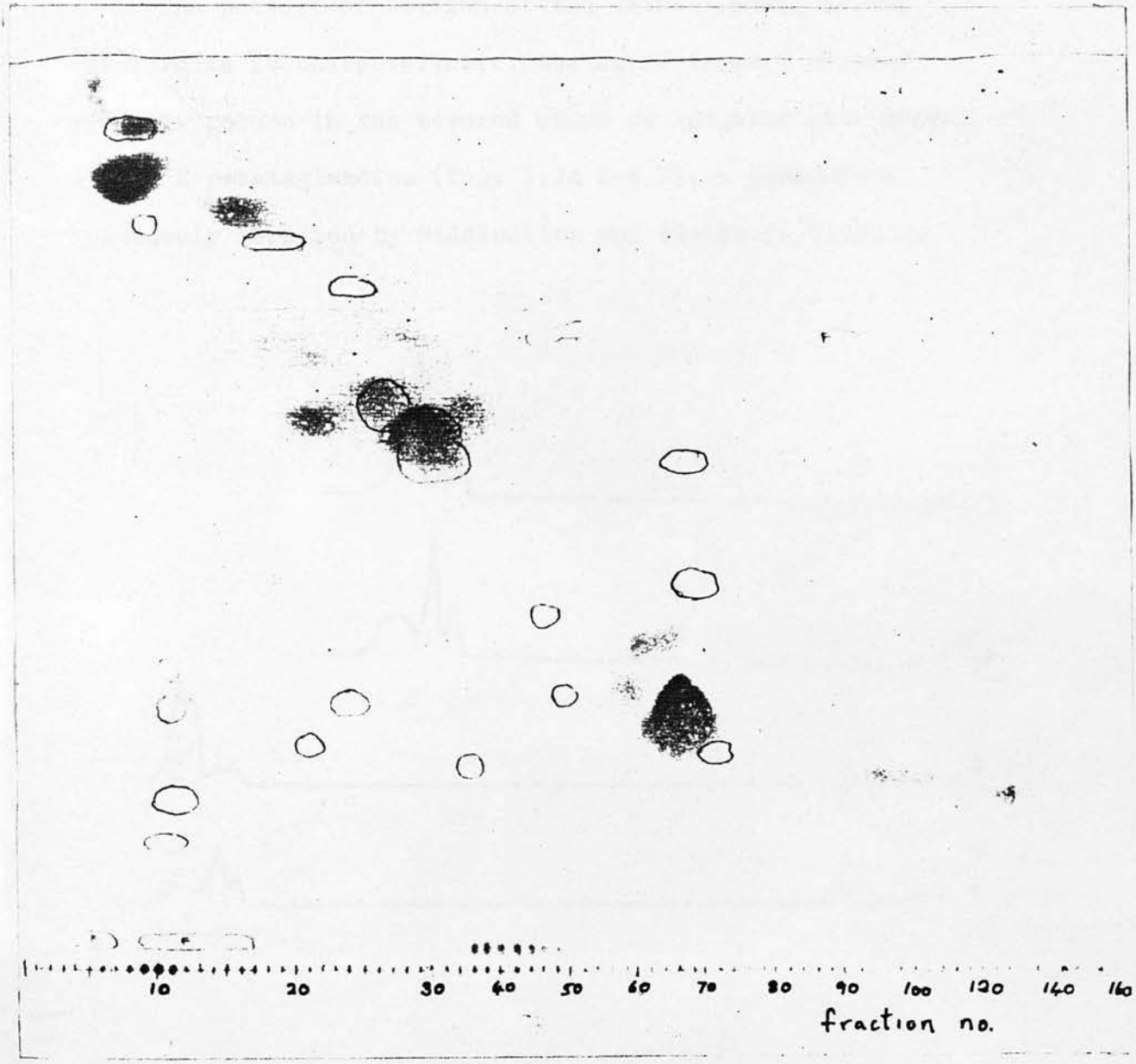


Key to spots identified.

1) Cholesterol
2) 8-iso PG Es
3) PG Es
4) Dinor PG Es
5) PG Fs
6) 8-iso PG Fs
7) Dinor PG Fs
8) 8-iso 19-hydroxy PG Es
9) 19-hydroxy PG Es
10) 19-hydroxy PG Fs
11) 8-iso 19-hydroxy PG Fs

(Spots ringed on the plate itself are visible under u/v)

Fig. 1.1 A two dimensional separation of the seminal prostaglandins.



Key to spots identified.

1) Cholesterol
2) 8-iso PG Es
3) PG Es
4) Dinor PG Es
5) PG Fs
6) 8-iso PG Fs
7) Dinor PG Fs
8) 8-iso 19-hydroxy PG Es
9) 19-hydroxy PG Es
10) 19-hydroxy PG Fs
11) 8-iso 19-hydroxy PG Fs

(Spots ringed on the plate itself are visible under u/v)

after derivatisation (MO/ME/TMS) on GLC.

The pattern of elution of the oxime isomers of the 8-iso PG Es is characteristic, the oxime isomers eluting from the column in the reverse order as compared with those of the E prostaglandins (fig. 1.2A and B), a phenomenon previously reported by Middleditch and Desiderio (1973a).

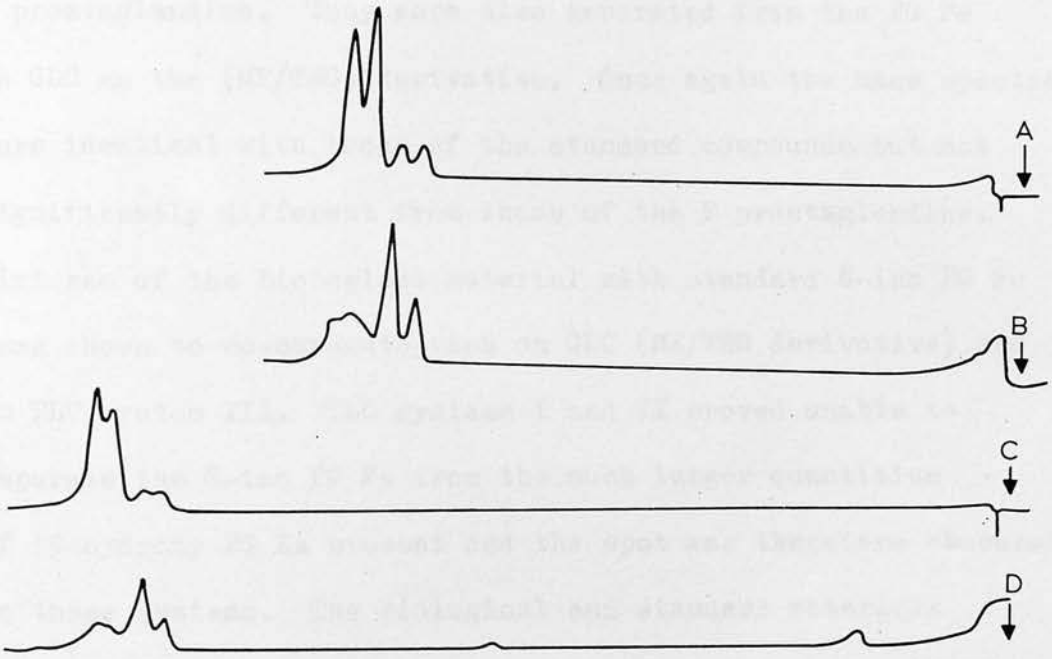


Fig. 1.2 GLC patterns of elution of 9-keto prostaglandins extracted from human semen. All compounds were derivatised as the MO/ME/TMS derivative, and the column temperature was programmed from 200°C at 2°/min. A. E prostaglandins; B. 8-iso E prostaglandins; C. 19-hydroxy E prostaglandins; D. 8-iso 19-hydroxy E prostaglandins. (Each pair of compounds produces four peaks owing to the syn/anti isomerism of the oxime derivative, the order of elution being E_2 E_1 E_2 E_1 in each case. The order of elution of the major and minor oxime isomers reverses between the parent and 8-isoprostaglandin in both cases.)

The mass spectra of the derivatised compounds were identical with those of the standards; however, the spectra did not differ significantly from those of the E prostaglandins, in agreement with the findings of Daniels et al (1968).

8-iso PG Fs. The 8-iso PG Fs were completely separated from the F prostaglandins by LH 20, although the fractions containing them also contained large amounts of 19-hydroxy E prostaglandins. They were also separated from the PG Fs on GLC as the (ME/TMS) derivative. Once again the mass spectra were identical with those of the standard compounds but not significantly different from those of the F prostaglandins. Mixtures of the biological material with standard 8-iso PG Fs were shown to co-chromatograph on GLC (ME/TMS derivative) and on TLC system III. TLC systems I and II proved unable to separate the 8-iso PG Fs from the much larger quantities of 19-hydroxy PG Es present and the spot was therefore obscured in these systems. The biological and standard materials were shown to have identical retention volumes on the LH 20 column.

8-iso 19-hydroxy PG Es. Fractions 65 - 72 were pooled and treated in a similar manner to those containing the E prostaglandins. Preparative TLC was carried out on the more polar system III, again yielding two fractions containing 19-hydroxy PG Es and 8-iso 19-hydroxy PG Es respectively. After derivatisation (MO/ME/TMS) the 8-iso compounds were shown to elute from the GLC column with the major oxime isomers first, in a pattern closely resembling that observed for the

8-iso PG Es (fig. 1.2C and D). The mass spectra of the four peaks were again identical with those of the 19-hydroxy E prostaglandins.

Treatment of the 8-iso 19-hydroxy E fraction with strong base yielded a pair of compounds identical with 19-hydroxy PG B_1 and 19-hydroxy PG B_2, as judged by comparison of their mass spectra, GLC retention times and R_f values on TLC with those of authentic compounds prepared in the same way from 19-hydroxy PG E_1 and 19-hydroxy PG E_2. Treatment of an aliquot from either the 19-hydroxy PG E fraction or the 8-iso 19-hydroxy PG E fraction with mild base yielded an equilibrium mixture of the two epimers containing about 10% 8-iso 19-hydroxy PG Es.

Treatment of an aliquot from the 8-iso 19-hydroxy PG E fraction with potassium borohydride gave a mixture of four compounds, separable into two groups by LH 20 chromatography or TLC. One of these groups was shown to contain F_α compounds, judged by their ability to form n-butyl boronate derivatives, and to be identical with the 8-iso 19-hydroxy PG F compounds described below on the basis of their mass spectra and chromatographic properties.

19-hydroxy F prostaglandins. Fractions 88 - 101 were shown to contain compounds whose mass spectra were consistent with a 19-hydroxy F prostaglandin structure (mass spectra described in section 2b). The identity of the 19-hydroxy PG $F_{2\alpha}$ compound was confirmed by comparison of its mass spectrum, GLC retention time and R_f values on TLC with

those of authentic 19-hydroxy PG $F_{2\alpha}$. 19-hydroxy PG $F_{2\alpha}$ was accompanied by an approximately equal amount of the analagous 1 series compound, 19-hydroxy PG $F_{1\alpha}$.

8-iso 19-hydroxy PG Fs. As with the 8-iso PG Fs the 8-iso 19-hydroxy PG Fs were completely separated from their parent compounds on LH 20. Fractions 121 - 137 were pooled and examined by TLC and GCMS. Once again the mass spectra of the 8-iso 19-hydroxy PG Fs were very similar to those of the parent compounds. The compounds could be separated from the 19-hydroxy PG Fs by GLC as the (ME/TMS) or (BB/ME/TMS) derivatives, or by TLC systems II and III.

The R_f values on TLC and retention times on GLC of all the 8-isoprostaglandins and their respective parent compounds are given in chapter 2 (page 166).

Other isoprostaglandins. During the preparation of the 8-iso E prostaglandins described above a third spot was observed on the TLC plate used to separate 8-iso PG E from PG E. Elution of this area of the plate followed by derivatisation (MO/ME/TMS) and GCMS analysis showed the presence of a pair of compounds whose mass spectra closely resembled those of PG E_1 and PG E_2. The separation between these compounds and the E prostaglandins was sufficient to ensure that the unknown isoprostaglandins were not the result of contamination by the parent compounds (R_f of the PG Es was 0.22, that of the unknown compounds 0.46 in TLC system I).

Each compound appeared to give only a single peak on the GLC trace corresponding in retention time to that of

the second oxime isomer of the parent E prostaglandins. The quantity of the unknown isoprostaglandins present was extremely small, and it was necessary to derivatise and inject the entire preparation (corresponding to 68 ml semen) in order to obtain satisfactory mass spectra. Inspection of the TLC plate used to separate the 8-iso 19-hydroxy E prostaglandins from their parent compounds showed the presence of analagous 19-hydroxy isoprostaglandins. In view of the very small quantities of these compounds present no further investigation of their structure was undertaken.

Dinor E prostaglandins. Examination of fractions 36-43 by GCMS showed the presence of compounds corresponding to the eighteen carbon prostaglandins dinor PG E_1 and dinor PG E_2. These compounds were separated on the LH 20 column, the 1 series compound eluting first in fractions 36 - 40, followed by dinor PG E_2 in fractions 40 - 43. Examination of the mass spectra of these compounds showed them to resemble those of the E prostaglandins, with the exception that all fragments derived from the C_1 side chain were shifted downward in mass by 28 a.m.u.

The spectrum of authentic dinor PG E_2 showed the same peaks as those of the biological material, however, there were differences in the proportions of the various ions, and repeated scanning of the natural and standard materials under identical conditions showed that these differences were real, and not attributable to statistical variations in the ion current during scanning. It must be concluded therefore that

the dinor E prostaglandins of semen are isomeric with the authentic compounds. The nature of this isomerism remains to be established. (Mass spectra of the dinor prostaglandins E are discussed in detail in chapter 2).

Dinor F prostaglandins. Further GCMS analysis of the fractions containing the 8-iso PG Fs (57 - 64) showed the presence of a peak with a much shorter retention time on GLC than that of the 8-iso PG Fs (ME/TMS). Unlike other prostaglandins studied so far the 1 and 2 series dinor F prostaglandins did not separate from each other on GLC. Examination of the mass spectrum produced by the single GLC peak showed that all fragments which include the C_1 side chain produce double peaks separated by two atomic mass units. The spectrum may therefore be interpreted as a mixed spectrum of two compounds differing from one another by the presence of one double bond in the C_1 side chain. The mass spectrum of the (ME/TMS) derivative closely resembles those of PG $F_{1\alpha}$ and PG $F_{2\alpha}$ except that all fragments which include the C_1 side chain are shifted downward in mass by 28 a.m.u.

The GLC retention time of these compounds was identical with that of authentic dinor PG $F_{2\alpha}$ and as far as can be judged from a mixed spectrum the mass spectrum closely resembles that of the authentic compound. However, in view of the inability to separate the two compounds, and of demonstrated differences between the natural and authentic dinor E compounds described above, it would be premature at this stage to conclude

that the structure of these compounds has been definitely established.

Prostaglandin D_2. The radioactive peak corresponding to PG D_2 was located in fractions 24 - 26 and the recovery of radioactivity was 16%. There was no detectable amount of PG D_2 present in these fractions by single ion monitoring GCMS. After correcting for losses using the figure for recovery of radioactivity the measured level in the original semen was less than 100 ng/ml. Examination of fraction 27 (where PG D_1 would be expected) showed a significant quantity of PG E_2, in the presence of which low levels of PG D_1 would not be measurable.

Dihydroxy E prostaglandins. No appreciable quantity of dihydroxy E prostaglandins could be detected in human semen using the methods described. In the absence of standard compounds it is not possible to quote a "less than" figure for the dihydroxy PG Es, but if present at all they can be no more than a minor component of the human seminal prostaglandins.

Incubation of semen with labelled prostaglandins. There was very little conversion of the labelled prostaglandins in this experiment (fig 1.3). In particular, the results of the LH 20 separation show no peaks corresponding to dinor PG Es, dinor PG Fs, 8-iso PG Fs or any 19-hydroxy prostaglandins. The radioactivity corresponding to the E prostaglandins migrated as a single band on TLC, and elution of the area of the plate corresponding to the 8-iso E prostaglandins showed less than 2% of the recovered radioactivity to be

present, a quantity comparable to that obtained when the separation was performed with unincubated ^{3}H PG E_1.

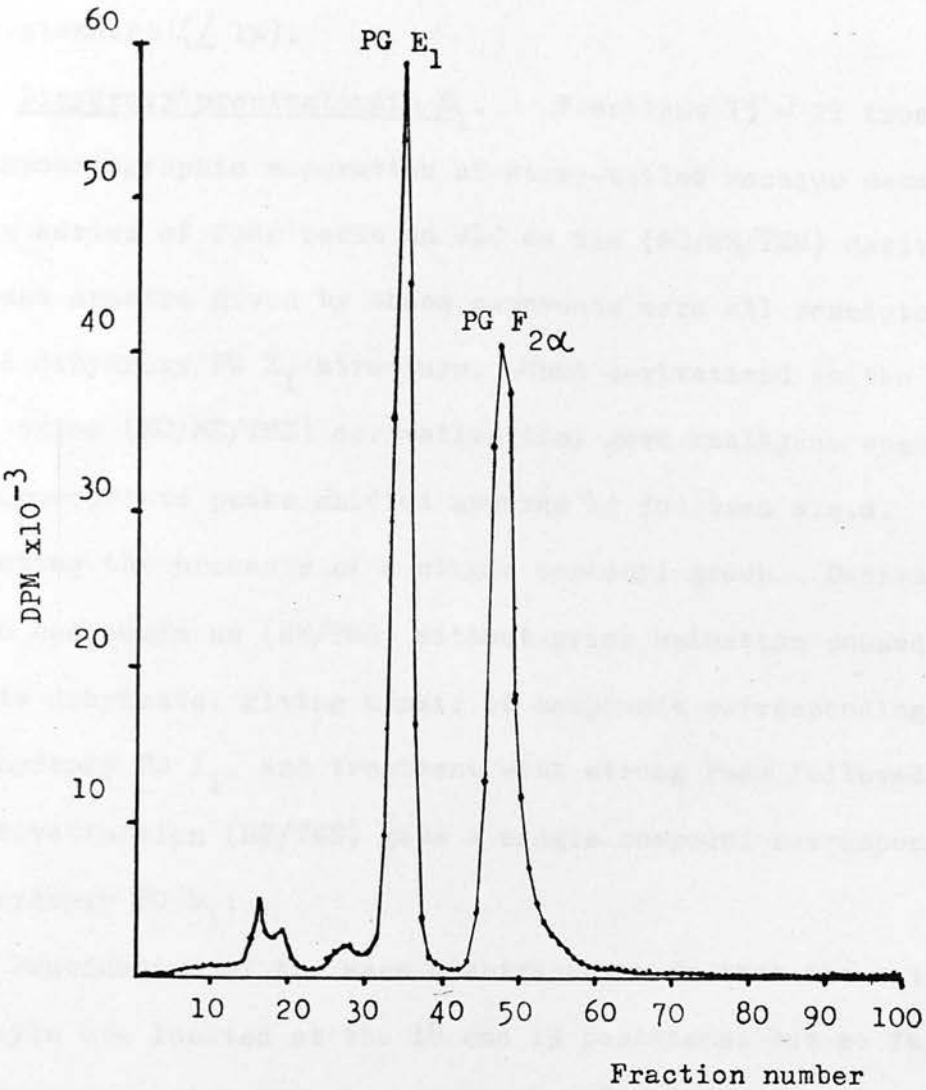


Fig. 1.3 Incubation of labelled PG E_1 and PG $F_{2\alpha}$ with human semen, showing no conversion of these compounds to compounds more polar than PG E_1.

Preparation of 19-hydroxy PG E_1 from the semen of Macaca arctoides.

One milligram of 19-hydroxy PG E_1 was obtained after recrystallisation, the mother liquor yielding 15 mg of less pure material on drying. 19-hydroxy PG E_1 crystallises from acetone / heptane in small colourless monoclinic crystals

which melt with decomposition at 129 - 131 °C. GLC analysis of the purified 19-hydroxy PG E_1 showed no measurable amounts of 19-hydroxy PG E_2 (< 5%), 19-hydroxy PG Fs (< 5%), E or F prostaglandins (< 1%).

Dihydroxy prostaglandin E_1. Fractions 15 - 22 from the chromatographic separation of stump-tailed macaque semen gave a series of four peaks on GLC as the (MO/ME/TMS) derivative. The mass spectra given by these compounds were all consistent with a dihydroxy PG E_1 structure. When derivatised as the ethyl oxime (EO/ME/TMS) derivative they gave analagous spectra, with appropriate peaks shifted upwards by fourteen a.m.u. indicating the presence of a single carbonyl group. Derivatisation of the compounds as (ME/TMS) without prior oximation caused them to dehydrate, giving a pair of compounds corresponding to dihydroxy PG A_1, and treatment with strong base followed by derivatisation (ME/TMS) gave a single compound corresponding to dihydroxy PG B_1.

Examination of the mass spectra suggests that the extra hydroxyls are located at the 18 and 19 positions, but so far attempts to confirm this by making the 18,19 butyl boronate or cleaving the C_{18-19} bond with periodate have yielded no identifiable compounds. Identification of these compounds therefore rests solely on the interpretation of the mass spectra, which are discussed in detail in chapter 2.

Discussion.

Evidence has been presented for the presence of a large number of previously undiscovered prostaglandins in semen. In the case of the compounds for which authentic reference standards were available, 19-hydroxy PG $F_{2\alpha}$, 8-iso PG E_1, 8-iso PG E_2, 8-iso PG $F_{1\alpha}$ and 8-iso PG $F_{2\alpha}$ the evidence may be regarded as conclusive, since the compounds were shown to be identical with the reference standards both by mass spectrometry and by co-chromatography in several systems. The established identity of 19-hydroxy PG $F_{2\alpha}$ makes the structure of the accompanying 19-hydroxy PG $F_{1\alpha}$ certain.

Evidence for the structure of the 8-iso 19-hydroxy prostaglandins is circumstantial, although extremely strong. The close similarity between the mass spectra of these compounds and their parent 19-hydroxy prostaglandins indicates that they are isomeric with the parent compounds, and that they are epimers rather than positional isomers. The conversion of the iso 19-hydroxy E prostaglandins to iso 19-hydroxy PG Fs by borohydride reduction indicates that the same type of isomerism is present in both groups of compounds. Dehydration of the iso 19-hydroxy PG Es to 19-hydroxy PG Bs by base treatment indicates that the site of isomerism is at C_8, C_{12} or C_{11}, and the ability of the compounds to isomerise back to the parent prostaglandins in the presence of mild base indicates that the site of epimerisation is adjacent to an enolysable carbonyl, strongly suggesting C_8. This assignment is supported

by the observation that the pattern of elution of the oxime isomers on GLC resembles that of the 8-iso E prostaglandins. Rigorous proof of the structure of these prostaglandins awaits either the synthesis of authentic 8-iso 19-hydroxy prostaglandins, or the isolation from semen of sufficient quantities of the pure compounds to enable physical methods such as optical rotatory dispersion spectrometry to be used.

Evidence for the structure of the dihydroxy E prostaglandins, detected in the semen of the stump-tailed macaque but not in human semen, is based entirely on interpretation of the mass spectra of the compounds and of their dehydrated derivatives. In the absence of chemical degradation studies such evidence cannot be regarded as conclusive, and the postulated location of the two extra hydroxyls at C_{18} and C_{19} must, for the time being, be regarded as merely the most probable out of several possible structures. Two isomeric compounds corresponding to dihydroxy PG E_1 were detected, and although the nature of this isomerism has not been specifically investigated some conclusions can be drawn from the evidence presented. Once again the close similarity between the mass spectra of the two compounds indicates that they are epimers, and the loss of this epimerisation on dehydration to the equivalent dihydroxy PG B_1 implies that the chiral centre is at C_8, C_{12} or C_{11}. Since dehydration to dihydroxy PG A_1 gives a pair of compounds C_{11} may be eliminated. Of the remaining two possibilities, C_{12} seems the more probable, since the pattern of elution of the oxime isomers does not resemble

that observed in the case of 8-iso PG E nor 8-iso 19-hydroxy PG E (fig 1.2).

Mass spectral evidence indicates that the compound provisionally identified as dinor PG E_2 is not identical with authentic dinor PG E_2. There is no evidence of positional isomerism, and it must be concluded once more that the compound present in semen is an epimer of dinor PG E_2. The pattern of elution of the oxime isomers of the seminal dinor E prostaglandins resembles that of the E prostaglandins rather than that of the 8-iso PG Es, and it seems improbable therefore that the site of epimerisation is located at C_6 in this case. Further work is required to establish the configuration of the seminal dinor E prostaglandins.

The seminal dinor F prostaglandins similarly require further study, since the lack of separation between the 1 and 2 series compounds on GLC implies structural differences between these compounds and the known prostaglandins. The structures assigned to these eighteen-carbon F prostaglandins must meanwhile be regarded as tentative.

The results of the incubation of semen with PG E_1 and PG $F_{2\alpha}$ indicate that none of the compounds described above are artifacts produced from the classical prostaglandins by the techniques employed in storage and handling of the semen extracts. Furthermore, if the new prostaglandins are metabolites of the classical prostaglandins, they are already present at the time of ejaculation, and are not produced by the action of enzymes present in the seminal plasma.

Of the compounds described, 8-iso PG E_1 has been reported previously as a product of the incubation of eicosa-8,11,14-trienoic acid with seminal vesicle homogenates (Daniels et al, 1968), and dinor PG $F_{1\alpha}$ has been reported to be a urinary metabolite of PG $F_{1\alpha}$ in the rat (Granstrom et al, 1965), although neither compound has been previously described in semen. Parts of the work described above have been published or submitted for publication (Taylor and Kelly, 1975; Taylor and Kelly, 1979; Taylor, 1979).

Estimates have been made of the levels of all of the compounds mentioned in semen, although the quality of these measurements is variable. In some cases measurements have been made on fertile semen, using well-characterised assays calibrated against authentic reference compounds (Templeton et al, 1978). In other cases no suitable reference compounds were available, and only preliminary estimates on pooled semen originating from the infertility clinic could be made. In the case of the 8-isoprostaglandins, the ratio of the GLC detector response $\frac{\text{8-iso PG}}{\text{parent PG}}$ was measured, and the level of the 8-isoprostaglandin calculated from published measurements of the parent prostaglandins. Some measurements of this type are included in the synoptic table which follows, the figure quoted being preceded by "ca", indicating that it should be regarded merely as a first order approximation.

Table 1.1 summarises current knowlege of structures and levels of prostaglandins in human semen. The structural formulae, systematic and trivial names are given in the first

two columns, and since the table includes all compounds mentioned in the text of this thesis some compounds are included which have not been shown to be present in semen. The third column, titled "status", gives an indication of the strength of evidence for the quoted formula, and the fourth column cites the appropriate reference to support this. The column titled "concentration" gives the measured level (in fertile human semen unless otherwise indicated). The column titled "n" gives the number of samples on which the measurements were made, or in the case of pooled semen originating from the R.I.E. infertility clinic the abbreviation "IC" indicates this. The final column cites references to support the levels quoted.

A key to the status codes and reference numbers is given at the end of the table.

Table 1.1 Synopsis of seminal prostaglandins

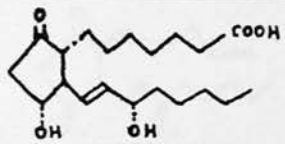
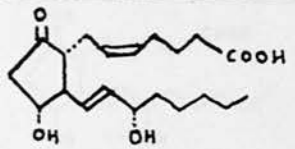
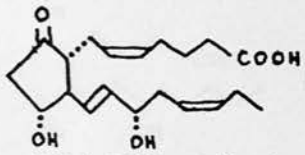
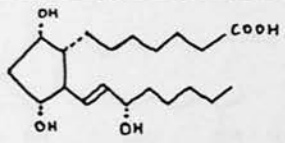
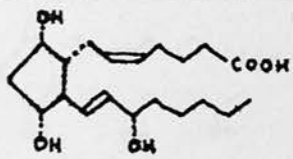
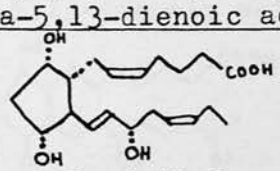
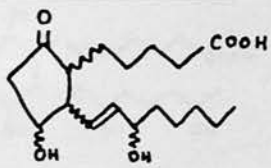
Structure and systematic name	Trivial name	Status	Ref	Concn $\mu g/ml \pm \sigma$	n	Ref
 9 keto 11α,15S, dihydroxy prosta-13-enoic acid	PG E_1	P	6	40 ± 35	17	1
				84 ± 63	10	2
				73 ± 72	22	3
 9 keto 11α,15S, dihydroxy prosta-5,13-dienoic acid	PG E_2	P	7	58 ± 39	10	2
				47 ± 45	17	1
 9 keto 11α,15S, dihydroxy prosta-5,13,17-trienoic acid	PG E_3	P	8	5.5	6	5
 9α,11α,15S, trihydroxy prosta-13-enoic acid	PG $F_{1\alpha}$	P	6	1.1 ± 1.2	17	1
				2.1 ± 1.8	17	3
 9α,11α,15S, trihydroxy prosta-5,13-dienoic acid	PG $F_{2\alpha}$	P	7	0.91 ± 0.73	17	1
 9α,11α,15S, trihydroxy prosta-5,13,17-trienoic acid	PG $F_{3\alpha}$	P	15	NI		
 7 keto 9,13, dihydroxy dinor isoprosta-11-enoic acid	dinor iso-PG E_1	C	4	ca 5	IC	1

Table 1.1 Synopsis of seminal prostaglandins (cont.)

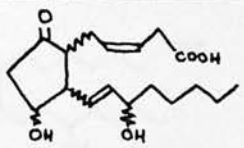
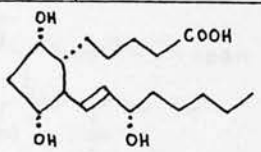
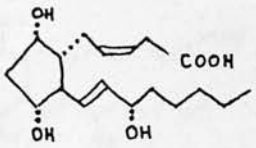
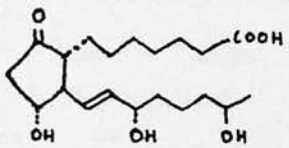
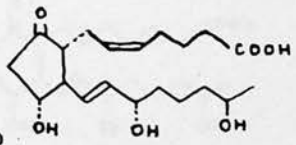
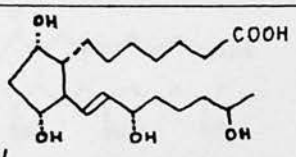
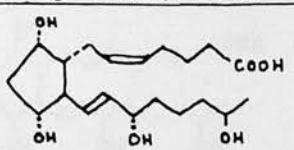
Structure and systematic name	Trivial name	Status	Ref	Concn $\mu g/ml \pm \sigma$	n	Ref
 7 keto 9,13, dihydroxy dinor-isoprosta-3,11-dienoic acid	dinor iso-PG E_2	C	4	ca 3	IC	1
 7α,9α, 13S, trihydroxy dinor-prosta-11-enoic acid	dinor PG $F_{1\alpha}$	C	4	ca 3	IC	1
 7α,9α, 13S, trihydroxy dinor-prosta-3,11-dienoic acid	dinor PG $F_{2\alpha}$	C	4	3.8 ± 2.9	6	1
 9 keto 11α,15S,19R, trihydroxy prosta-13-enoic acid	19-hydroxy PG E_1	P	9	160 ± 151 138 ± 100 267 ± 240	17 10 22	1 2 3
 9 keto 11α,15S,19R, trihydroxy prosta-5,13-dienoic acid	19-hydroxy PG E_2	P	9	144 ± 116 389 ± 275	17 10	1 2
 9α,11α, 15S,19R, tetrahydroxy prosta-13-enoic acid	19-hydroxy PG $F_{1\alpha}$	P	10	5.7 ± 3.6 18 ± 14	16 16	1 3
 9α,11α, 15S,19R, tetrahydroxy prosta-5,13-dienoic acid	19-hydroxy PG $F_{2\alpha}$	P	10	12.6 ± 11	16	1

Table 1.1 Synopsis of seminal prostaglandins (cont.)

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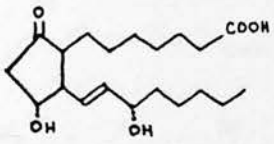
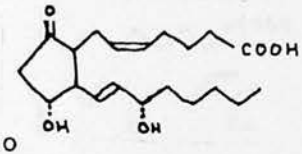
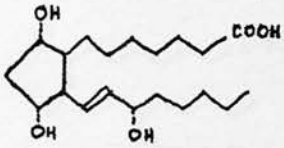
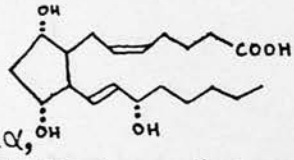
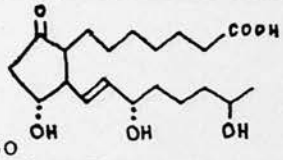
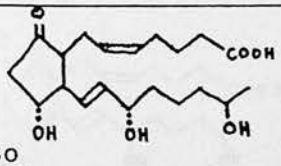
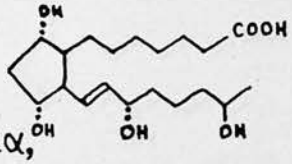
 9 keto 11α,15S,dihydroxy 8-isoprosta-13-enoic acid	8-iso PG E_1	P	11	ca 4	IC	12
 9 keto 11α,15S, dihydroxy 8-iso-prosta-5,13-dienoic acid	8-iso PG E_2	P	12	ca 2.5	IC	12
 9α,11α,15S, trihydroxy 8-isoprosta-13-enoic acid	8-iso PG $F_{1\alpha}$	P	12	ca 1	IC	12
 9α,11α, 15S, trihydroxy 8-iso-prosta-5,13-dienoic acid	8-iso PG $F_{2\alpha}$	P	12	ca 0.2	IC	12
 9 keto 11α,15S,19R, trihydroxy 8-isoprosta-13-enoic acid	19-hydroxy 8-iso PG E_1	C	12	ca 11	IC	12
 9 keto 11α,15S,19R, trihydroxy 8-iso prosta-5,13-dienoic acid	19-hydroxy 8-iso PG E_2	C	12	ca 5	IC	12
 9α,11α, 15S,19R, tetrahydroxy 8-isoprosta-13-enoic acid	19-hydroxy 8-iso PG $F_{1\alpha}$	C	12	ca 12	IC	12

Table 1.1 Synopsis of seminal prostaglandins (cont.)

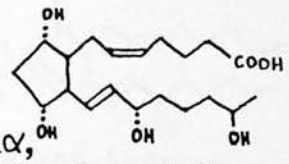
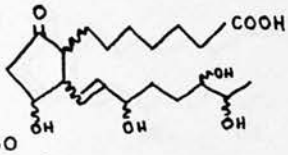
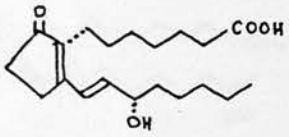
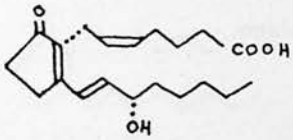
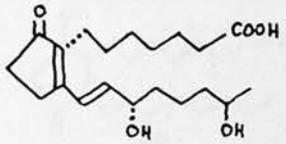
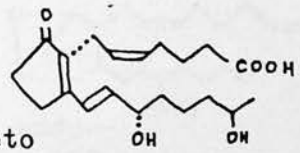
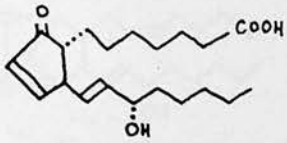
Structure and systematic name	Trivial name	Status	Ref	Concn $\mu g/ml \pm \sigma$	n	Ref
 9α,11α, 15S,19R, tetrahydroxy 8-iso-prosta-5,13-dienoic acid	19-hydroxy 8-iso PG $F_{2\alpha}$	C	12	ca 5	IC	12
 9 keto 11,15,18,19 tetrahydroxy prosta-13-enoic acid	di-hydroxy PG E_1	C	4	ND (human) ca 10 (Macaca arctoides)		4
 9 keto 15S hydroxy prosta-8(12),13-dienoic acid	PG B_1	P	13	Probably absent from fresh human semen		14
 9 keto 15S hydroxy prosta-5,8(12),13-trienoic acid	PG B_2	P	13			
 9 keto 15S,19R dihydroxy prosta-8(12),13-dienoic acid	19-hydroxy PG B_1	P	13			
 9 keto 15S,19R dihydroxy prosta-5,8(12),13-trienoic acid	19-hydroxy PG B_2	P	13			
 9 keto 15S hydroxy prosta-10,13-dienoic acid	PG A_1	P	13			

Table 1.1 Synopsis of seminal prostaglandins (cont.)

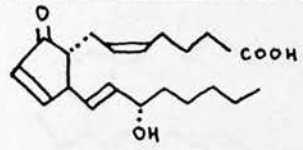
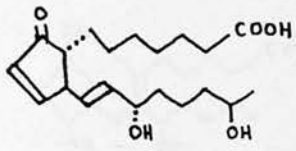
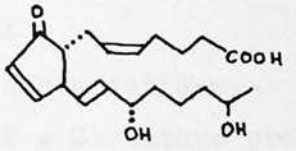
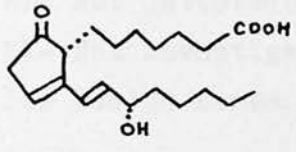
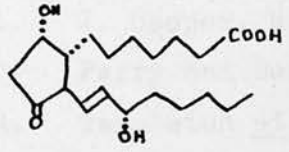
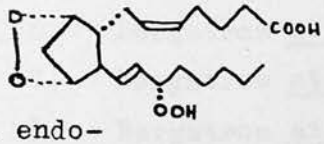
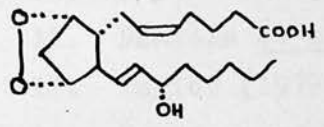
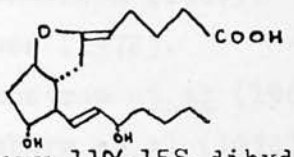
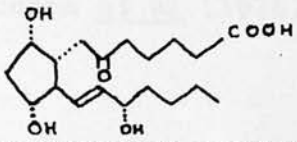
Structure and systematic name	Trivial name	Status	Ref	Concn $\mu g/ml \pm \sigma$	n	Ref
 9 keto 15S hydroxy prosta-5,10,13-trienoic acid	PG A_2	P	13	Probably absent from fresh human semen		14
 9 keto 15S,19R dihydroxy prosta-10,13-dienoic acid	19-hydroxy PG A_1	P	13			
 9 keto 15S,19R dihydroxy prosta-5,10,13-trienoic acid	19-hydroxy PG A_2	P	13			
 9 keto 15S hydroxy prosta-11,13-dienoic acid	PG C_1	P	16			
 11 keto 9α,15S dihydroxy prosta-13-enoic acid.	PG D_1	P	17	< 0.1	IC	4
 9,11 endo-peroxy 15S hydroperoxy prosta-5,13-dienoic acid	PG G_2	P	18	ND	1	19
 9,11 endoperoxy 15S hydroxy prosta-5,13-dienoic acid	PG H_2	P	18	ND	1	19

Table 1.1 Synopsis of seminal prostaglandins (cont.)

Structure and systematic name	Trivial name	Status	Ref	Concn ug/ml $\pm\sigma$	n	Ref
 6(9) epoxy 11α,15S dihydroxy prosta-5,13-dienoic acid	PG I_2	P	20	ND	IC	19
 6 keto 9α,11α,15S trihydroxy prosta-13-enoic acid	6 keto PG $F_{1\alpha}$	P	20	ND	IC	19

Notes.

Abbreviations:-

P = Structure proven.

C = Structure depends on circumstantial evidence.

ND= Not detected.

NI= Not investigated.

IC= Pooled semen from infertility clinic.

References:-

1. I. Cooper, R.W. Kelly and A. Templeton (unpublished).
2. Perry and Desiderio (1977).
3. Templeton et al (1978).
4. P.L. Taylor (this thesis).
5. Bygdeman and Samuelsson (1966).
6. Bergstrom et al (1962c)
7. Bergstrom et al (1962a), Samuelsson (1963b, c)
8. Bergstrom et al (1962a), Samuelsson (1963a).
9. Taylor and Kelly (1974).
10. Taylor and Kelly (1975).
11. Daniels et al (1968).
12. Taylor (1979).
13. Hamberg and Samuelsson (1966a, b).

Continued overleaf.

14. Jonsson *et al* (1976).
15. Samuelsson (1964).
16. Jones (1972).
17. Granstrom *et al* (1968).
18. Hamberg *et al* (1974).
19. R.W. Kelly (unpublished).
20. Johnson *et al* (1976).

Chapter 2. The physical properties of the seminal prostaglandins.

Introduction.

Since much of the work described in this thesis has involved mass spectrometry, the bulk of this chapter will be taken up with descriptions of the mass spectra of the newly discovered prostaglandins. My main concern, in interpreting these mass spectra, has been to identify the parts of the prostaglandin molecule responsible for the peaks observed, thus providing evidence to support the postulated structure. The actual mechanism of fragmentation and rearrangement together with the final structures of the ions have in general not been considered, being outside the scope of this thesis. In the tables which follow some ions are represented by structural formulae: these are used simply as a shorthand method of depicting the original location of the atoms in the prostaglandin molecule and do not necessarily represent the structure of the ion as it arrives at the collector.

All of the mass spectra depicted in figures 2.1 - 2.6 were recorded by scanning GLC peaks, and in most cases the background spectrum has been subtracted. This method of operation introduces certain unavoidable distortions into the recorded spectra, while also providing more than commensurate advantages. The use of the GLC inlet system was mandatory, since only very rarely were pure compounds available for analysis. With the mass spectrometer and data

system used, the maximum practicable scanning speed was sixteen seconds per decade, and the time required for a full scan from about m/e 800 to m/e 28 was therefore approximately thirty seconds. In most cases, this represented a substantial fraction of the time taken for the GLC peak to pass through the system, and the amount of sample present in the source therefore varied considerably throughout the duration of the scan. By choosing to start each scan at or near the maximum of the GLC peak it was possible to emphasise the high-mass end of the spectrum, increasing the probability of detecting the molecular ion and other useful high mass fragments, and all the spectra reproduced here are biassed in this fashion. Background subtraction produces cleaner spectra, but inevitably sample ions whose masses coincide with those of the background are reduced or lost in the process, this applying particularly to m/e 207 in the spectra of PG E_1 and 19-hydroxy PG E_1 (MO/ME/TMS) and m/e 73, 75 and 77 in (TMS) spectra generally.

Identification of the peaks in mass spectra was performed by comparison of the spectra of different derivatives of the same compound (a shift of 14 a.m.u. in a peak derived from an ethyl ester derivative compared with that of a methyl ester indicates that the ion includes the carboxyl group for example), by comparison of closely related compounds (a shift of 2 a.m.u. in the spectrum of a 1 series prostaglandin compared with that of the 2 series compound indicates an ion which includes C_{5-6}) or simply assumed to arise by mechanisms

analagous to those known to occur in related compounds (m/e 117 in the spectra of (TMS) derivatives of 19-hydroxy prostaglandins arises by cleavage of the 18-19 bond; a prostaglandin with hydroxyls at 18 and 19 might be expected to show both this peak and a peak at m/e 219 arising by an analagous cleavage of C_{17-18}). In no case has the identification of an ion been proved rigorously, as this would require the synthesis of isotopically labelled prostaglandins. The structures quoted therefore represent a series of working hypotheses, and in the description of the mass spectra I have tried to indicate as far as possible the strength of the circumstantial evidence in favour of each assignment.

a) The mass spectra of the 19-hydroxy prostaglandins E.

The mass spectra of the 19-hydroxy PG Es as the (MO/ME/TMS) derivative have already been briefly described (Taylor and Kelly, 1974). In this section a fuller description is given and the spectra are compared with those of the E prostaglandins. In addition, the spectra of the 19-hydroxy PG Es derivatised in several other ways are described.

Formation of the oxime of a ketoprostaglandin leads to two isomeric compounds as a result of the asymmetry of the nitrogen atom. The two oxime isomers are distinguished here by the Roman subscripts (I) and (II), referring to the order in which the isomers elute from the GLC column.

The mass spectra of the 19-hydroxy PG Es as the (MO/ME/TMS) derivative are given in fig. 2.1, and a list of peaks identified in the spectra and in those of the E PGs is given in table 2.1, along with postulated structures for the ions. The mass spectra of the E prostaglandins as this derivative have been examined by Green (1969), and structures assigned by him to the ions are for the most part retained here. One exception is the ion at m/e 133 which Green did not interpret. Middleditch and Desiderio (1973a) have suggested that this may arise by a migration of the methoxy group from the oxime to C_{11} followed by fragmentation to give an ion of the following structure:- $TMS\text{-}O\overset{+}{=}CH\text{-}OCH_3$

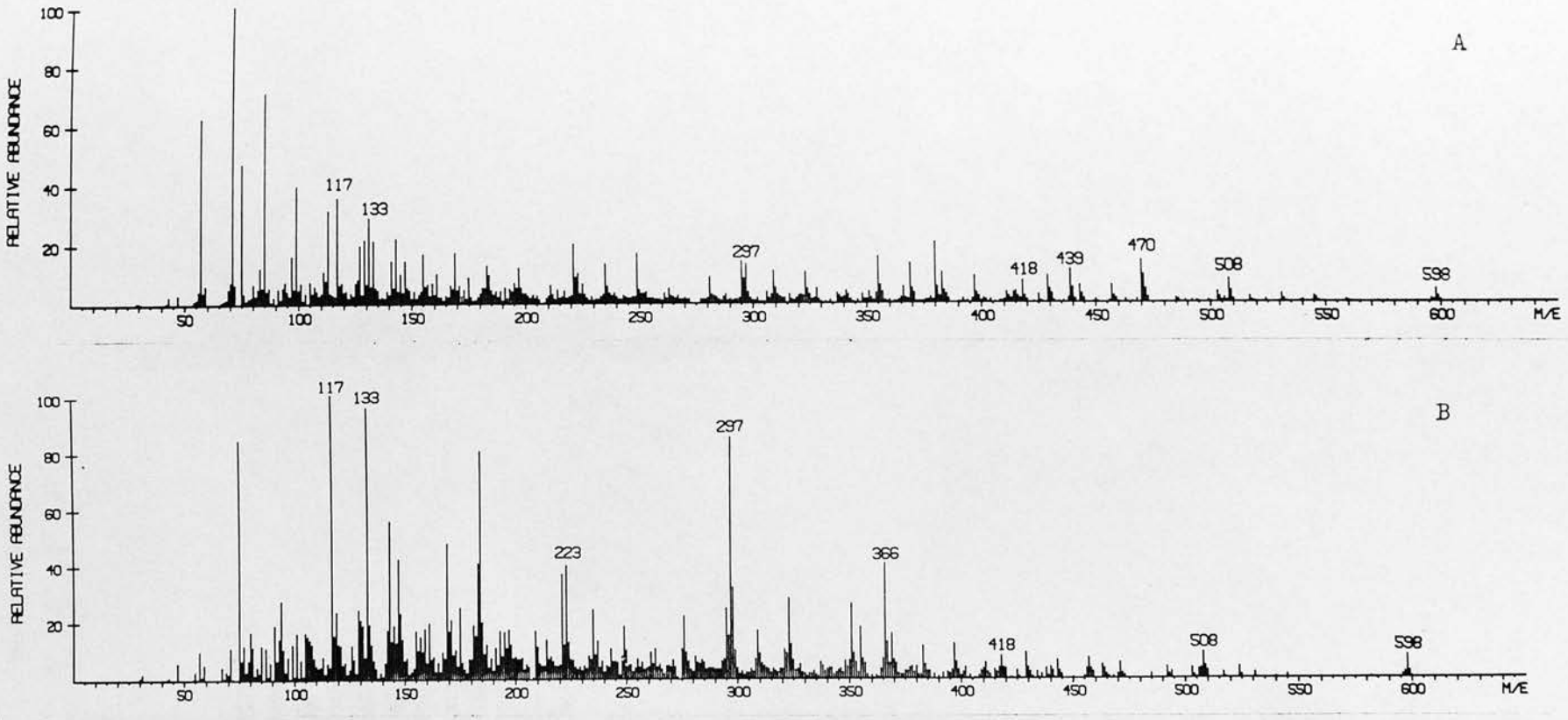


Fig. 2.1 Mass spectra of 19-hydroxy E prostaglandins (MO/ME/TMS derivative).

A. 19-hydroxy PG E_1 (I)·

B. 19-hydroxy PG E_1 (II)·

(continued overleaf)

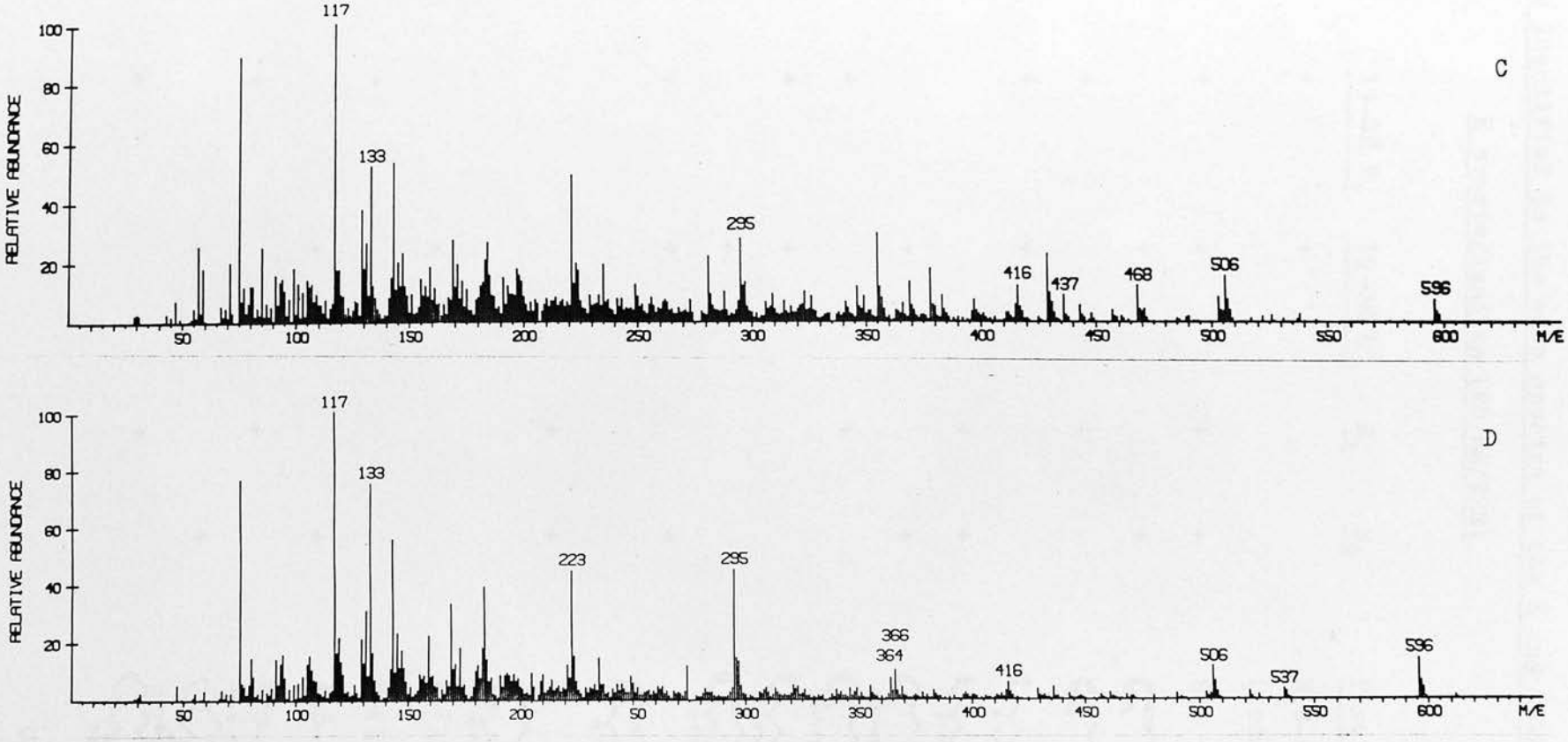


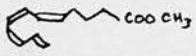
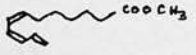
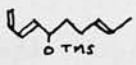
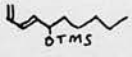
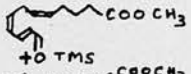
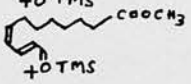
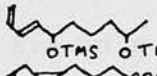
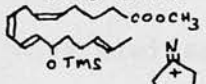
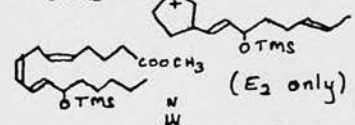
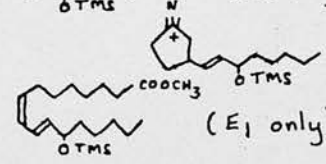
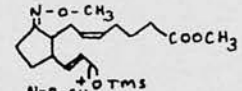
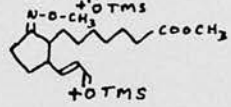
Fig. 2.1 (continued)

C. 19-hydroxy PG E_2 (I)·

D. 19-hydroxy PG E_2 (II)·

Table 2.1

Peaks identified in the mass spectra of the E and 19-hydroxy E prostaglandins (MO/ME/TMS).

m/e	19-OH E_1	19-OH E_2	E_1	E_2	Possible structure.
117	+	+			$H_3C-CH_2=O^+Si(CH_3)_3$ (from C_{19}- C_{20})
133	+	+	+	+	See text.
205		+		+	
207	+		+		
223	+	+			
225			+	+	
295		+		+	
297	+		+		
313	+	+			
364		+			
366	+	+		+	 (E_2 only)
368			+	+	 (E_1 only)
416		+			M-(31+90+90)
418	+				M-(31+90+90)
437*		+		+	468-31
439*	+		+		470-31
468*		+		+	
470*	+		+		

Cont/

Table 2.1 continued.

m/e	19-OH E_1	19-OH E_2	E_1	E_2	Possible structure.
506		+			M-(90+31)
508	+				M-(90+31)
508				+	M-31
510			+		M-31
537		+			M-90
539	+				M-90
596		+			M-31
598	+				M-31

* Ions arising from the loss of the C_{16}-C_{20} fragment are only strong in the (I) isomers; otherwise, the isomer pairs have very similar spectra.

The ion occurs in the spectra of both the 19-hydroxy PG Es and is frequently the base peak, but does not occur (at an analagous m/e value) in the spectra of any oxime derivative other than the methyl oxime. The ion does not appear in the spectrum of the saturated prostaglandin E_0 (prepared by hydrogenation of the natural compounds), which may indicate that the C_{13-14} unsaturated bond is involved in the fragmentation mechanism.

The characteristic patterns produced by trimethyl silyl derivatives are present in the spectra of both E and 19-hydroxy E prostaglandins. m/e 73 ($H(CH_3)_2Si\overset{+}{=}CH_2$), m/e 75 ($(CH_3)_2Si{=}O\overset{+}{-}H$) and m/e 77 ($H(CH_3)_2SiO^+H_2$) are well represented in all the compounds. At the other end of the spectrum the loss of trimethyl silanol gives rise to peaks at M - 90. The methyl oxime characteristically loses a methoxy radical giving rise to peaks at M - 31 in all the compounds. A small peak at m/e 205, arising from the loss of C_{16-20} plus C_{9-11} is found in the spectra of almost all the 2 series prostaglandins as (ME/TMS) derivatives, and is present in the spectra of 19-hydroxy PG E_2 and PG E_2. The analagous peak in the 1 series compounds (m/e 207) tends to be obscured in GCMS spectra by the strong column bleed peak at this mass.

Many of the strong peaks in the body of the spectrum involve breakage of the ring and loss of C_{9-11} (e.g. m/e 223, 225, 295 and 297) and ions arising from the loss of C_{16-20} are stronger in the (I) isomers of both groups of

compounds. The molecular ions, 541 and 539 for the PG Es and 629 and 627 for the 19-hydroxy PG Es are small, and absent from all but the strongest spectra. A strong peak at m/e 117 is present in all 19-hydroxy prostaglandin (TMS) spectra. This fragment is characteristic of compounds with the $-CH(OSi(CH_3)_3)CH_3$ group and probably has the structure $CH_3-CH=O^{+}-Si(CH_3)_3$.

The mass spectra of the 19-hydroxy PG Es as the dimethyl silyl derivative (MO/ME/DMS) are less informative. The spectra are more complex than those of the (MO/ME/TMS) derivative, with fewer high mass peaks and the majority of the ion current concentrated in the mass range below m/e 300. However, some peaks analagous to those in the spectrum of the (MO/ME/TMS) derivative can be identified. m/e 103, analagous to 117 in the earlier spectra is present, although not as striking. The molecular ions, m/e 587 and 585 are once again small or absent. The loss of the methoxy radical from the methyl oxime gives rise once more to a peak at M - 31 (m/e 556, 554) and the loss of dimethyl silanol to a small peak at M - 76 (m/e 511, 554) and a larger one at M - (31+76) (m/e 480, 478). Loss of the C_{16-20} fragment gives a peak at m/e 442 and 440.

The tertiary butyl dimethyl silyl derivatives (tBDMS) of these compounds display characteristically simple spectra. Loss of the t-butyl group gives rise to a very strong ion at M - 57 in the spectra of (tBDMS) derivatives of most

compounds which form them (Kelly and Taylor, 1976a). The t-butyl dimethyl silyl oxime derivative (O/ME/tBDMS) of the 19-hydroxy PG Es gives a particularly striking spectrum, consisting almost entirely of a single peak at m/e 799 (19-OH PG E_1) or m/e 797 (19-OH PG E_2). Loss of the t-butyl group plus t-butyl dimethylsilanol from the methyl oxime derivative (MO/ME/tBDMS) gives a strong peak at M - (57+132) (m/e 566, 564) in addition to the usual M - 57 at m/e 698 and m/e 696.

The concentration of a large proportion of the total ion current into one or two very intense high mass peaks makes these derivatives particularly suitable for the assay of the 19-hydroxy E prostaglandins by single ion monitoring GCMS analysis.

b) The mass spectra of the 19-hydroxy prostaglandins F.

The structure of the 19-hydroxy F prostaglandins was originally established by comparison of the mass spectrum of the (ME/TMS) derivative of natural 19-hydroxy PG $F_{2\alpha}$ with that of the synthetic compound (Taylor and Kelly, 1975). The mass spectra of these derivatives (fig. 2.2) show a clear relationship to those of the F prostaglandins, and a list of the major ions given by both groups of compounds as this derivative, together with their postulated structures is given in table 2.2.

The (ME/TMS) derivative has been widely used for the identification and assay of the F prostaglandins, and many groups have published full or partial mass spectra (see Crain et al, 1975; Horvath, 1976 for references). I have, however, been unable to locate any detailed analysis of the mass spectra of this derivative combination in the literature, and structural assignments given here are therefore either original or drawn by analogy from the published spectra of related derivatives. In this connection, the spectra of the F prostaglandins as the (TMS ether, TMS ester) derivative, analysed by Middleditch and Desiderio (1973b), have proved extremely useful, containing many peaks identical with or analagous to those present in the (ME/TMS) derivative.

As usual, the typical pattern produced by (TMS) derivatives is present, with strong ions at m/e 73, 75 and 77.

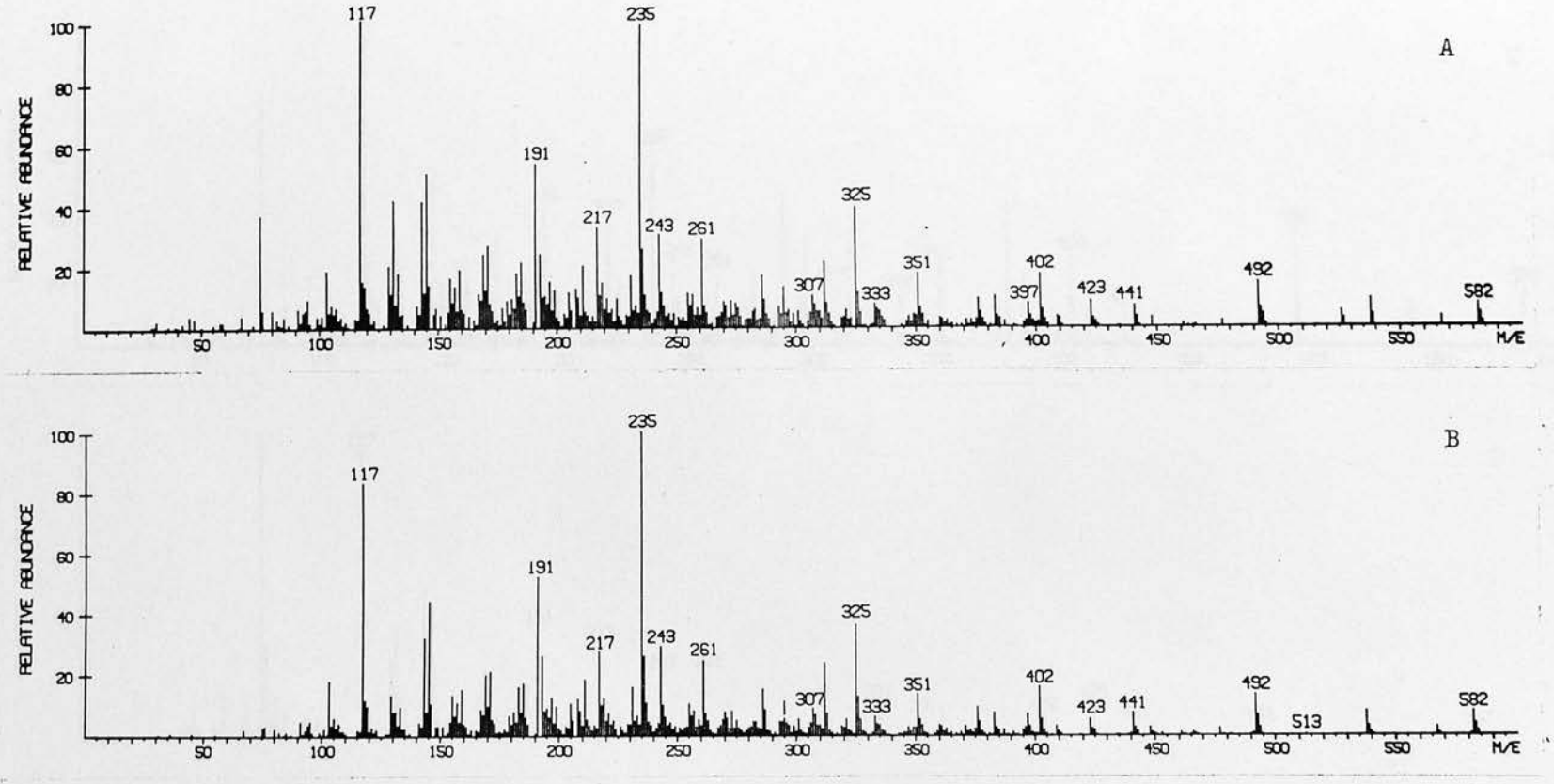


Fig. 2.2 Mass spectra of 19-hydroxy F prostaglandins (ME/TMS derivative).

A. 19-hydroxy PG $F_{2\alpha}$ (ex human semen).

B. 19-hydroxy PG $F_{2\alpha}$ (authentic).

(continued overleaf)

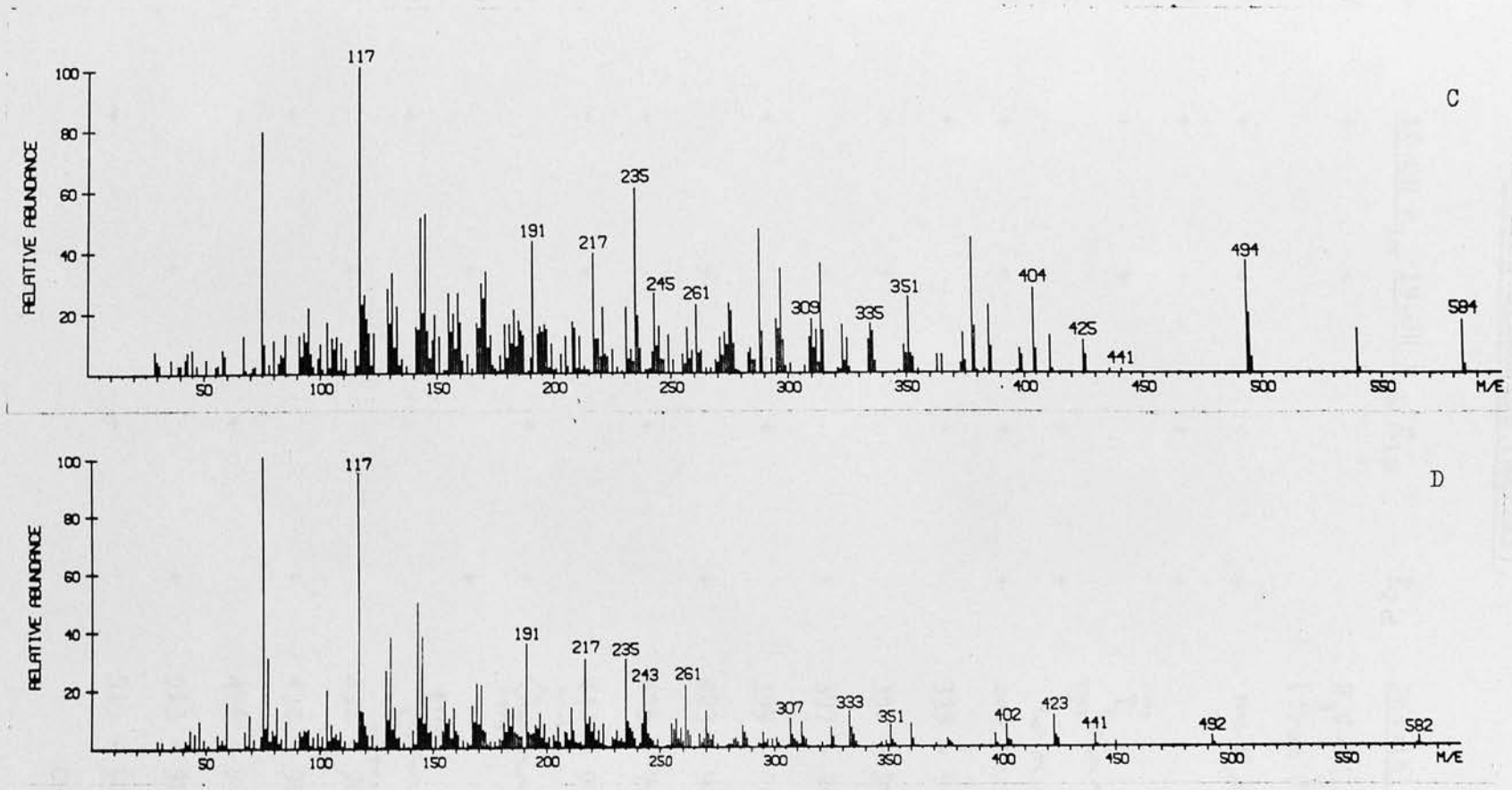


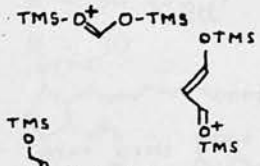
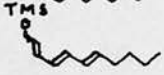
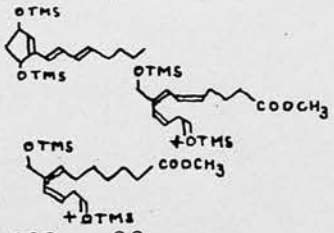
Fig. 2.2 (continued)

C. 19-hydroxy PG $F_{1\alpha}$ (ex human semen).

D. 8-iso 19-hydroxy PG $F_{2\alpha}$ (ex human semen).

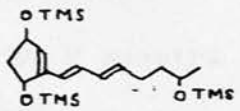
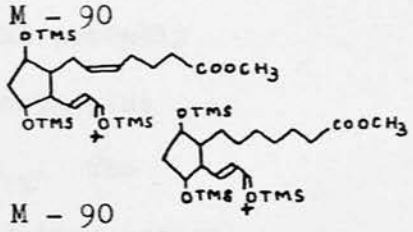
Table 2.2

Peaks identified in the mass spectra of the F and 19-hydroxy F prostaglandins (ME/TMS).

m/e	19-OH $F_{1\alpha}$	19-OH $F_{2\alpha}$	$F_{1\alpha}$	$F_{2\alpha}$	Possible structure.
117	+	+			$H_3C-CH_2=O^{+}Si(CH_3)_3$ (from C_{19-20})
191	+	+	+	+	
217	+	+	+	+	
235	+	+			
237			+	+	
243	+	+	+	+	see text
245	+		+		335 - 90
261	+	+			351 - 90
307		+		+	397 - 90
309	+		+		399 - 90
333		+		+	423 - 90
335	+		+		425 - 90
351	+	+			441 - 90
353			+	+	
397		+		+	
399	+		+		
402		+			492 - 90
404	+			+	494 - 90
406			+		496 - 90
423		+		+	513 - 90
425	+		+		515 - 90

Cont/

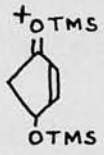
Table 2.2 continued.

m/e	19-OH $F_{1\alpha}$	19-OH $F_{2\alpha}$	$F_{1\alpha}$	$F_{2\alpha}$	Possible structure.
441	+	+			
492		+			M - 180
494	+			+	M - 180 (19-OH $F_{1\alpha}$)
					M - 90 ($F_{2\alpha}$)
496			+		M - 90
513		+		+	
515	+		+		
582		+			M - 90
584	+				M - 90

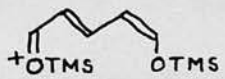
At the high mass end of the spectrum the loss of trimethylsilanol provides peaks at M-90 and M-180 in both groups of compounds. The base peak of the 19-hydroxy PG F spectra is m/e 117, typical of the (TMS) derivatives of ω-hydroxy prostaglandins.

Fragmentation of the ring gives rise to an intense ion at m/e 235 (19-OH Fs) or m/e 237 (Fs). This probably arises from the loss of the entire C_{1-7} side chain plus C_{10-11} with the TMS groups from C_{11}, C_{15} and C_{19}. The other example of ring fragmentation is afforded by a series of peaks at m/e 397/399 and 307/309 which may arise from the loss of C_{16-20} plus two of the ring carbons, either C_{9-10} or C_{10-11}. This group of peaks is somewhat stronger in PG $F_{2\alpha}$ and 19-hydroxy PG $F_{2\alpha}$ than in the corresponding F_1 series compounds, presumably because the longer series of conjugated double bonds confers extra stability on the fragment.

A peak at m/e 243 in both groups of compounds appears to arise by at least three mechanisms. Two of these routes lead to ions of empirical formula $C_{11}H_{23}O_2Si_2$, and Middleditch and Desiderio (1973b) have suggested that these arise from the loss of both side chains (i) or from C_{11-15} (ii).



(i)



(ii)

In PG $F_{2\alpha}$ and 19-hydroxy PG $F_{2\alpha}$ an ion of the same nominal mass may arise from the loss of C_{16-20} plus three molecules

of trimethylsilanol. Evidence for this mechanism is provided by the presence of the analagous ion at m/e 245 in the spectra of the F_1 series compounds.

The ions of m/e 191 (TMS-O^{+}=CH-O-TMS) and 217 (TMS-O^{+}=CH$(CH_2)_2$-O-TMS) have also been discussed by Middleditch and Desiderio (1973b). The former has been observed in the mass spectra of polyhydroxylated steroids (TMS), and probably arises by several routes, while the latter is derived from C_{9-11}. These authors also attributed the ion of m/e 173 present in the spectra of (TMS) derivatives of many prostaglandins to a fragment consisting of C_{15-20}. An analagous ion at m/e 261 is present in the spectra of the 19-hydroxy prostaglandins, although in the case of the 19-hydroxy PG Fs an ion of the same nominal mass may arise by the loss of C_{1-7} plus two molecules of trimethylsilanol.

Many of the strong peaks present in both groups of spectra may be ascribed to the loss of fragments from the two side chains. Loss of C_{16-20} (plus molecules of trimethylsilanol) leads to identical series of peaks in the spectra of both PG Fs and 19-hydroxy PG Fs (m/e 513/515, 423/425, and 333/335). Loss of C_{1-7} gives a small peak at m/e 441 which is present only in the spectra of the 19-hydroxy PG Fs, and peaks at m/e 351 (19-OH PG Fs) and 353 (PG Fs).

The mass spectra of the 19-hydroxy F prostaglandins as alkyl boronates (ME/BB/TMS or ME/tBB/TMS) closely resemble each other and those of the F prostaglandins. The

mass spectra of these derivatives are dominated by the loss of the C_{16-20} group, giving rise to a single intense peak at m/e 437 ($F_{1\alpha}$ and 19-OH $F_{1\alpha}$) or 435 ($F_{2\alpha}$ and 19-OH $F_{2\alpha}$). (The mass spectrum of PG $F_{2\alpha}$ ME/BB/TMS has been described by Kelly, 1973). The molecular ions are absent, the highest mass peaks being usually m/e 506/504, arising from the loss of trimethylsilanol. The spectra of the methyl boronate derivatives (ME/MB/TMS) are very similar, with the strong ion in this case located at m/e 395/393.

The t-butyl dimethylsilyl derivatives (ME/tBDMS) as usual display a very intense peak at M-57 owing to the loss of a t-butyl group (Kelly and Taylor, 1976a), together with a somewhat smaller peak at M-(57+132) from the additional loss of t-butyl dimethylsilanol.

c) The mass spectra of the 8-isoprostaglandins.

The spectra of the 8-isoprostaglandins as all derivatives so far studied (ME/TMS, ME/BB/TMS, ME/tBDMS, MO/ME/TMS or MO/ME/tBDMS as appropriate) are extremely similar to those of the parent compounds. In some cases there is a suggestion that the 8-isoprostaglandin spectrum contains a greater proportion of its total ion current in the low mass range (see for example the spectra of 19-hydroxy PG $F_{2\alpha}$ and 8-iso 19-hydroxy PG $F_{2\alpha}$ (ME/TMS) in fig 2.2 a and 2.2 d). In no case, however, is the difference great enough to allow the 8-isoprostaglandin to be differentiated from its parent compound on the basis of the mass spectrum alone.

d) The mass spectra of the dinor prostaglandins E.

The mass spectra of the dinor E prostaglandins are closely analagous to those of the PG Es, all the peaks attributable to fragments including the C_{1-7} side chain being shifted downward in mass by 28 a.m.u. The spectra of both dinor E prostaglandins extracted from semen and that of authentic dinor PG E_2 are given in fig. 2.3. It will be seen that the spectrum of the authentic material differs from that of the biological extract as was pointed out in chapter 1. In general the same ions occur in both spectra, but the proportion of the total ion current concentrated in the base peak of the compound found in semen is much greater. Repeated scanning of the spectra of the two compounds under identical conditions showed that this difference was reproducible, and not due to variations in the source conditions or to the statistical nature of the ionisation process. The similarities between the mass spectra indicate that the compounds are isomeric with one another, but the nature of this isomerism remains to be investigated.

Table 2.3 lists the ions identified in the spectra of the (MO/ME/TMS) derivatives of the dinor E prostaglandins. The molecular ions are weak, only the dinor PG E_2 from semen giving a detectable peak. In the other two cases the highest mass peak was m/e 498/496 corresponding to the loss of a methyl group. Loss of a methoxy radical gives a peak at M - 31 (482/480) and loss of trimethylsilanol a small peak

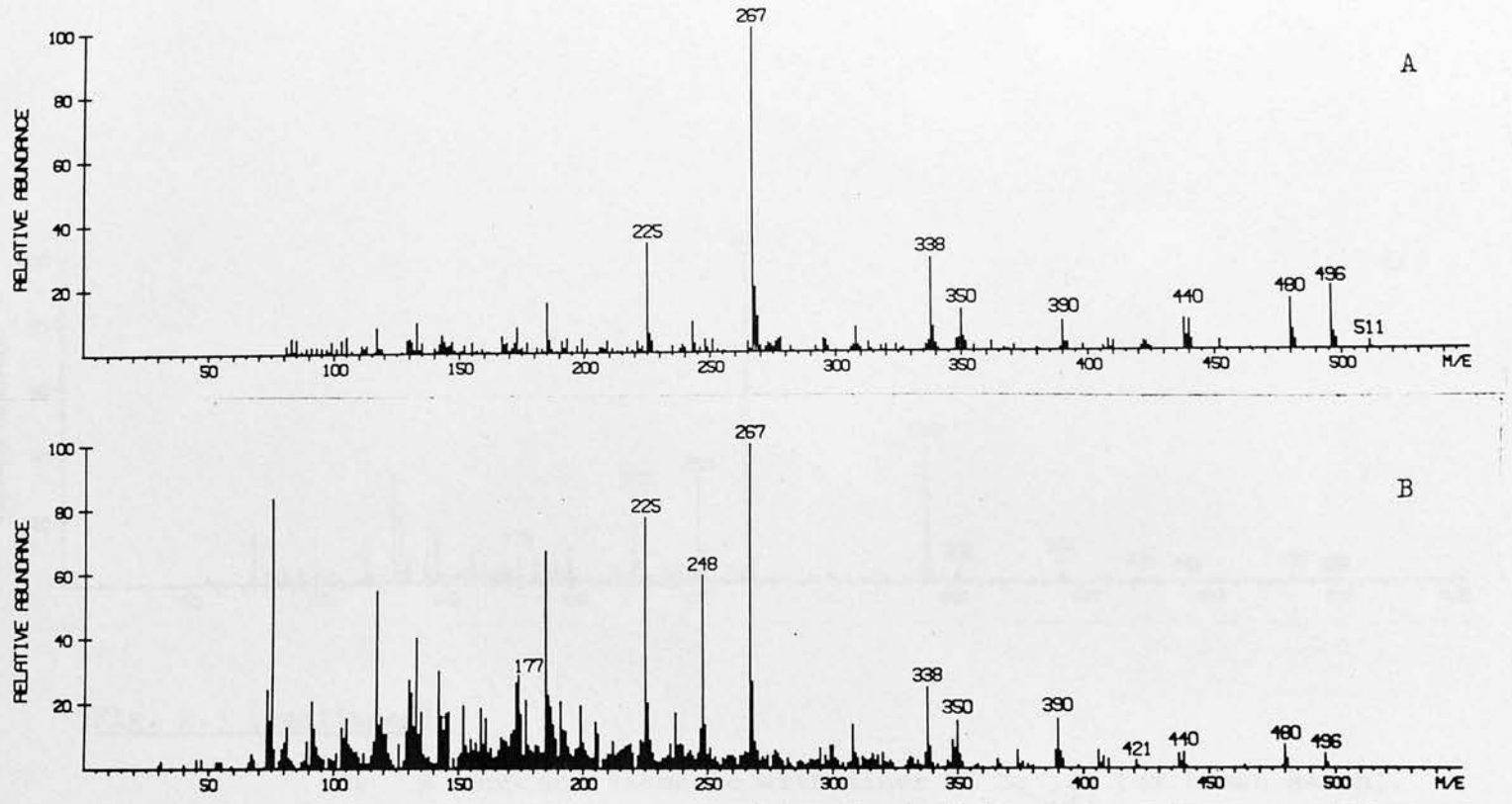


Fig. 2.3 Mass spectra of dinor E prostaglandins (MO/ME/TMS derivative).

A. A compound isomeric with dinor PG E_2 (II) (ex human semen).

B. Dinor PG E_2 (II) (authentic).

(continued overleaf)

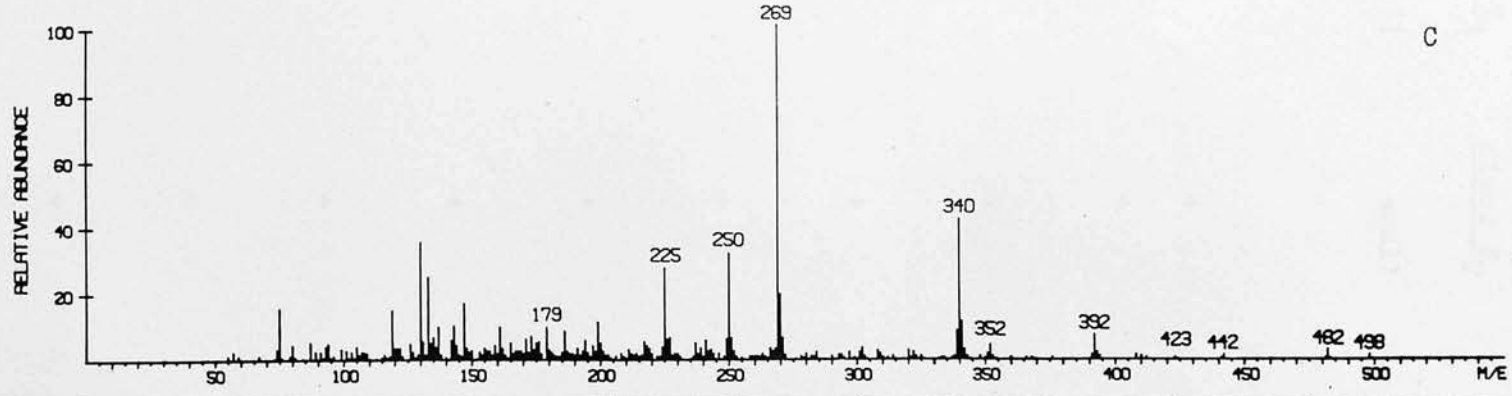
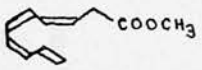
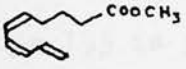
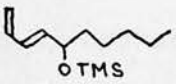
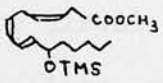
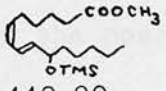


Fig. 2.3 (continued)

C. A compound isomeric with dinor PG $E_{1\ (I)}$ (ex human semen).

Table 2.3

Peaks identified in the mass spectra of the dinor E prostaglandins (MO/ME/TMS).

m/e	dinor E_1	dinor E_2	Possible structure.
133	small	small	See section 2a.
177		+	
179	+		
225	+	+	
248		+	338-90
250	+		340-90
267		+	
269	+		
338		+	
340	+		
350*		+	440-90
352*	+		442-90
390		+	M-(90+31)
392	+		M-(90+31)
421		+	M-90
423	+		M-90
440*		+	M-71
442*	+		M-71
480		+	M-31
482	+		M-31
496		+	M-15
498	+		M-15
511 /513	absent	absent	M.

*Peaks arising from the loss of the C_{14-18} fragment are stronger in the (I) isomers.

at M - 90 (423/421). Once again, we find that the ions involving the loss of the C_{16-20} fragment are much stronger in the (I) oxime isomers (m/e 442/440 and 352/350). As with the E and 19-hydroxy E prostaglandins, strong ions in the body of the spectrum generally involve fragmentation of the ring, and all the peaks analagous to those in the spectrum of the E prostaglandins are present. The elusive m/e 133 is present but small in comparison with the PG Es and 19-hydroxy PG Es.

The mass spectrum of the dinor E prostaglandins as the (O/ME/tBDMS) derivative consists virtually of a single peak at M - 57 (m/e 640/638) making this derivative highly suitable for single ion monitoring. This derivative has also been used to obtain a spectrum of dinor PG E_1 by direct derivatisation of fresh semen, eliminating the possibility of the compound arising as an artifact of storage.

e) The mass spectra of the dinor prostaglandins F.

The dinor F prostaglandins of semen are not separable from each other, either by LH 20 Sephadex chromatography or by GLC as either of the derivatives so far studied. The mass spectra obtained therefore contain peaks from both dinor PG $F_{1\alpha}$ and dinor PG $F_{2\alpha}$.

Fig. 2.4 gives the mass spectrum of the (ME/TMS) derivative and table 2.4 lists the ions identified. For convenience, peaks due to dinor PG $F_{1\alpha}$ and dinor PG $F_{2\alpha}$ have been listed in separate columns as in the previous tables in this chapter. The spectrum shows an obvious relationship with those of the PG Fs, as many of the ions arise by loss of the C_{1-5} side chain, giving rise to peaks at identical m/e values in both groups of compounds (m/e 191, 237, 353). The molecular ions are once more absent, but a series of peaks arising from the loss of successive trimethylsilanol groups (M - 90, M - 180) is present, together with a similar series involving the loss of the pentyl side chain (M - 71, M - (71+90) etc.).

As the (ME/tBDMS) derivative, the compounds give the usual strong ion at M - 57 (m/e 627/625) and a smaller peak at M - (57+132) (m/e 495/493).

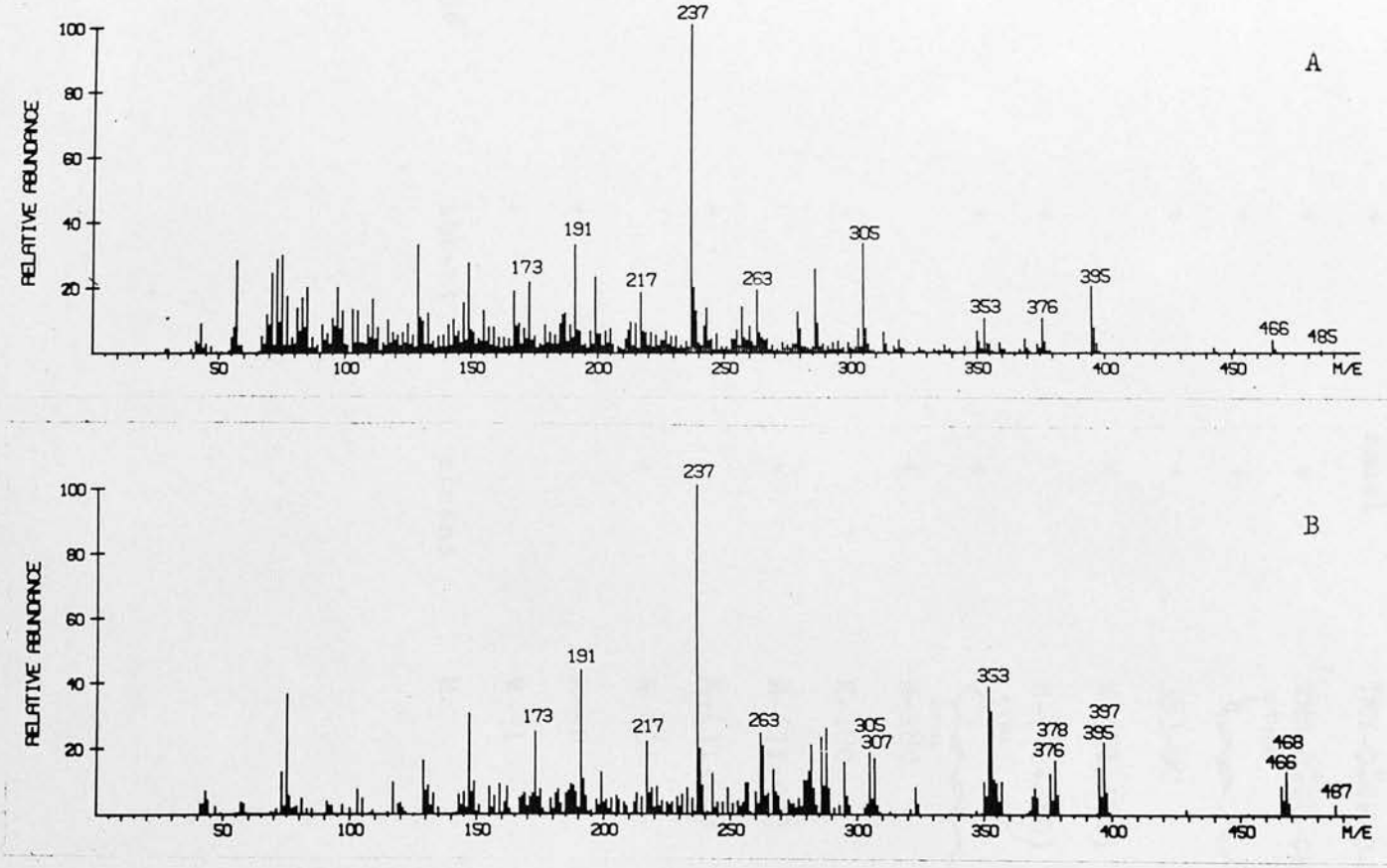


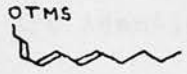
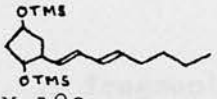
Fig. 2.4 Mass spectra of dinor F prostaglandins (ME/TMS derivative).

A. Dinor PG $F_{2\alpha}$ (authentic).

B. Mixed spectrum of dinor PG $F_{1\alpha}$ and dinor PG $F_{2\alpha}$ (ex human semen).

Table 2.4

Peaks identified in the mass spectra of the dinor F prostaglandins (ME/TMS).

m/e	dinor $F_{1\alpha}$	dinor $F_{2\alpha}$	Possible structure.
173	+	+	263-90
191	+	small	TMS-O^{+}=CH-O-TMS
217	+	+	TMS-O^{+}=CH-CH=CH-O-TMS
237	+	+	
263	+	+	353-90
305		+	M-(71+180)
307	+		M-(71+180)
353	+	+	
376		+	M-180
378	+		M-180
395		+	M-(71+90)
397	+		M-(71+90)
466		+	M-90
468	+		M-90
487	+		M-71
558/556	absent	absent	M.

f) The mass spectrum of dihydroxy prostaglandin E_1.

Two compounds provisionally identified as 18,19 dihydroxy prostaglandin E_1 and an isomeric prostaglandin have been partially isolated from the semen of Macaca arctoides. Since the mass spectra of the two compounds are identical they will be discussed together.

Table 2.5 lists the ions identified in the spectra of the (MO/ME/TMS) derivative of these compounds, and the spectra are reproduced in fig. 2.5.

Peaks arising by the loss of the C_{16-20} fragment are identical with those produced by PG E_1 and 19-hydroxy PG E_1 (m/e 297, 470), and as in the case of these compounds m/e 470 is stronger in the spectrum of the (I) isomer. Although the molecular ion (m/e 717) is absent, a series of peaks arising by the loss of trimethylsilanol or a methoxy radical (M - 31, M - 90, M - (31+90), M - 180 etc.) act as pointers to its mass. The presence of m/e 117 indicates that the compound possesses a β-hydroxyl group. A strong ion at m/e 219 is interpreted as being derived from C_{18-20} by a mechanism analagous to that which produces m/e 117 from C_{19-20}. A small peak at m/e 247 may arise from C_{16-20} (An analagous peak at m/e 159 is present in the spectra of the 19-hydroxy prostaglandins). Loss of C_{19-20} by a mechanism analagous to that which produces m/e 470 gives rise to a small peak at m/e 600 and the additional loss of trimethylsilanol gives stronger peaks at m/e 510 and 420. These peaks are stronger

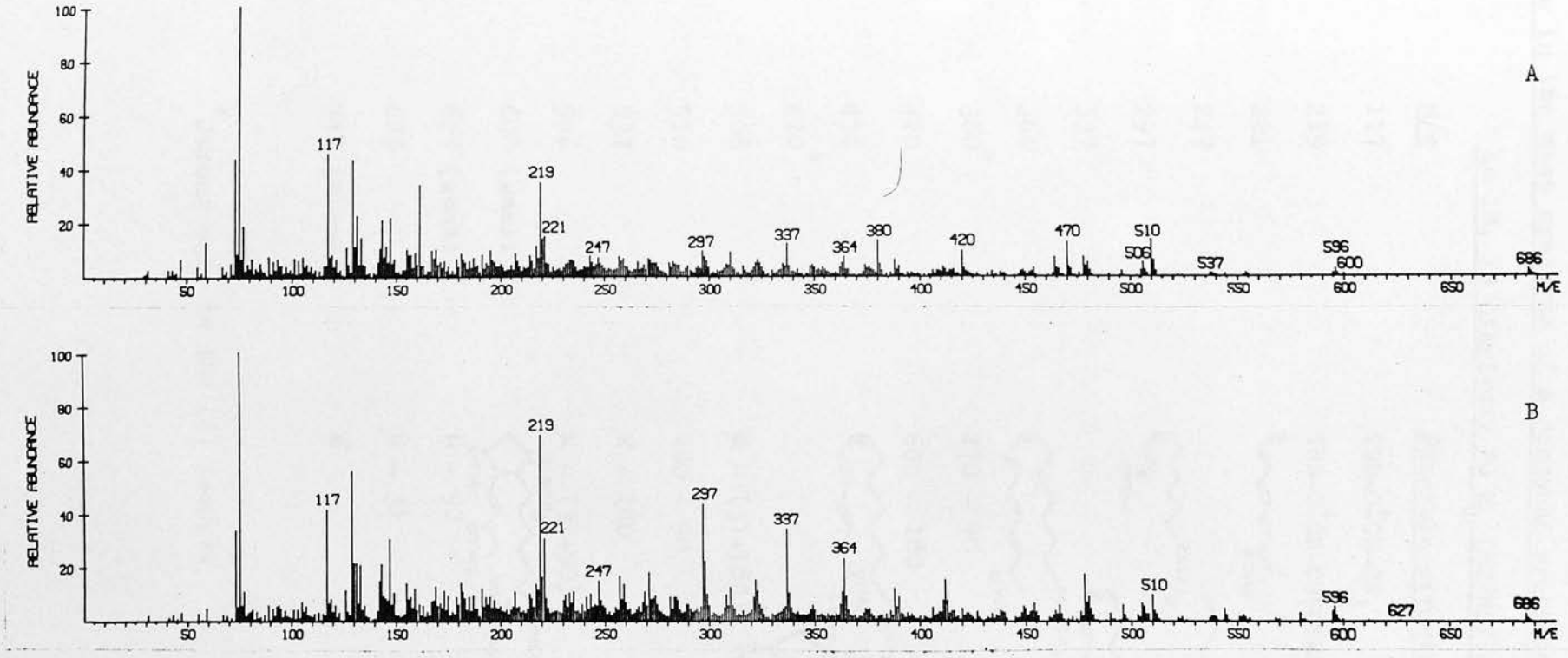


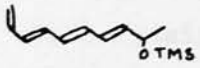
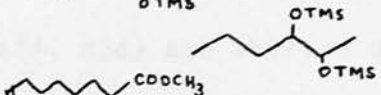
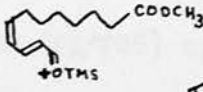
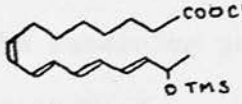
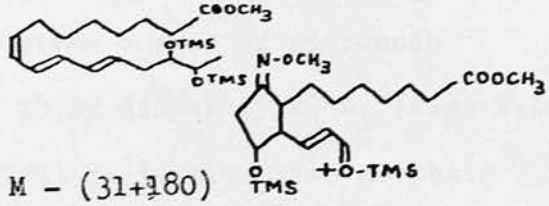
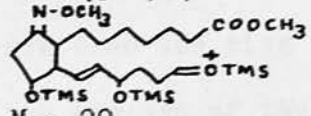
Fig. 2.5 Mass spectra of 18,19-dihydroxy E prostaglandins (MO/ME/TMS derivative).

A. 18,19-dihydroxy PG E_1 (I).

B. 18,19-dihydroxy PG E_1 (II).

Table 2.5

Peaks in the mass spectrum of a compound provisionally identified as 18, 19 dihydroxy PG E_1 (MO/ME/TMS).

m/e	Possible structure.
117	TMS-O^{+}=CH-CH_3
219	TMS-O^{+}=CH-CH(OTMS)-CH_3
221	
247	
297	
337	
364	
380*	470 - 90
420	600 - 180
454	
470*	
506	M - (31+180)
510	600 - 90
537	M - 180
596	M - (31+90)
600 (small)	
627 (small)	M - 90
686	M - 31
717 (absent)	M

*Strong only in the (I) isomer.

in the (I) isomers, lending weight to the interpretation that they arise by a mechanism analagous to that which produces m/e 470.

In the spectrum of the ethyl oxime derivative (EO/ME/TMS), peaks corresponding to fragments which retain the ethoxy group (m/e 641, 614, 551, 524, 484, 434) are shifted upward by 14 a.m.u. compared with the (MO/ME/TMS) spectrum, indicating the presence of a single carbonyl and confirming the structural interpretation of these ions. The remaining peaks in the spectrum of this derivative are unchanged.

Derivatisation of the dihydroxy E prostaglandins as (ME/TMS) without prior oximation gives a pair of compounds whose mass spectra correspond to 18,19 dihydroxy PG A_1 (fig. 2.6a). The A prostaglandins as the (ME/TMS) derivative have as their major mode of fragmentation a mechanism giving rise to a strong ion from the loss of C_{16-20} (m/e 351 in the spectra of PG A_1 and 19-hydroxy PG A_1) and a smaller fragment from the further loss of carbon monoxide from the ring (m/e 323)(Throck-Watson and Sweetman 1974). In the spectra of the isomeric B prostaglandins the relative sizes of the two peaks are reversed, with m/e 323 being the base peak in the spectra of PG B_1 and 19-hydroxy PG B_1. In the spectrum of dihydroxy PG A_1 these ions are present, m/e 351 being the larger of the pair. The major mode of ionisation in this case however is the analagous mechanism which involves the loss of C_{19-20}. This gives rise to a peak at m/e 481, and a further loss of trimethylsilanol gives the base peak of the spectrum at

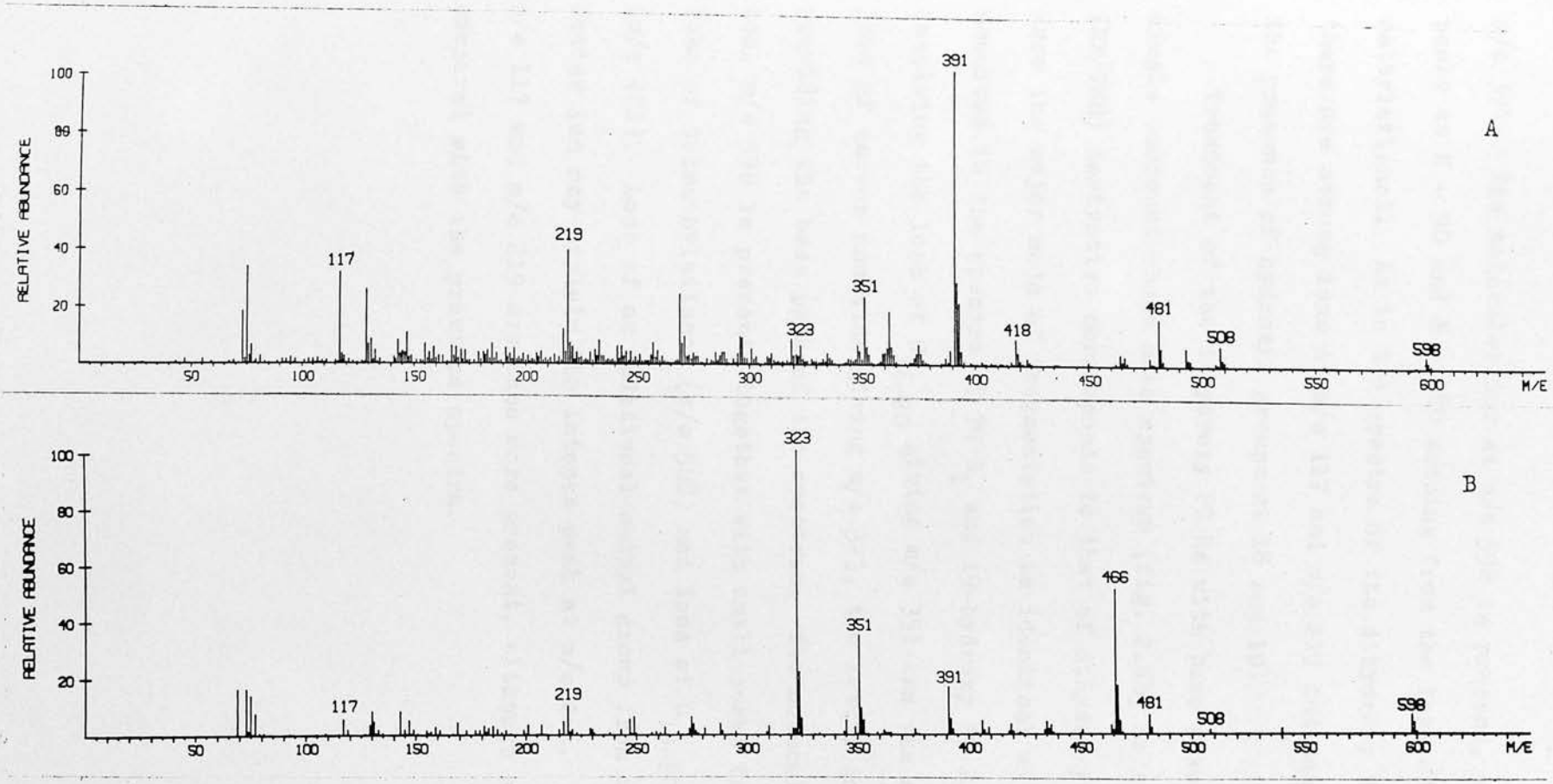


Fig. 2.6 Mass spectra of dehydrated prostaglandins derived from 18,19-dihydroxy PG E_1 (ME/TMS derivative).

A. 18,19-dihydroxy PG A_1.

B. 18,19-dihydroxy PG B_1.

m/e 391. The molecular ion at m/e 598 is present, as are peaks at M - 90 and M - 180 arising from the loss of trimethylsilanol. As in the spectra of the dihydroxy PG Es there are strong ions at m/e 117 and m/e 219 indicating the presence of hydroxyl groups at 18 and 19.

Treatment of the dihydroxy PG Es with base gives a single compound whose mass spectrum (fig. 2.6b) as the (ME/TMS) derivative corresponds to that of dihydroxy PG B_1. Here the major mode of fragmentation is identical with that observed in the spectra of PG B_1 and 19-hydroxy PG B_1, involving the loss of C_{16-20} giving m/e 351 and the subsequent loss of carbon monoxide giving m/e 323, the latter ion providing the base peak of the spectrum. The molecular ion, m/e 598 is present, together with small peaks from loss of trimethylsilanol (m/e 508) and loss of C_{19-20} (m/e 481). Loss of an additional methyl group from the latter ion may explain the intense peak at m/e 466. m/e 117 and m/e 219 are once more present, although small compared with the previous spectra.

g) Chromatographic data.

Table 2.6 gives GLC retention time data for the prostaglandins measured, the results being quoted as equivalent carbon values relative to the series of n-paraffins.

Table 2.7 gives TLC R_f values for the underivatised compounds in three TLC systems.

Table 2.6

Gas liquid chromatographic retention indices.

Compound	Derivative	Carbon value	
PG $F_{1\alpha}$	ME/TMS	27.0	
PG $F_{2\alpha}$	"	26.8	
8-iso PG $F_{1\alpha}$	"	26.2	
8-iso PG $F_{2\alpha}$	"	26.0	
PG $F_{1\beta}$	"	26.3	
PG $F_{2\beta}$	"	26.2	
dinor PG $F_{1\alpha}$	"	25.2	
dinor PG $F_{2\alpha}$	"	25.2	
19-OH PG $F_{1\alpha}$	"	28.8	
19-OH PG $F_{2\alpha}$	"	28.5	
8-iso 19-OH PG $F_{1\alpha}$	"	28.3	
8-iso 19-OH PG $F_{2\alpha}$	"	28.0	
PG E_1	MO/ME/TMS	26.8 (I)	27.4 (II)
PG E_2	"	26.7	27.2
PG E_3	"	26.6	27.2
PG D_2	"	26.5	26.8
8-iso PG E_1	"	26.8	27.4
8-iso PG E_2	"	26.7	27.2
dinor PG E_1	"	25.2	25.7
dinor PG E_2	"	24.9	25.5
19-OH PG E_1	"	28.9	29.5
19-OH PG E_2	"	28.7	29.3
8-iso 19-OH PG E_1	"	28.9	29.4
8-iso 19-OH PG E_2	"	28.7	29.2

Table 2.7 R_f values in three TLC systems.

Compound	R_f values.		
	System I	System II	System III*
PG E_1	0.22	0.63	0.63
8-iso PG E_1	0.30	0.62	0.71
8-iso PG E_2	0.31	0.62	0.68
PG $F_{2\alpha}$	0.10	0.50	0.49
8-iso PG $F_{1\alpha}$	0.10	0.46	0.47
8-iso PG $F_{2\alpha}$	0.09	0.47	0.47
19-OH PG E_2	0.04	0.45	0.27
8-iso 19-OH PG E_{1+2}	0.09	0.42	0.40
19-OH PG $F_{2\alpha}$	0.03	0.29	0.23
8-iso 19-OH PG $F_{1\alpha+2\alpha}$	0.03	0.25	0.20
15R 19-OH PG $F_{2\alpha}$	0.05	0.37	0.33
15R 19-OH PG E_2	0.08	0.46	0.38

* R_f values in this system are not reproducible owing to the difficulty of ensuring removal of all the solvent between runs. Values quoted are typical and show the separations obtained.

Chapter 3.

The effect of 19-hydroxy prostaglandin E_1 on isolated myometrium.

It proved difficult to obtain reproducible results in this series of experiments since the response of the tissue to prostaglandins was extremely variable. Sensitivity toward prostaglandins varied with time, being low initially but rising to a peak about one hour after the start of the experiment and then declining along with the activity of the muscle. It was difficult therefore to compare results obtained from different preparations, or even from the same preparation at different times. The human preparations were short lived (about six hours) and sluggish (2 - 15 contractions per hour) and so relatively few measurements could be made within the life of each preparation.

A total of 15 strips from 5 non-pregnant human uteri, 9 strips from 3 pregnant human uteri and 2 strips from 2 pregnant marmoset uteri yielded results. On many additional occasions tissue supplied proved inactive and yielded no results.

a) Non-pregnant human tissue.

Table 3.1 gives a summary of the results achieved with non-pregnant human myometrial strips using prostaglandin E_1, synthetic 19-hydroxy PG E_1 and natural 19-hydroxy PG E_1. The results are extremely scattered, for the reasons given above, but it is clear that all three compounds inhibit the activity of the strips, and that PG E_1 is about four times as effective as natural 19-hydroxy PG E_1 and eight times

Table 3.1

The effects of prostaglandins on the contractile activity of non-pregnant human myometrial strips.

a. Prostaglandin E_1

Dose (ng/ml)	1.0	2.5	5.0	10	20
Effect	-13	75	100	31	82
(% inhibition)	24	40	100	78	40
		20	100	53	100
		49	100		100
		47	100		53
		70	88		
		57	66		
		7			
Mean	5.5	45	93	54	75
σ		22	12		

b. Synthetic 19-hydroxy PG E_1.

Dose (ng/ml)	1.0	2.5	5.0	10	20	40	50
Effect	35	46	2	61	37	54	29
(% inhibition)		24	61	20	25	83	43
		3		32	39	70	
				10	34		
				35	45		
				32	81		
				7	1.5		
					52		
					40		
					8		
					20		
Mean	35	24	32	28	35	69	36
σ				17	21		

Continued/

Table 3.1 cont.

c. Natural 19-hydroxy E_1

Dose (ng/ml)	5.0	10	20	30	40	50
Effect (% inhibition)	38	53	82	71	51	25
		51	59		84	
		0	38			
		92	100			
		25	89			
		31	9			
		21	30			
		14	71			
		61	66			
		13	53			
		58	13			
		38	18			
Mean	38	38	52	71	68	25
σ		25	29			

as effective as the synthetic compound. It should be possible to obtain more precise results by comparing the effects obtained within the set of results for each individual strip with each other, thus eliminating variation between strips. Taking the ratio of 1:4:8 as the hypothetical equipotent molar ratio, and looking within the set of results for each strip for cases where pairs of injections have been made with the prostaglandins in the correct ratio, we should find that the ratio of the effect produced is near unity.

Taking the pair: natural 19-OH E_1 / E_1 we find nine cases where these were injected in the ratio 4:1. The ratios of effects obtained in the nine cases were as follows:-

1.25, 0.7, 0.9, 0.4, 1.0, 0.8, 1.0, 1.6, 1.1

Mean ratio = 0.97 σ = 0.3

In the case of the pair: synthetic 19-OH E_1 / natural 19-OH E_1 we find again that there were nine cases where these were injected in the ratio 2:1. Effects ratios obtained were as follows:-

0.7, 1.3, 1.6, 1.25, 0.9, 0.7, 0.2, 1.2, 1.0

Mean ratio = 0.98 σ = 0.4

As a final check we may take the pair: E_1 / synthetic 19-OH E_1. There were six cases where these compounds were injected in the ratio 1:8. The ratios of the effects obtained were as follows:-

1.1, 0.97, 1.4, 0.86, 2.0, 1.6

Mean ratio = 1.32 σ = 0.4

It is therefore concluded that the EPMR for E_1 : 19-OH E_1 : synthetic 19-OH E_1 is 1:4:8.

b. Pregnant human tissue.

The pregnant human myometrium gave less clear cut results than the non-pregnant material. Table 3.2 lists the entire set of results for prostaglandins E_1 and 19-hydroxy E_1 (natural compound). As the supply of tissue was strictly limited the effect of the synthetic compound was not investigated in this case. As the experiment proceeded, it became clear that in order to obtain a statistically significant result with the available material it would be necessary to concentrate on one dose level, and restrict the objective of the experiment to proving the existence of an effect at that level. 50 ng/ml was selected as a dose which gave a clear effect with 19-hydroxy PG E_1, and the majority of results quoted are at this level.

Results obtained with PG E_1 (table 3.2a) were equivocal, in line with the finding of Embrey and Morrison (1968) that the response of lower segment human myometrial strips to PG E *in vitro* is erratic. In six out of sixteen cases, at a dose level of 50 ng/ml PG E_1 caused stimulation of the muscle; in two cases it produced an inhibition of greater than 50%, and in the remaining cases a more moderate degree of inhibition.

In general, the response to 19-hydroxy PG E_1 was one of inhibition, although in a few cases stimulation was observed. At a dose level of 50 ng/ml, complete inhibition of motility of the muscle was observed in three out of eleven cases, contractions resuming when the prostaglandin was washed

Table 3.2

The effects of prostaglandins on the contractile activity of pregnant human myometrial strips.

a. Prostaglandin E_1

Dose (ng/ml)	20	25	32	50	100
Effect	9	5	-22	-28	57
(% inhibition)	14	37	-40	77	
			4.5	24	
				-14	
				38	
				-23	
				23	
				15	
				19	
				44	
				-71	
				-81	
				44	
				67	
				-17	
				27	
Mean	11	21	-19	9	57
σ				45	

Cont./

Table 3.2 cont.

b. 19-hydroxy E_1

Dose (ng/ml)	20	25	32	50	100
Effect	9	18	-9	30	36
(% inhibition)		13	38	57	
		-11	25	100	
			10	100	
				-54	
				1.6	
				10	
				41	
				100	
				-14	
				25	
Mean	9	10	16	36	36
σ				50	

out. In two cases the muscle responded with an increase in activity, the remaining six being cases of intermediate inhibition (table 3.2b).

It was noted that an initially quiescent strip could often be caused to start contracting by injecting prostaglandin E_1, although contractions could not be sustained by further injections, the strip requiring a background of oxytocin for sustained activity. In two cases where strips were started by PG E_1 alone, the effect of PG E_1 was reversed, and the activity of the muscle abolished by injection of an equal quantity of 19-hydroxy PG E_1 (fig. 3.1).

Statistical analysis was performed using the paired t test. The activity of the muscle during exposure to prostaglandin was compared to the mean of its activity before injection and its activity after washing the prostaglandin out, the null hypothesis being that the activity of the muscle in the presence of prostaglandin was equal to that mean. Table 3.3 gives the statistical analysis of results achieved at the 50 ng/ml dose level.

The paired t test shows that the activity of the muscle in the presence of prostaglandin E_1 was not significantly different from control values ($p \angle 0.2$), while that in the presence of 19-hydroxy PG E_1 was significant ($p \angle 0.05$). A non-parametric test was also run on the same data, as there was some doubt as to whether the distribution of muscle activities was normal, and an anormal distribution might invalidate the t test. The Wilcoxon rank test requires no assumptions

Table 3.3

Statistical analysis of the effects of prostaglandins on human pregnant myometrium at 50 ng/ml.

a. Effect of prostaglandin E_1

Control activity	Activity during treatment	Difference	Rank no.	Background treatment*
X_1	X_2	$D = X_1 - X_2$		
84	71	13	1	0.25
66	48	18	2	0.25
80	103	-23	3	0.125
153	179	-26	4	0.125
114	141	-27	5	0.25
117	90	27	6	0.25
44	80	-36	7	none
150	103	47	8	0.5
83	142	-59	9	none
144	80	64	10	0.25
256	162	94	11	0.125
429	326	103	12	0.25
168	55	113	13	0.25
423	541	-118	14	0.25
392	216	176	15	0.25
294	69	225	16	0.25

Paired t test

$\bar{d} = 36.93$

$s_D^2 = 8010$

$s_D = 89.50$

$s_{\bar{d}}^2 = 534$

$s_{\bar{d}} = 23.1$

$t = 1.6$ (d.f. = 15)

$p \angle 0.2$

Wilcoxon rank test

Sum of ranks corresponding to negative differences = 42

not significant.

* Figures refer to the concentration of oxytocin present in mU/ml.

Continued/

Table 3.3 cont.

b. Effect of 19-hydroxy E_1

Control activity	Activity during treatment	Difference	Rank no.	Background treatment*
X_1	X_2	$D = X_1 - X_2$		
92	90	2.0	1	0.25
182	163	19	2	0.75
163	185	-22	3	0.25
154	116	38	4	0.125
111	66	45	5	E_1
59	0	59	6	E_1
129	198	-69	7	0.25
524	368	156	8	0.25
175	0	175	9	0.125
388	166	222	10	0.25
355	0	355	11	0.25

Paired t test

$\bar{d} = 89.09$

$s_D^2 = 15552$

$s_{\bar{d}}^2 = 1414$

$s_{\bar{d}} = 37.60$

$t = 2.37$ (d.f. = 10)

$p < 0.05$

Wilcoxon rank test

Sum of ranks corresponding to negative differences = 10

$p < 0.05$

* Figures refer to concentration of oxytocin present in mU/ml. When prostaglandin E_1 was used the concentration was 50 ng/ml.

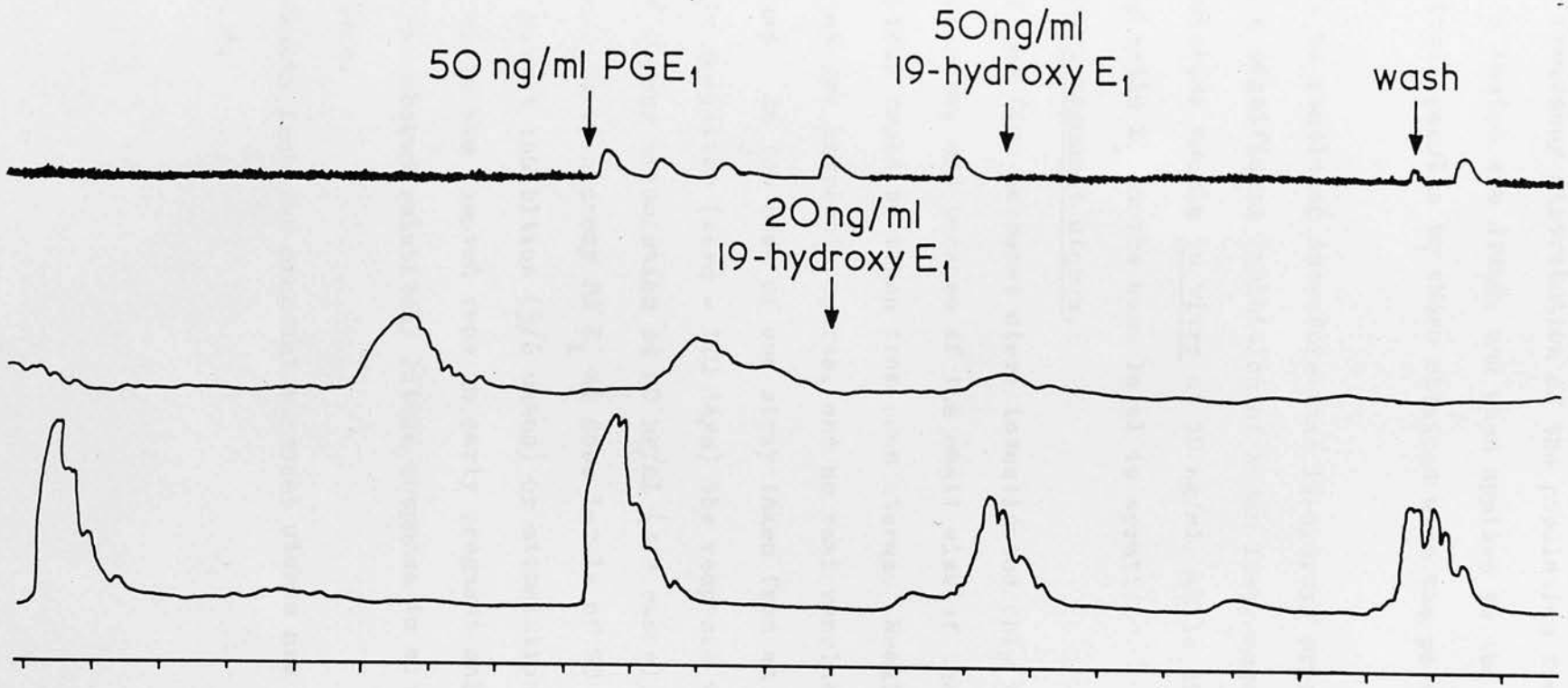


Fig. 3.1 Effect of 19-hydroxy prostaglandin E_1 on human myometrium.

Upper trace - Reversal of effect of PG E_1 on pregnant myometrium by 19-hydroxy PG E_1.
Middle and lower traces - Simultaneous recording from two non-pregnant strips in the same bath, showing effect of 20 ng/ml 19-hydroxy PG E_1.
Time marker (lower margin) indicates minutes.

about the frequency distribution of the population from which the samples tested are drawn, and when applied to the data gave identical results to those obtained with the paired t test.

It is concluded therefore that 19-hydroxy prostaglandin E_1 causes a significant inhibition of human lower-segment pregnant uterine muscle *in vitro* at 50 ng/ml, while the effect of prostaglandin E_1 at the same level is erratic.

c) Pregnant marmoset uterus.

Of the five marmoset uteri investigated only two provided results, and because of the small size of the animals only one strip could be taken from each uterus. Results in this section are extremely sparse, and no real conclusions can be drawn. In the case of one strip taken from an animal at 130 days gestation (term = 140 days) the response to PG E_1 was one of strong stimulation at 20 ng/ml (3/3 cases), while the response to 19-hydroxy PG E_1 at dose levels of 20 - 100 ng/ml was slight inhibition (5/6 cases) or stimulation (1/6). The other strip was removed from an early pregnant animal (40 days) and showed relatively little response to either prostaglandin.

Results from the pregnant marmoset uterus are listed in table 3.4.

Table 3.4

The effects of prostaglandins on the motility of pregnant marmoset myometrial strips.

a) Effect of PG E_1 on 130 day gestation strip.

Dose	20 ng/ml
Effect (% inhibition)	-89, -132, -85.

b) Effect of 19-hydroxy PG E_1 on 130 day gestation strip.

Dose (ng/ml)	20	50	100
Effect (% inhibition)	-13 35 17	28 16	7

c) Effect of PG E_1 on 40 day gestation strip.

Dose (ng/ml)	20	50	100	200	400
Effect (% inhibition)	-4	2	-9	11	19

d) Effect of 19-hydroxy PG E_1 on 40 day gestation strip.

Dose (ng/ml)	50	100	200	400
Effect (% inhibition)	-5	-23	-10	12

Discussion.

The effect of the classical prostaglandins on the non-pregnant human uterus *in vitro* has been shown to vary with the stage of the menstrual cycle of the donor of the tissue (Bygdeman and Eliasson, 1963; Bygdeman 1964) and it was originally intended that the results quoted here should be examined for similar effects. Unfortunately, in two cases this information was not available, and the results were therefore pooled and presented together. In the case of the three uteri for which we did have information, one was from a patient in the proliferative stage, and two were secretory. There were no obvious differences in response to prostaglandins between any of the uteri, the variation between strips from the same uterus being as large as the variation between uteri.

The effect of 19-hydroxy PG E_1 on the non-pregnant uterus is not unexpected in view of the findings of other workers that the 19-hydroxy PG Es were less potent than, but similar in action to the E prostaglandins. The measured EPMR PG E_1 / 19-hydroxy PG E_1 = 1:4 is (coincidentally?) equal to the mean ratio of these compounds found in human semen, and if their effect on the uterus represents a physiological effect of semen, then in an average ejaculate about half of the effect of the 9-ketoprostaglandins is due to PG Es and half to 19-hydroxy PG Es.

One point of interest concerns the measured EPMR for natural / synthetic 19-hydroxy PG E_1 = 1:2. The synthetic compound used was a racemic mixture in two senses, firstly

about all optically active centres, and secondly and independently about the 19 carbon. There were therefore four molecular species present, only one of which was identical with the natural compound (fig. 3.2).

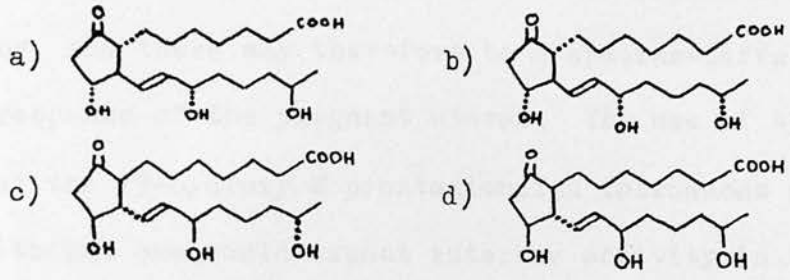


Fig. 3.2 Isomers of 19-hydroxy PG E_1
a) is the natural compound.

One would expect to find an EPMR of 1:4, and the findings indicate that at least one of the other isomers is active. It seems unlikely that the enantiomeric compounds c) and d) are active, since they differ from the naturally occurring prostaglandins at the 8, 11, 12 and 15 positions. The implication is that the activity of isomer b) is roughly equal to that of isomer a), and that the uterus is indifferent to the configuration at C_{19}. Spilman *et al* (1977) have shown that the methyl ester of compound b) is about half as active as that of compound a) in stimulating contraction of the pregnant rhesus monkey uterus *in vivo*, results which are not incompatible with those reported above.

The finding that 19-hydroxy PG E_1 inhibits oxytocin-induced contractions, and reverses the stimulation induced by PG E_1 in the pregnant uterus *in vitro* is interesting, since the effect of the 19-hydroxy compound is here quali-

-tatively different to that of PG E_1. This finding is at variance with those of Spilman et al (1977), although there are several possible explanations for this. The semen of the rhesus monkey contains large amounts of 19-hydroxy PG E_1, but unlike the human, rhesus monkeys do not copulate during pregnancy, and there may therefore be a species-difference in the response of the pregnant uterus. The use of the methyl esters of the 19-hydroxy E prostaglandins introduces complications, since although one would expect esterase activity in the blood to release the native prostaglandin, the methyl esters themselves may not be without activity, and this activity may be qualitatively different from that of the native compounds. Since the compounds were introduced by intravenous injection, the observed effects may have been due to metabolites formed during circulation or to stimulation of the release of oxytocin from the pituitary gland. Differences of effect of prostaglandins between in vitro and in vivo tests are not without precedent, since the E prostaglandins have been shown to inhibit the contraction of the non-pregnant human uterus in vitro (Sandberg et al, 1963, 1964; Bygdeman, 1964) while stimulating the same organ in vivo (Roth-Brandel et al, 1970; Coutinho and Maia, 1971; Karim et al, 1971; Martin and Bygdeman, 1975) and these observations have yet to be reconciled.

The results obtained with the marmoset uterus were insufficient to allow any conclusions to be drawn, but in the case of the late pregnant uterus do not contradict those obtained with human material.

Chapter 4.

In vitro biosynthesis of 19-hydroxy prostaglandins.

Results - series 1.

The weights of organs, homogenate volumes and measured concentrations of prostaglandins are listed in table 4.1. The extracts of the reproductive organ homogenates contained large amounts of interfering compounds, and under these conditions prostaglandins could not be identified with certainty below 2 μg/ml. Only 19-hydroxy E prostaglandins could be identified, and these only in the extract prepared from the seminal vesicle homogenate. The proportions of the two 19-hydroxy E compounds were 20% 19-hydroxy PG E_2 and 80% 19-hydroxy PG E_1, proportions similar to those found in the semen of the stump-tailed macaque.

Table 4.1

Prostaglandin content of Macaca arctoides reproductive organs.

Organ	Weight (g)	Homogenate vol. (ml)	Concentration of prostaglandins PG Es (μg/ml)	19-OH Es (μg/ml)	Weight of 19-OH PG Es per organ (μg)
Seminal vesicle (1)	32.5	150	∠2	10	1500
Testis (1)	23.5	100	∠2	∠2	∠200
Epididymis (1)	5.0	25	∠2	∠2	∠50
Prostate	6.5	25	∠2	∠2	∠50
Ampullae (2)	1.2	25	∠2	∠2	∠50

The results of the incubations performed with the seminal vesicle homogenate are listed in table 4.2. No prostaglandins were detected in the homogenates of the other organs, and these are therefore omitted.

Table 4.2

Prostaglandin content of fresh seminal vesicle homogenate after incubation.

Additions	PG Es (μg/ml)	19-OH PG Es (μg/ml)
None	/2	12.7
Eicosa-11,14,17-trienoic acid	2.7	19.9
Arachidonic acid	2.5	15.2
Eicosa-5,8,11,14,17-pentaenoic acid	/2	20.3

The addition of all three polyunsaturated fatty acids appeared to stimulate the synthesis of 19-hydroxy E prostaglandins. The amount of E prostaglandins was small, and too near the detection limit of the assay for the results to be of significance. There was no difference in the ratio of 19-hydroxy PG E_1 to 19-hydroxy PG E_2 attributable to the substrate added.

Experiments with frozen seminal vesicle homogenate - series 1.

Time course of 19-hydroxy PG E synthesis. (29 days storage)

The results of this experiment (fig. 4.1) show that 19-hydroxy PG E levels rose on incubation from an initial level of 1.5 μg/ml to 3.5 μg/ml over a period of one hour, remaining near this level for a further three hours and finally declining to near the detection limit of the assay after 8.5 hours.

Fig. 4.1 Time course of 19-hydroxy PG E synthesis in vitro.

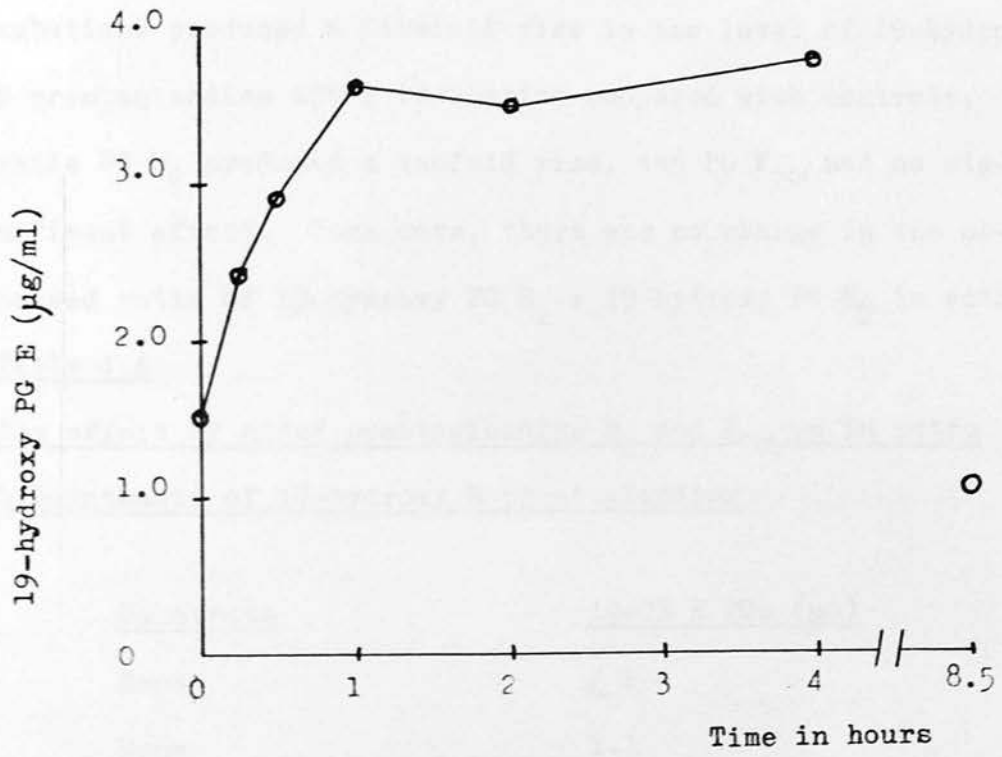


Table 4.3

The effect of added prostaglandin E_1 on levels of 19-hydroxy E prostaglandins in incubated seminal vesicle homogenate.

Additions	19-OH PG Es (µg).
none	1.3
none	1.7
PG E_1	7.9
PG E_1	7.0

Incubations with PG E_1 (43 days storage) and with PG E_2 and PG $F_{2\alpha}$ (46 days storage).

The results of these experiments are given in tables 4.3 and 4.4 respectively. The addition of PG E_1 to the incubations produced a fivefold rise in the level of 19-hydroxy E prostaglandins after incubation compared with controls, while PG E_2 produced a twofold rise, and PG $F_{2\alpha}$ had no significant effect. Once more, there was no change in the observed ratio of 19-hydroxy PG E_1 : 19-hydroxy PG E_2 in either case.

Table 4.4

The effect of added prostaglandins E_2 and $F_{2\alpha}$ on in vitro biosynthesis of 19-hydroxy E prostaglandins

Substrate	19-OH E PGs (µg)
None	< 1
None	1.1
PG $F_{2\alpha}$	1.4
PG $F_{2\alpha}$	< 1
PG E_2	2.3
PG E_2	2.6

Results - series 2

The measured levels of prostaglandins in the reproductive organs are given in table 4.5, and broadly agree with those measured in series 1. The higher sensitivity of the single ion monitoring method allowed E and 19-hydroxy E prostaglandins to be detected in all the tissues examined, with the exception of the prostate, where PG E levels were below the detection limit of the assay. Very high levels of 19-hydroxy E prostaglandins were present only in the seminal vesicle, although traces were found in the other organs. It should be pointed out, however, that the organs were handled together and homogenised using the same homogeniser, and under these conditions the possibility of cross-contamination cannot be ruled out. In particular, the minute quantities of 19-hydroxy E prostaglandins detected in the testis seem likely to have arisen from this source.

Incubation of the seminal vesicle homogenate with ^{14}C eicosatrienoic acid or arachidonic acid yielded a pattern of products not significantly different to that observed with bovine or ovine seminal vesicle homogenates. Neither of these substrates, nor PG E_1 gave rise to a significant incorporation of radioactivity into the 19-hydroxy prostaglandin fractions (table 4.6). The highest level of incorporation (3.3%) was obtained using eicosatrienoic acid in the presence of testosterone, however, it cannot be concluded that the steroid exerted a significant effect, since there was no evidence of increased incorporation in the presence

of testosterone when arachidonic acid was the substrate.

Incubations performed with the seminal vesicle homogenate after storage under liquid nitrogen likewise showed no evidence of incorporation of label into the 19-hydroxy prostaglandin fractions. The effects of the various cofactors added were similar to those reported for prostaglandin synthetase preparations from other sources. Tryptophan stimulated production of PG E and PG D, while reduced glutathione strongly increased the yield of PG E at the expense of PG D and PG F. Cyclic AMP, ascorbic acid and the nicotinamide nucleotides (reduced or oxidised) had little effect at the levels tested, or were inhibitory. Cyanide, interestingly, stimulated the production of PG D, and to a lesser extent PG F. None of the compounds tested could be shown to stimulate incorporation of the label into the 19-hydroxy prostaglandin fractions and PG $F_{3\alpha}$ could not serve as a substrate for hydroxylation in this system (table 4.7).

The production of 19-hydroxy PG E_1 from endogenous precursors was much more rapid in this preparation than in that studied in series 1, and it proved necessary to dilute the homogenate tenfold in order to produce the time course shown in fig. 4.3. In the absence of indomethacin, the level of 19-hydroxy PG E_1 in the diluted homogenate rose more than twofold over the first ten minutes of incubation and declined thereafter. In the presence of indomethacin (20 μMolar) levels did not change significantly during the first ten minutes, but then declined rapidly.

Table 4.5

Prostaglandin content of reproductive organs- series 2.

Organ	Weight (g)	Vol. (ml)	Prostaglandin content (µg/organ) PG E_1	PG E_2	19-OH E_1	19-OH E_2
Testis (1)	23.5	150	0.79	1.1	0.28	0.02
Prostate	6.3	25	/0.04	/0.08	2.0	0.13
Ampulla (1)	1.0	25	1.2	1.4	6.9	0.82
Seminal vesicle (1)	27.2	150	90	64	1220	164

Table 4.6

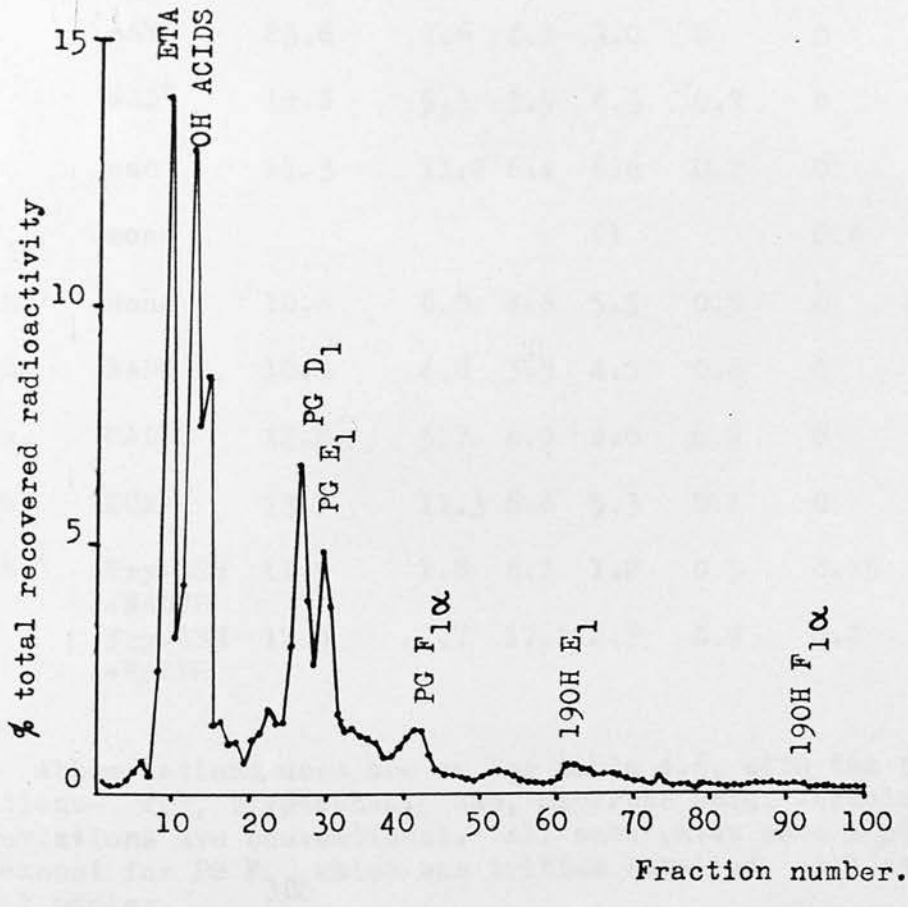
Incubation of labelled precursors with seminal vesicle homogenate.

Substrate	Additions	% radioactivity recovered as OH acid	PGD	PGE	PGF	19OHE	19OHF	Recovery
ETA	none	30.8	12.2	11.3	5.7	1.0	0.1	68%
ETA	T	36.9	16.1	12.6	6.4	3.3	0.4	50%
ETA	PG E_2	31.4	7.8	8.0	4.1	0.3	0.2	49%
Arach.	none	19.5	6.2	6.7	3.9	1.2	0.6	62%
Arach.	T	31.2	7.1	6.3	3.3	0.5	0	39%
PG E_1	none			69		2.2	1.4	17%
PG E_1	T			88		1.2	0.1	43%

The above incubations were carried out using fresh homogenate immediately after preparation. Abbreviations used in the table are as follows:- ETA, ^{14}C eicosa-8,11,14-trienoic acid; T, testosterone. PG E_1 was tritium labelled. Unlabelled PG E_2 was 50 µg/ml and testosterone 1 mMolar. All radioactive substrates were 1 µCi

Fig. 4.2

Incubation of labelled precursors with seminal vesicle homogenate, separation of products on LH 20 Sephadex column.



Seminal vesicle homogenate (1 ml) incubated with eicosa-8, 11,14-trienoic acid (1 μCi) and testosterone (1 mMolar). The peak labelled 19-OH PG E_1 may also contain the (hypothetical) 19-hydroxy prostaglandin D_1.

Table 4.7

Incubation of labelled precursors with seminal vesicle homogenate after storage under liquid nitrogen.

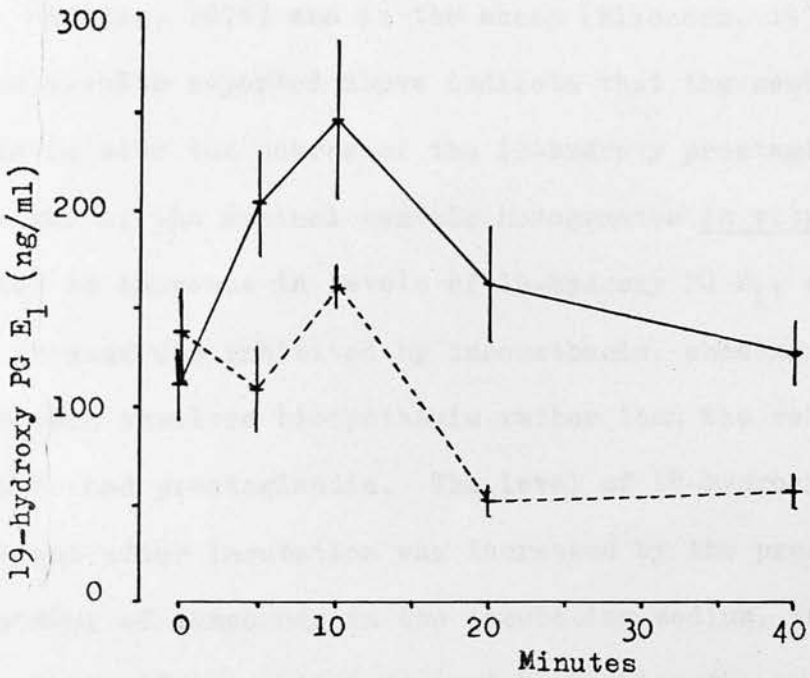
Substrate	Additions	% radioactivity recovered as						Recovery
		OH acid	PGD	PGE	PGF	19OHE	19OHF	
ETA	none	19.0	14.2	8.3	9.4	0	0	38%
ETA	Try	16.0	20.0	13.0	7.6	0.6	0	60%
ETA	GSH	24.3	3.4	26.4	4.2	0.3	0	60%
ETA	cAMP	23.6	7.6	4.1	3.0	0	0	45%
ETA	NAD^+	19.6	5.3	5.9	6.3	0.7	0	50%
ETA	asc	17.3	11.2	6.4	6.6	0.2	0	60%
PG $F_{3\alpha}$	none				91		0.6	90%
Arach.	none	10.4	6.8	4.6	5.5	0.5	0	76%
Arach.	NADH	10.3	4.8	3.5	4.5	0.6	0	72%
Arach.	NADPH	12.8	5.7	6.9	4.0	0.9	0	95%
Arach.	KCN	13.1	11.3	6.6	5.3	0.1	0	106%
Arach.[1]	Try+GSH +NADPH	11.5	1.8	6.1	1.2	0.5	0.15	80%
ETA[1]	Try+GSH +NADPH	12.8	2.7	17.1	2.2	0.9	0.2	94%

Abbreviations used are as for table 4.6, with the following additions- Try, tryptophan; asc, ascorbic acid. Remaining abbreviations are conventional. All substrates were 1 μCi ^{14}C except for PG $F_{3\alpha}$ which was tritium labelled. All additions were 1 mMolar.

[1] The final two incubations were performed using only 0.3 ml homogenate, remainder 1.0 ml.

Fig. 4.3

Time course of 19-hydroxy PG E_1 production in vitro (series 2).



Incubation of seminal vesicle homogenate (diluted x 10) with reduced glutathione (1 mMolar) and tryptophan (1 mMolar), total volume 2.5 ml.

Incubation as above plus indomethacin (20 μMolar)

Bars indicate standard deviation of replicate injections from a single sample (n = 6). (Since this standard deviation does not include all sources of variance it may not be used to infer statistical significance.)

Discussion.

The seminal vesicle has been shown to be the source of the classical prostaglandins of semen both in man (Eliasson, 1959; Hamberg, 1976) and in the sheep (Eliasson, 1959), and the results reported above indicate that the seminal vesicle is also the source of the 19-hydroxy prostaglandins. Incubation of the seminal vesicle homogenates *in vitro* produced an increase in levels of 19-hydroxy PG E_1, and this increase was inhibited by indomethacin, showing that the process involves biosynthesis rather than the release of pre-formed prostaglandin. The level of 19-hydroxy PG E_1 present after incubation was increased by the presence of a number of compounds in the incubation medium, including eicosa-11,14,17-trienoic acid (which, lacking the unsaturated group at C_8, cannot be a substrate for prostaglandin biosynthesis) and PG E_2. It seems probable that this effect was due to inhibition of the breakdown of 19-hydroxy PG E_1 formed from endogenous precursors, since neither arachidonic acid nor PG E_1, which were both shown to raise 19-hydroxy PG E_1 levels in the first series of incubations, could serve as precursors when isotopically labelled.

Three routes are suggested for the biosynthesis of 19-hydroxy PG E_1 from esterified eicosa-8,11,14-trienoic acid which is presumed to be the original precursor (fig. 4.4). Route A involves hydroxylation of PG E_1, and a prostaglandin hydroxylase capable of forming 19-hydroxy and 20-

hydroxy prostaglandins by this route has been shown to be present in guinea pig liver (Israelsson et al, 1969; Kupfer et al, 1978). In the seminal vesicle homogenates, however, no incorporation of label into 19-hydroxy PG E_1 could be detected when ^3H PG E_1 was used as a substrate, effectively eliminating this pathway as a possibility.

A microsomal oxidase capable of hydroxylating fatty acids at the ω position, as required by route B, has been shown to exist in rat liver (Hamberg and Bjorkem, 1971) but the failure of the seminal vesicle homogenate to produce labelled 19-hydroxy PG E_1 in significant quantities in the presence of ^{14}C ETA indicates that this is not an important pathway in the seminal vesicle.

GCMS analysis of macaque seminal vesicle homogenates has shown no evidence for the presence of 19-hydroxy fatty acids, however, there is also very little free eicosatrienoic or arachidonic acid, so the esterase step may be rate-limiting, leading to low levels of the precursors for both E and 19-hydroxy E prostaglandins. Sih et al (1969) incubated 19-hydroxy arachidonic acid with bovine seminal vesicle microsomes, and showed that 19-hydroxy PG E_2 was produced only in very low yield; it may be however, that there are species differences in the specificity of the cyclo-oxygenase enzyme towards these hydroxylated substrates.

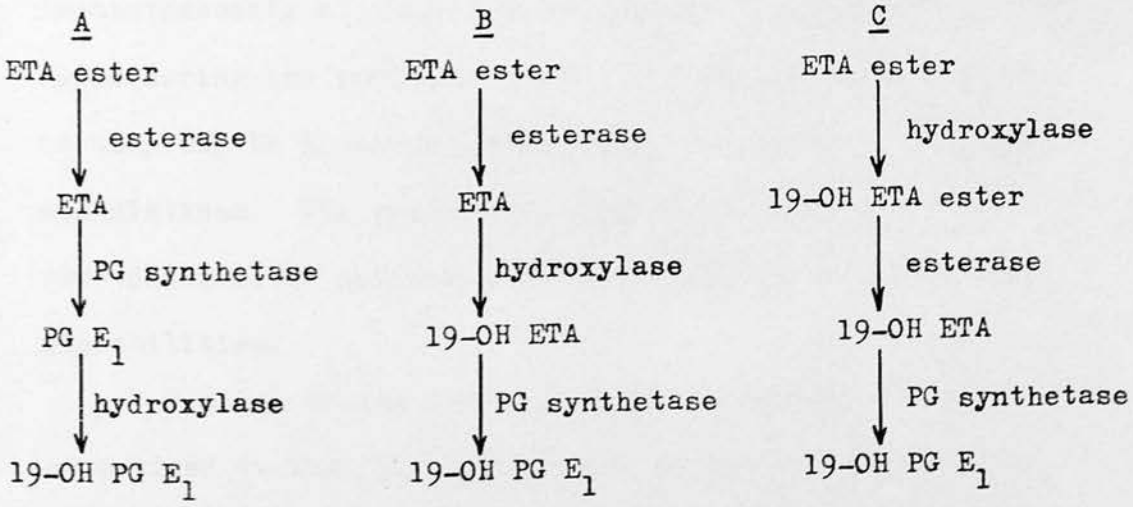
In the absence of any positive evidence, it cannot be concluded that the remaining pathway, route C, is identified as the biosynthetic pathway involved. The definitive

experiment, incubation of seminal vesicle homogenate with phospholipase, lipase or other esterases in the presence of indomethacin, followed by isolation of the released 19-hydroxy eicosatrienoic acid, must await the supply of further primate seminal vesicles.

It should be noted that a small amount of a compound having a retention volume on LH 20 Sephadex similar to that of 19-hydroxy PG E was formed from eicosatrienoic acid, however, the low yield compared with that of PG E suggests that a roundabout route was involved, possibly involving esterification of the acid as in route C.

Fig. 4.4

Possible routes for the biosynthesis of 19-hydroxy PG E_1.



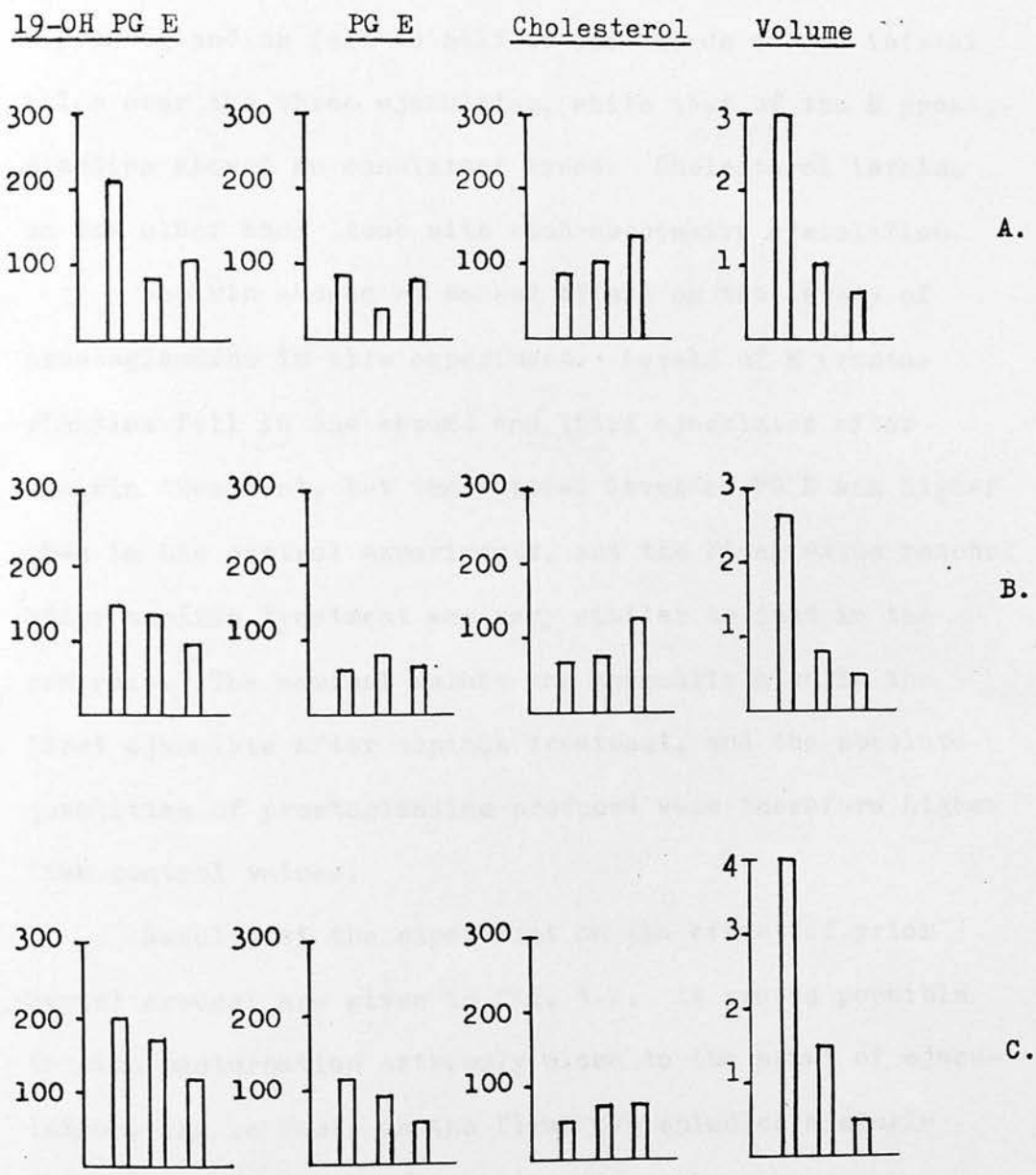
Chapter 5. Dynamic aspects of seminal prostaglandin biosynthesis in man.

Early work by Eliasson (1959) showed that the prostaglandin content of human semen (as measured by bioassay) was not affected when ejaculates were produced at 24 hour intervals, but beyond this the time course of biosynthesis of the seminal prostaglandins has so far received little attention. In most tissues, prostaglandins are not stored, but synthesised rapidly in response to a stimulus. In the case of the seminal prostaglandins, however, it is possible that the very large amounts produced in an ejaculate may require storage. There are essentially three possibilities for the time course of seminal prostaglandin biosynthesis: the prostaglandins may be synthesised almost instantaneously at the point of orgasm; they may be synthesised during the period of sexual arousal preceding orgasm; or they may be produced continuously in the period between ejaculations. The experiments described in this section were designed to contribute to an evaluation of these three possibilities.

Results of the serial ejaculate experiments are summarised in fig. 5.1. There was no change in the ratio PG E_1 : PG E_2 or 19-OH PG E_1 : 19-OH PG E_2 and for the sake of clarity results are quoted in terms of total PG E and total 19-OH PG E.

In terms of the absolute quantities produced, the amount of all three compounds measured fell with each successive ejaculation. There was, however, a parallel fall

Fig. 5.1 Serial ejaculate results.



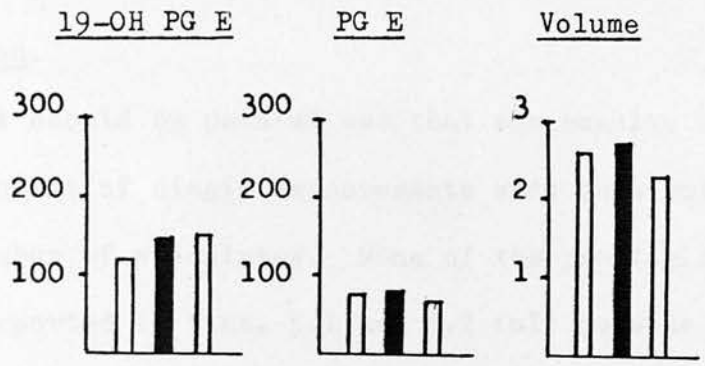
Each cell of the diagram gives the concentration of the compounds identified at the top of the page (μg/ml) or seminal volume (ml) for three successive ejaculates taken at half hour intervals. A and B are duplicate control experiments, C gives results obtained after aspirin ingestion.

in seminal volume, and when this is taken into account the concentrations of the measured compounds can be seen to behave differently. The concentration of the 19-hydroxy E prostaglandins fell to half to two thirds of its initial value over the three ejaculates, while that of the E prostaglandins showed no consistent trend. Cholesterol levels, on the other hand rose with each successive ejaculation.

Aspirin showed no marked effect on the levels of prostaglandins in this experiment. Levels of E prostaglandins fell in the second and third ejaculates after aspirin treatment, but the initial level of PG E was higher than in the control experiments, and the final value reached after aspirin treatment was very similar to that in the controls. The seminal volume was unusually high in the first ejaculate after aspirin treatment, and the absolute quantities of prostaglandins produced were therefore higher than control values.

Results of the experiment on the effect of prior sexual arousal are given in fig. 5.2. It proved possible to stop masturbation extremely close to the point of ejaculation, and in fact, in the first two episodes a single drop of fluid was produced before masturbation ceased. Subjectively, this process produces extremely unpleasant symptoms, including a diffuse ache in the lower pelvis, enlarged and tender testes and a sensation as of incipient diarrhoea. All of these symptoms were immediately relieved by complete ejaculation. Neither the prostaglandin

Fig. 5.2 Effect of prior sexual arousal on seminal prostaglandin levels.



Each cell of the diagram gives the prostaglandin concentration (μg/ml) or seminal volume (ml) for three ejaculates spaced at 24 hour intervals. Open bars (days 1 and 3) represent control ejaculates. Solid bars (day 2) represent an ejaculate produced after two prior episodes of masturbation stopping short of ejaculation.

levels nor the seminal volume were affected by previous sexual arousal, and the ejaculate produced was similar in all respects to the control ejaculates produced on the previous and following days.

Discussion.

It should be pointed out that the results reported above consist of single measurements made on a relatively small number of ejaculates. None of the prostaglandin levels reported in figs. 5.1 and 5.2 fall outside the normal range of levels for fertile semen as reported by Templeton et al (1978), and the results cannot be considered statistically significant. In view of the obvious experimental difficulties attendant upon this work, it was not possible to obtain sufficient samples for statistical analysis, and any conclusions drawn from these results must be considered speculative.

Chronic aspirin ingestion, sufficient to depress seminal prostaglandin levels, showed no effect on seminal volume (Collier and Flower, 1971; Horton et al, 1973), and therefore presumably the reduced availability of prostaglandins in closely spaced ejaculates is not the factor determining reduced seminal volume. The relatively constant levels of E prostaglandins observed in serial ejaculates indicates the presence of a regulatory mechanism responding to the available volume of fluid. The falling levels of 19-hydroxy E prostaglandins suggest that in this

case the demand was sufficient to outstrip the biosynthetic capacity of the system, and that 19-hydroxy PG Es are replaced more slowly than the E prostaglandins relative to their normal levels.

It had been hoped that the administration of a single large dose of aspirin shortly before a series of ejaculates would allow differentiation between the various possibilities for the time course of seminal prostaglandin biosynthesis. If the prostaglandins present in each ejaculate were synthesised during the processes of sexual arousal and ejaculation, aspirin present at the time should depress prostaglandin levels in all three ejaculates, whereas if prostaglandin synthesis was a steady-state process taking place in the resting period between ejaculates, the prostaglandin levels in the first ejaculate should be unaffected, while those in the second and third ejaculates should be depressed.

In practice, the initial levels of E prostaglandins present were higher than in the controls, and although levels fell in the subsequent ejaculates the final concentration was similar to that in the controls. Levels of 19-hydroxy E prostaglandins in all three ejaculates after aspirin treatment were not notably different from control values. The lack of action of aspirin in this experiment may be due to a slow uptake into the seminal vesicles, since the dose employed (2.1 g) was similar to that which produced a significant depression of seminal prostaglandin levels when administered in distributed doses (2.4 g/day

Collier and Flower, 1971; 3.6 and 7.2 g/day, Horton et al, 1973).

The failure of previous sexual arousal to depress 19-hydroxy PG E levels in a subsequent ejaculate may indicate that the seminal prostaglandins are not synthesised during sexual arousal, however, this result must be treated with caution, since control levels on days one and three were rather low compared with those measured in the serial ejaculate experiments. The subjective symptoms reported by the subject during the course of this experiment could possibly be due to leakage of the synthesised prostaglandins from the seminal vesicles, and the results of this experiment must be considered inconclusive.

Chapter 6.

The species distribution of the 19-hydroxy E prostaglandins.

Table 6.1 gives the concentration of 19-hydroxy PG E_1 and 19-hydroxy PG E_2 found in the semen of species examined so far. In addition to those species listed we have examined a small sample (ca. 50 µl) of semen obtained from the Barbary ape (Macaca sylvestris) by electroejaculation. Although containing motile sperm, this sample contained no detectable prostaglandins, and it is probable that ejaculation was not complete in this case. Similarly, in the case of the electroejaculated gorilla semen listed in table 6.1 the seminal volume was extremely small (ca. 100 µl), and it is suspected that the measured concentration of prostaglandins is not representative of that produced during coitus.

We have also attempted to obtain semen samples from the marmoset (Callithrix jacchus) by injection of a synthetic prostaglandin analogue (ICI 81008, fig. 6.1) which is said to induce ejaculation in some species. We were unable to induce ejaculation in this case. However, examination of the urine produced by the animal after injection of the drug showed the presence of 19-hydroxy F prostaglandins and 19-hydroxy PG A_1. It is possible that in this case the ICI compound induced retroejaculation, and that the 19-hydroxy PG E_1 present dehydrated to 19-hydroxy PG A_1 during exposure to the urine. Recovery of semen from the vagina of a marmoset after coitus gave no detectable prostaglandins.

Table 6.1 Concentrations of 19-hydroxy E prostaglandins in non-human semen.

Species	Source of semen	No. of samples	19-OH E_1 (μg/ml)	19-OH E_2 (μg/ml)
Chimpanzee (Pan troglodytes)	M	2	390	84
Gorilla (Gorilla gorilla)	E	1	5	NI
Orang-utan (Pongo pygmaeus)	E	2	54	NI
Rhesus monkey (Macaca mulatta)	M	8	420	84
Stump-tailed macaque (Macaca arctoides)	M	1	780	150
Horse (Equus caballus)	AV	1	ND	ND
Cow (Bos taurus)	AV	3	ND	ND
Sheep (Ovis aries)	AV	8	ND	ND
Pig (Sus scrofa)	AV	2	ND	ND
Rabbit (Oryctolagus cuniculus)	AV	3	ND	ND
Chicken (Gallus domesticus)	LM	1	ND	ND

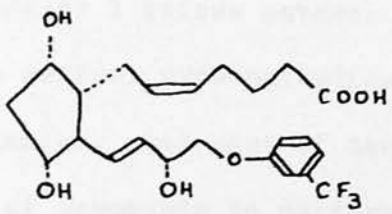
Abbreviations -

M Masturbation
E Electroejaculation
AV Artificial vagina
LM Lumbar massage
NI Not identified (mass spectra unobtainable due to interfering compounds or low levels of PG)
ND Not detectable (< 1 μg/ml)

GLC measurements were performed by Dr R.W. Kelly.

Fig. 6.1

ICI 81008 - a prostaglandin analogue capable of inducing ejaculation.



Discussion.

The results of the analyses of non-human semen have shown that the 19-hydroxy prostaglandins are characteristic of the semen of primates, although since this work was performed a report has appeared describing the presence of 19-hydroxy F prostaglandins in the semen of a marsupial, Trichosurus vulpecula (Marley et al, 1977). Measurable levels of E and F prostaglandins were found only in human and in ram semen, other primates' semen containing only a trace of these components. No prostaglandins were detected in the semen of non-primates other than the ram. The absence of prostaglandins from bull semen is in accordance with earlier reports (Eliasson, 1959; Voglmayr, 1973), but is nonetheless surprising, since bull seminal vesicles are a rich source of prostaglandin synthetase, and are used commercially as a source of this enzyme.

General discussion.

The pattern of prostaglandins present in human semen, as revealed in chapter 1 raises several interesting questions. Assuming that the seminal prostaglandins represent part of a functional mechanism, what sort of mechanism requires such a diversity of compounds to perform its function? The answer which springs most readily to mind is that the pattern of prostaglandins which we see in human semen represents mainly metabolites, and that the functional compounds are much fewer in number. Many of the compounds described in chapter 1 have been shown to be produced (by other tissues) as metabolites of the classical prostaglandins - dinor PG $F_{2\alpha}$ as a urinary metabolite of PG $F_{2\alpha}$ in the rat (Granstrom et al, 1965), 8-iso PG E_2 and 8-iso PG $F_{2\alpha}$ as minor products of the incubation of PG E_2 with guinea pig liver homogenates (Hamberg and Israelsson, 1970) and 19-hydroxy PG E_1 as a product of the incubation of PG E_1 with guinea pig liver microsomes in the presence of NADPH (Kupfer et al, 1978).

The balance of evidence presented here, however, indicates that the compounds described are not metabolites of PG E or PG F, although they can all be regarded in a sense as metabolites of the appropriate endoperoxide precursors. The incubation of semen with isotopically-labelled PG E_1 and PG $F_{2\alpha}$ has shown that none of the prostaglandins described are formed in semen as metabolites of these compounds (chapter 1), and the incubation of seminal vesicle homogenates

with labelled PG E_1, PG $F_{2\alpha}$ and PG $F_{3\alpha}$ indicates that these compounds are not metabolised to 19-hydroxy prostaglandins in the seminal vesicle (chapter 4). The inability of the seminal vesicle homogenates to synthesise significant quantities of 19-hydroxy prostaglandins from added arachidonic acid or eicosatrienoic acid while retaining the capacity to do so from endogenous precursors indicates that the pathway leading to the 19-hydroxy prostaglandins is completely separate from that leading to the classical prostaglandins.

One possible explanation for the high levels of 19-hydroxy prostaglandins in primate semen concerns the solubility of these compounds. Although PG E and PG F are quite soluble in water, their immediate precursors, PG G and PG H are considerably less polar (Hamberg et al, 1974) and could be expected to show lower solubility in aqueous solvents. Hydroxylation of the fatty acid precursor prior to cyclisation would increase the polarity of the endoperoxides and render them more soluble. The presence of 19-hydroxy prostaglandins may therefore represent an adaptation permitting higher concentrations of the endoperoxide precursors to be present in the seminal vesicles.

Another possible explanation for the presence of 19-hydroxy prostaglandins in primate semen is connected with the effects of the seminal prostaglandins on the pregnant uterus. In man, and in some other primate species, copulation serves a social as well as a reproductive function, and

continues to occur during pregnancy. The pregnant uterus is therefore exposed to frequent doses of seminal prostaglandins. The results reported in chapter 3 of this thesis indicate that 19-hydroxy PG E_1 antagonises the effects of both PG E_1 and oxytocin on the human pregnant uterus *in vitro*. It is possible therefore that the 19-hydroxy E prostaglandins represent an evolutionary adaptation in primates, serving to counteract the deleterious effects of the seminal prostaglandins on the pregnant female.

Neither of these explanations offer us any clues about the primary function of the seminal prostaglandins however. Why should sheep and primates require relatively huge amounts of these compounds in their semen when most other mammals manage perfectly well without? A particularly interesting paradox arises in the case of the bull, an animal which possesses large seminal vesicles which are richly endowed with prostaglandin synthetase. Bull seminal vesicle microsomes are commercially available as a source of prostaglandin synthetase, and are used for the commercial production of prostaglandins, yet bull semen contains almost no prostaglandins (Voglmayr, 1973; Kelly *et al*, 1976).

One possible explanation for this is that the prostaglandin synthetase of bull seminal vesicles represents a vestigial character whose function has been lost in the animal's evolutionary development. Kunze and Vogt (1971) have shown that incubation of bull seminal vesicles yields

very little prostaglandin (unlike those of the sheep) unless phospholipase A is added to the medium. However, acetone powders prepared from bull seminal vesicle were shown to contain more phospholipase A activity than those prepared from the sheep seminal vesicle, so the lack of activity in the intact cell presumably represents an effect of compartmentalisation. It seems possible then, that the bovine seminal vesicle prostaglandin synthetase system is disabled *in vivo* and that the species has lost the capacity to synthesise seminal prostaglandins even though retaining the appropriate enzymes.

Homogenisation of the seminal vesicle prior to incubation with substrates for PG synthesis undoubtedly introduces artifacts. The major prostaglandin product of such incubations with the stump-tailed macaque seminal vesicle in the absence of added cofactors was PG D (chapter 4), a prostaglandin shown to be absent from human semen (chapter 1) and certainly not present at high levels in the semen of the stump-tailed macaque. Similarly, the hydroxy acids produced by the prostaglandin synthetase *in vitro* have not been reported to be present in semen. These effects may be due to changes in availability of cofactors resulting from the destruction of compartments during homogenisation, or alternatively may be due to selective passage of the various products of the prostaglandin synthetase system across the cell membrane *in vivo*.

Another possible solution to the paradox of the bull

lies in the observations that mammalian spermatozoa can bind prostaglandins (Bartoszewicz et al, 1975; Mercado et al, 1978) and that bull semen contains an extremely high concentration of spermatozoa (Mann, 1964). It is possible, therefore, that the function of the seminal prostaglandins is connected with their ability to bind to spermatozoa, and that the free prostaglandins which we can detect in semen simply represent excess production. Early observations by Sturde (1968) showed significantly higher prostaglandin activity in the semen of azoospermic men. Recent measurements of E and 19-hydroxy E prostaglandins (Kelly et al, 1979) have shown that the converse is also true, that men with very high sperm-counts (more than $3x10^8$/ml) have significantly lower seminal prostaglandin levels. Seminal prostaglandins have also been shown to rise after vasectomy (Brummer, 1973).

The complex pattern of prostaglandins observed in semen is somewhat simplified when we consider the endoperoxide precursors. Prostaglandins D, E and F have all been shown to arise from the same pool of endoperoxide (Wlodawer and Samuelsson, 1973). Work reported here (chapter 4) indicates that the 19-hydroxy prostaglandins are probably synthesised via a different pathway. It is not known at present whether the 8-isoprostaglandins arise from the same endoperoxide precursors as their parent compounds or from 8-iso PG G and 8-iso PG H. Nonetheless, if binding of the prostaglandins to sperm takes place before the endoperoxide precursors

decay to PG E and PG F the picture of prostaglandin production in the seminal vesicle is considerably simplified. In addition, relatively minor non-functional byproducts of the prostaglandin synthetase may not be bound, and their levels in semen would therefore be selectively increased. Such a subtractive process would also go a long way towards explaining the enormous variation in prostaglandin levels found in the semen of fertile men (Templeton et al, 1978; Perry and Desiderio, 1977).

It is unfortunate that relatively little progress has been made in the elucidation of the biosynthetic pathway leading to the 19-hydroxy E prostaglandins, since a knowlege of this mechanism offers the possibility of a practical application of this work. The biosynthesis of 19-hydroxy prostaglandins appears to be confined to the seminal vesicle, and if these compounds are shown to be functional the biochemistry of the seminal vesicle therefore offers a point of pharmacological attack on the reproductive system. The prostaglandin synthetase system of bull seminal vesicles has been shown to utilise 19-hydroxy arachidonic acid only poorly (Sih et al, 1969), and this may be true for the prostaglandin synthetase of other organs which do not normally synthesise 19-hydroxy prostaglandins. It may therefore be possible to design a specific inhibitor for the 19-hydroxy prostaglandin synthetase of the primate seminal vesicle. Such an inhibitor would be a useful tool for the investigation of the function of the 19-hydroxy prostaglandins, and, should

the 19-hydroxy prostaglandins prove to be functional, it is just conceivable that it could function as a contraceptive.

A further possible practical application arises from the work presented in chapter 3. The inhibition of activity of pregnant human myometrium by 19-hydroxy PG E_1 suggests that this compound could be used in the treatment of premature labour. Results reported on the rhesus monkey *in vivo* (Spillman *et al*, 1977; M.J. Novy, unpublished) indicate that here the action is stimulatory, although this may be due to a species difference. Nevertheless, the lack of activity of 19-hydroxy PG E_1 on smooth muscle other than the human uterus, and the fact that the human female is frequently exposed as a result of intercourse to moderately large doses of this compound without ill effect implies that a low incidence of adverse side effects may be expected.

List of abbreviations and symbols.

a.m.u.	Atomic mass units (Daltons).
asc.	Ascorbic acid.
ATP	Adenosine 5' triphosphate.
(BB)	n-butyl boronate derivative.
ca	Circa.
cAMP	Cyclic adenosine 3'5' monophosphate.
cGMP	Cyclic guanosine 3'5' monophosphate.
CPM	Counts per minute.
DPM	Disintegrations per minute.
(DMS)	Dimethyl silyl derivative.
(EE)	Ethyl ester derivative.
(EO)	Ethyl oxime derivative.
EPA	Eicosa-5,8,11,14,17-pentaenoic acid
EPMR	Equipotent molar ratio.
ETA	Eicosa-8,11,14-trienoic acid.
GCMS	Combined gas-liquid chromatography-mass spectrometry.
GLC	Gas-liquid chromatography.
GSH	Glutathione, γ-glutamyl cysteinyl glycine.
HHT	12-hydroxy heptadeca-5,8(cis),10(trans)-trienoic acid.
HSF-PG	A crude extract of semen containing PGs.
IR	Infra red spectrometry.
(MB)	Methyl boronate derivative.
(ME)	Methyl ester derivative.
m/e	Mass/charge ratio of ions. Since only singly charged ions are discussed m/e = mass in a.m.u.
MID	Multiple ion detector.
(MO)	Methyl oxime derivative.
NAD^+	β-Nicotinamide-adenine dinucleotide (oxidised form).
NADH	β-Nicotinamide adenine dinucleotide (reduced form).
$NADP^+$	β-Nicotinamide adenine dinucleotide phosphate (oxidised form).
NADPH	β-Nicotinamide adenine dinucleotide phosphate (reduced form)

Continued/

NMR	Nuclear magnetic resonance spectrometry.
(O)	Oxime derivative.
PG	Prostaglandin (systematic names of all prostaglandins are given in Table 1.1, P. 125).
T	Testosterone (Androst-4-ene 17-ol,3-one).
(tBB)	t-butyl boronate derivative.
(tBDMS)	t-butyl dimethyl silyl derivative.
TLC	Thin layer chromatography.
(TMS)	Trimethyl silyl derivative.
Try	Tryptophan (Indole-3-(2-amino)acetic acid).
α,β	When used in connection with PG F compounds refers to the steric configuration of the hydroxyl at C_9.
σ	Standard deviation.
ψ	Refers to the penultimate numbered carbon atom of a compound, ie C_{19} in the 20-carbon PGs, C_{17} in the dinor PGs.

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Appendix - computer programs.

Computer programs used in this work were written in the high level interpretive language 'Focal' (Digital equipment Co.) for use on the PDP 8I computer. Since only 4K of core was available the programs contain no comments in order to conserve space. A pragmatic attitude has been adopted in the writing of these programs; no claim is made that they represent the most economical or the most elegant solutions to the problems posed, it was sufficient that they work and give accurate results.

Programs as listed on the teletype cannot be bound into a thesis since the margins are too narrow. The programs listed here were therefore re-typed on a typewriter. Experience has shown that it is extremely difficult to transcribe programs in this way without error, and errors can only be detected by running and debugging the transcribed program. The program listings have been carefully checked against the originals, but may still contain some transcription errors, and in this respect I must crave the reader's indulgence.

GLC integrator program.

"Focal" is loaded together with the FADC patch (bin) and started (SA=Ø2ØØ). Program tape is inserted in the high speed reader and read in by typing *(CR). Type 'GO' to start the initial dialogue;-

GLC integrator program.

```
C-FOCAL,1969

Ø1.Ø5 E
Ø1.1Ø A "SLOPE SENSITIVITY" S;S S=1/S
Ø1.12 A "NO. OF SAMPLES" NS
Ø1.13 A "MAX +VE BASELINE SLOPE" G
Ø1.15 S T=Ø;F K=1,6;D 2.
Ø1.2Ø I (S-D)1.3Ø,1.4Ø,1.4Ø
Ø1.3Ø S C=1;S BL=B;S TS(C)=T;T "I";S I=0
Ø1.31 S I=I+R(6);D 2.
Ø1.32 I (D+S)1.5Ø,1.5Ø,1.31
Ø1.4Ø S Z=Z+R(6);D 2.;G 1.2Ø
Ø1.5Ø S T(C)=T;T %5.Ø T*NS/1ØØ
Ø1.55 D 1.31
Ø1.6Ø I (D+S/2)1.55,1.65,1.65
Ø1.65 I (((B-BL)*1ØØ/(T-TS(1)))-G)1.7Ø,1.75,1.75
Ø1.7Ø S TE(C)=T;S V=C;S I(C)=I;G 4.1Ø
Ø1.75 S TE(C)=T;S I(C)=I;S C=C+1;S TS(C)=T;T !,"S";D 2.;S I=Ø;G 1.32

Ø2.Ø5 S A=Ø;F N=1,NS;S A=A+FADC
Ø2.Ø6 I (A-8.8*NS)2.1Ø,2.Ø7,2.Ø7
Ø2.Ø7 T "+"
Ø2.1Ø F N=1,6;S R(N)=R(N+1)
Ø2.15 S R(6)=A
Ø2.17 S D=Ø
Ø2.2Ø F N=2,6;S D=D+R(N)-R(N-1)
Ø2.25 S T=T+1
Ø2.3Ø S B=R(5)

Ø4.1Ø T !;S BD=(B-BL)/(TE(V)-TS(1))
Ø4.15 F C=1,V;D 5.
Ø4.2Ø G 1.2Ø

Ø5.Ø5 S P=P+1;I (P-39)5.1Ø,5.Ø6,5.Ø6
Ø5.Ø6 D 9.;G 1.15
Ø5.1Ø S BS=BL+BD*(TS(C)-TS(1))
Ø5.15 S BE=BL+BD*(TE(C)-TS(1))
Ø5.2Ø S PT(P)=T(C)*NS/1ØØ
Ø5.3Ø S PV(P)=I(C)-((BS+BE)/2)*(TE(C)-TS(C)-1)

Ø9.1Ø F N=1,P;T %5.Ø PT(N);T %6.2 PV(N),!
```

Slope sensitivity. This is an arbitrary number determining the threshold slope at which the computer detects a peak. Values between 10 and 100 were used in this work.

No. of samples. The program samples the output of the A/D converted a predetermined number of times and the sum of these samples constitutes one data point. Ten samples per point were invariably used in this work.

Max. +ve baseline slope. At the end of the integration process for each peak the program checks to see if the baseline has fallen to near its original value. If not, it starts to integrate a new peak unresolved from the first. In order to cope with the rising baselines produced by temperature programming, the maximum possible rise in the baseline since the start of integration is calculated using this parameter. The figure entered is an arbitrary number determined by practice, and depends on the amount of column bleed and the rate of the temperature program. Values used here were 0.1 - 0.2.

After entering "max +ve baseline slope" the carriage return is not depressed until after the injection is made and the solvent peak passed. The program then begins to collect data points (para. Ø2.). If the output from the A/D converter approaches the maximum output of the total ion current monitor amplifier the program types a series of '+' signs to indicate overloading (lines Ø2.Ø6, Ø2.Ø7).

A stack consisting of the last six data points is held in memory, and this data is differentiated to give the slope (line ∅2.2∅).

The slope is compared with the reciprocal of "slope sensitivity" parameter (∅1.2∅) and if it exceeds it integration of the peak begins (∅1.3∅). The program types 'I' to indicate this. Integration continues until the slope goes negative by an amount equal to or greater than the reciprocal of slope sensitivity (∅1.32). Having thus detected the top of the peak, the program types the retention time (in arbitrary units)(∅1.5∅), and continues to integrate the peak until the (negative) slope falls to half the reciprocal slope sensitivity (∅1.6∅). The reciprocal slope sensitivity is here halved to allow for asymmetry of the GLC peak.

The value of the baseline is then compared with a value computed from its initial value at the beginning of integration and the "max +ve baseline slope" parameter (∅1.65). If the baseline has returned to permissible values the integrated value of the peak(s) is computed in para. ∅4. and ∅5. and the program returns to tracking baseline, issuing a carriage return to indicate this. If the baseline is high, the program types an 'S' to indicate a shoulder or unresolved peak and continues to integrate (∅1.75). Unresolved peaks are quantitated by dropping perpendiculars from the valleys to the computed value of the baseline at that point and integrating the areas so defined.

The run is terminated (CTRL/C) after all peaks have

passed and the program is restarted at line Ø9.1Ø to type out a table of retention times and integrated values for the peaks collected.

Validation.

Results obtained using the integrator program were compared with those obtained on the same samples by photocopying the chart record, cutting out the peaks and weighing them. Samples run included semen (MO/ME/TMS)(6 replicates from pooled semen), standard 19-hydroxy PG E_1 (MO/ME/TMS) 5.2 - 52 µg/injection and extracts of stump-tailed macaque seminal vesicle homogenate (MO/ME/TMS)(3 replicates).

In all cases, for total PG E, total 19-hydroxy PG E and cholesterol, the ratio of internal standard/sample was within 7% for the two methods. In addition, the integrator program gave slightly smaller standard deviations for replicate samples, and all results quoted here were therefore measured using it.

GLC retention indices program.

The program is loaded and started in tha same way as the GLC integrator. The initial dialogue in this case requires only one parameter, slope sensitivity. The program is started before injection of the sample, and commences to measure time on detecting the rise of the solvent peak (Ø1.2Ø). Data points are collected in a similar manner to the previous program. Detection of the top of the peaks (Ø1.26) takes place when the differentiated signal is equal to zero rather than minus the reciprocal of the slope sensitivity as in the GLC integrator. This gives a more accurate measure of retention time but is more prone to noise interference on very small peaks.

After the sample and standard peaks have passed the program is stopped (CTRL/C) and re-started at line Ø3.Ø1. The program requests "identify standards" and the operator enters the equivalent carbon values for any four peaks, identifying them by the reference numbers printed out during data collection. The computer then constructs the regression line for $\log_e$ CV vs. retention time (lines Ø4.1Ø-Ø4.46) and using this interpolates and prints out the measured equivalent carbon values for all the peaks detected, including the reference standards. Equivalent carbon values may be measured against any homologous series of compounds. In the present work the series of n-paraffins were used.

Validation.

The program is essentially self-validating, since the

GLC Retention indices program.

C-FOCAL,1969

```
ø1.ø5 E
ø1.1ø A "SLOPE SENSITIVITY" S;S S=1/S
ø1.2ø D 2.;I (S-D)1.25,1.3ø,1.3ø
ø1.25 S T=ø
ø1.26 D 2.;I (D)1.28,1.28,1.27
ø1.27 G 1.26
ø1.28 S RT(X)=T;T %2.ø X,!;S X=X+1;G 1.35
ø1.3ø G 1.2ø
ø1.35 D 2.;I (S-D)1.26,1.4ø,1.4ø
ø1.4ø G 1.35

ø2.ø5 S A=ø;F N=1,1ø;S A=A+FADC()
ø2.1ø F N=1,6;S M(N)=M(N+1)
ø2.15 S M(6)=A;S D=ø;F N=2,6;S+D=D+M(N)-M(N-1)
ø2.2ø S T=T+1

ø3.ø1 S Z=X
ø3.ø2 T "IDENTIFY STANDARDS",!
ø3.ø5 F N=1,4;A "PK NO." PK(N);A "CARBON NO." C(N),!

ø4.1ø S M(1)=(FLOG(RT(PK(4)))-FLOG(RT(PK(3))))/(C(4)-C(3))
ø4.15 S M(2)=(FLOG(RT(PK(4)))-FLOG(RT(PK(2))))/(C(4)-C(2))
ø4.2ø S M(3)=(FLOG(RT(PK(4)))-FLOG(RT(PK(1))))/(C(4)-C(1))
ø4.25 S M(4)=(FLOG(RT(PK(3)))-FLOG(RT(PK(2))))/(C(3)-C(2))
ø4.3ø S M(5)=(FLOG(RT(PK(3)))-FLOG(RT(PK(1))))/(C(3)-C(1))
ø4.35 S M(6)=(FLOG(RT(PK(2)))-FLOG(RT(PK(1))))/(C(2)-C(1))
ø4.37 S MX=ø;F N=1,6;S MX=MX+M(N)
ø4.38 S MX=MX/6
ø4.4ø F N=1,4;S I(N)=FLOG(RT(PK(N)))-MX*C(N)
ø4.45 S IX=ø;F N=1,4;S IX=IX+I(N)
ø4.46 S IX=IX/4
ø4.5ø F X=1,(Z-1);T %2.ø X;T %8.ø4 (FLOG(RT(X))-IX)/MX,!
ø4.6ø Q
```

re-calculation of the equivalent carbon value of the reference standards provides a built-in check on the accuracy of the regression line. The properties of the system were investigated by running paraffin wax, which contains all the n-paraffins from C_{14} - C_{40}. Identification of any four peaks allowed the measurement of peaks falling between these four to better than 0.1 CV accuracy. Accuracy falls off somewhat outside the four reference compounds, and so all measurements quoted in chapter 2 were made using paraffin reference compounds selected to straddle the sample peak.

Disintegrations-per-minute calculator program.

This program accepts paper tape punched by the Packard liquid scintillation spectrometer containing counts-per-minute data for ^{14}C and tritium and the external standardisation ratio. Using standards of known counting efficiency it constructs the curve of efficiency vs. standardisation ratio for each isotope and interpolates the measured standardisation ratio to obtain the efficiency for each sample. It then corrects the measured counts for efficiency and prints out the result as DPM for the two isotopes. A running total of counts is also kept to enable total recoveries to be calculated.

DPM calculator program.

```
C-FOCAL,1969

Ø1.1Ø A "CALIBRATE? Y/N" Q; I (Q-ØY)1.2Ø,8.1Ø,1.2Ø
Ø1.2Ø I (Q-ØN)1.1Ø,1.3Ø,1.1Ø
Ø1.3Ø * T "TUBE NO.     3H          14C",!
Ø1.31 A RX;I (RX-1)1.32,1.33,1.33
Ø1.32 I (RX-Ø.1)1.33,1.33,1.35
Ø1.33 T " ",!;G 1.31
Ø1.35 D 2.
Ø1.37 A Z;T %3.Ø Z; F N=1,4; A Z
Ø1.4Ø A CT;T %8.Ø4 CT*1ØØ/ETX
Ø1.42 S TT=TT+CT*1ØØ/ETX
Ø1.43 A "   "Z
Ø1.45 A CC; T CC*1ØØ/ECX,!
Ø1.46 S TC=TC+CC*1ØØ/ECX
Ø1.47 F N=1,2;A Z
Ø1.5Ø G 1.31

Ø2.1Ø S N=1
Ø2.15 I (RT(N)-RX)2.2Ø,2.2Ø,2.3Ø
Ø2.2Ø S N=N+1;G 2.15
Ø2.3Ø S ETX=((ET(N)-ET(N-1))*(RX-RT(N-1))/(RT(N)-RT(N-1)))+ET(N-1)
Ø2.4Ø D 2.1Ø
Ø2.45 I (RC(N)-RX)2.5Ø,2.5Ø,2.55
Ø2.5Ø S N=N+1;G 2.45
Ø2.55 S ECX=((EC(N)-EC(N-1))*(RX-RC(N-1))/(RC(N)-RC(N-1)))+EC(N-1)
Ø2.6Ø R

Ø8.1Ø T !;S TT=Ø;S TC=Ø
Ø8.19 *
Ø8.2Ø F N=1,8;A RT(N);A ET(N)
Ø8.35 F N=1,8;A RC(N);A EC(N)
Ø8.36 S RT(9)=1;S RC(9)=1;S ET(9)=ET(8);S EC(9)=EC(8)
Ø8.39 *
Ø8.4Ø A "READY ?" Z;T !,!,!,;G 1.3Ø

Ø9.1Ø T "TOTAL COUNTS",!
Ø9.15 T "3H" TT,!
Ø9.2Ø T "14C" TC,!
```