PITUITARY, OVARIAN AND UTERINE FUNCTION IN DYSFUNCTIONAL UTERINE BLEEDING

IAN STEWART FRASER

DOCTOR OF MEDICINE
UNIVERSITY OF EDINBURGH
1985

CONTENTS 2

This thesis has been composed entirely by myself. The work described in Sections 2 and 3 was carried out as part of a research team to which I made a major contribution through planning, execution, analysis and publication of the investigations. I was wholly responsible for the planning and mainly responsible for execution and analysis of studies described in Sections 4 and 5. All statistical analyses were carried out by myself, except where noted in Chapter 8. My contribution to each investigation has been described in individual chapters.

LIST OF TABLES

LIST OF FIGURES

Figure 3 Comparison of endometrial blood flow measurements with plasma oestradiol concentration.

Figure 4 Comparison of endometrial blood flow during the menstrual cycle as measured by Xe-133 clearance in normal women with data from rhesus monkeys and from heat clearance in women.

PREFACE:

Disturbances of the menstrual cycle are relatively common in modern industrialised societies, and excessively heavy menstrual bleeding is one of the most important of these in terms of potential health disturbance and social distress. In spite of the large literature on the topic there are still major gaps in our understanding of the underlying causes and mechanisms of abnormal bleeding and of the ways in which they may be satisfactorily managed.

This thesis describes a series of investigations which have been carried out over a period of 12 years. They have been devised to characterise certain abnormalities of pituitary, ovarian and uterine function in a particular type of excessive menstrual bleeding termed 'dysfunctional uterine bleeding'. In several cases studies of normal women have been carried out in a parallel for comparison.

Dysfunctional uterine bleeding occurs most commonly in women in the later reproductive years and may be seen in women who are still ovulating or have become anovulatory. The investigations described in this thesis have addressed several aspects of the condition which have not previously been studied and have resulted in a range of new findings. Some of the studies have involved fairly complex methodology and considerable inconvenience for the subjects, all of whom participated entirely voluntarily after receiving detailed information on the projects.

These studies have led to many new questions, and to continuing investigations of the condition in the 3 centres where the work described in this thesis was carried out.

ABSTRACT

This thesis describes a series of investigations of a rather poorly defined group of menstrual disturbances known as dysfunctional uterine bleeding (DUB). This condition is characterised by excessively heavy menstrual bleeding and, although commoner at ages 30-50 years, may occur at any time during reproductive life. The large published literature has been thoroughly reviewed and areas of ignorance highlighted.

The investigations described have been devised to elucidate several aspects of pituitary, ovarian and uterine function in women of different ages with ovulatory and anovulatory DUB and compare these with normal women. All have raised questions requiring future study. The first section describes the use of a combination of isotope dilution techniques and direct sampling of ovarian venous blood and follicular fluid to study the ovarian blood flow and the ovarian secretion and metabolism of oestradiol and oestrone in women with normal menstrual function or DUB. In normal women 95% of circulating oestradiol was secreted by the developing follicle or corpus luteum. In women with DUB oestrogen metabolism was normal but the dynamics of oestrogen secretion was sometimes disturbed. In some cases multiple follicle growth and inappropriately high oestradiol secretion was observed. In the follicular phase most large follicles were functionally active and contained very high concentrations of oestradiol (>1250 ng/ml).

Most adolescents with DUB are anovulatory and a very small proportion develop the more extreme endometrial changes of cystic glandular hyperplasia (CGH). Follow up of the group of 17 of these adolescents over 10 years revealed a high incidence of long term menstrual and reproductive disturbances. Detailed endocrine assessment over 3 cycles in 4 young women with DUB & CGH, and dynamic testing with oestrogen provocation and gonadotrophin-releasing hormone stimulation in a further 9 young women revealed a failure of positive oestrogen feedback as a cause of the anovulation. All exhibited prolonged follicular activity with excessive oestradiol secretion.

A careful study of perception of menstrual bleeding indicated that many women perceived their menstrual blood loss to be much heavier than objective measurements demonstrated. Only 38% of women with a convincing clinical history of menorrhagia had a measured blood loss of greater than 80 mls. These women also demonstrated some difficulty in assessing month to month and even day to day changes in blood loss volume. In a different group of 28 women it was found that only 36% of the menstrual discharge (range 1.6-81.7%) consisted of blood, and the remainder of the fluid is probably an endometrial transudate. This may contribute to difficulties in perception.

The final section describes the development and application of two inert gas clearance techniques (with Krypton-85 and Xenon-133) for the measurement of endometrial blood flow (EBF) in women. The techniques have been validated by comparison with radioactive-labelled microspheres in sheep. Cyclical fluctuations in EBF were seen during the menstrual cycle with a preovulatory peak, early luteal fall and gradual sustained rise up to the onset of menses. The pattern was similar in ovulatory DUB, while anovulatory women showed variable rates.

SECTION 1

GENERAL REVIEW OF THE LITERATURE

Chapter 1

DYSFUNCTIONAL UTERINE BLEEDING:

A CONCEPT REQUIRING CLEARER DEFINITION AND MORE LOGICAL MANAGEMENT.

> "THE VAPOURS FROM HER NATURAL DISEASE RISE TO HER HEAD, SUFFOCATE HER SPIRITS WITH TOO MUCH MOISTURE, OFFEND THE CHAMBER OF REASON AND INFECT THE PARLOUR OF HER PASSIONS, THE BRAINE AND HART"
>
> (Purchas 1619)

INTRODUCTION:

Dysfunctional Uterine Bleeding is a common but confusing condition which merits consideration from several different viewpoints. The aim of this review is to place present knowledge of the condition in perspective with relevant aspects of normal menstrual function and other menstrual disorders. The published literature on matters relevant to this thesis is enormous and over 2,000 references have been consulted during its preparation. In the thesis reference has only been made to publications of direct relevance. More detailed discussion of certain aspects appears in individual chapters in the thesis. This introductory review also incorporates data which have been published as a result of work described in the thesis.

BACKGROUND:

In most present-day cultures women are conditioned to expect a state of regular menstrual bleeding at approximately monthly intervals throughout the major part of their reproductive lives, although Homo Sapiens has until very recently existed in a state where late menarche, early first pregnancy, prolonged lactation and early menopause ensured that regular menstrual cycles were few and far between (Short 1976). Menstrual disorders have been a source of great concern to the medical profession and to women since earliest recorded history. Remedies for menorrhagia are mentioned in the Ebers and other ancient Egyptian papyri (Ghalioungui

1963). The social problems which menorrhagia and other disorders are likely to have caused in early societies can be gauged from the taboos and constraints which have often been placed on all menstruating women (Koetsawang 1980; Crawford 1981; Snowden and Christian 1983).

Nowadays, any spontaneous or contraceptive-induced deviation from the regular pattern may be interpreted as undesirable (Snowden and Christian 1983) although individual women will often tolerate major changes without undue alarm. It follows that complaints of abnormal menstruation or menstrually-related symptoms are highly subjective and greatly influenced by the local social and cultural environment.

Dysfunctional uterine bleeding (DUB) is an example of a condition which is subject to substantial psychological influences although there is little doubt that it has a biochemical or endocrine basis in many cases. This menstrual symptom is generally mediated through dysfunction of one or more structures within the uterus itself.

Somewhat surprisingly, present understanding of the sequence of events which occurs during normal menstruation is still relatively poor and a discussion of present knowledge of this complex process is fundamental to a rational consideration of dysfunctional uterine bleeding.

NORMAL MENSTRUATION AND THE NORMAL CYCLE:

In women, normal menstruation consists of the loss of blood, endometrial tissue and tissue fluid through the cervix and vagina following the withdrawal of trophic hormone support from the corpus luteum. The volume of blood lost per cycle appears to vary in different countries from a median of 20 mls in Egypt through 30-40 mls in Northern Europe to 50 mls in China (Hallberg et al 1966; Cole et al 1971; Hefnawi et al 1980; Gao et al 1981). The proportion of the total volume of menstrual flow contributed by tissue and tissue fluid is uncertain (Hahn 1980), but work described in Chapter 10 of this thesis indicates that the proportion contributed by blood is generally less than 40% (Fraser et al 1984b).

Most authors seem to be in agreement that an arbitrary upper limit for normal menstrual blood loss can be set at about 80 mls, since iron deficiency anaemia appears to become much more common in European women with repeated blood loss in this range (Hallberg et al 1966). Women with menorrhagia will lose in excess of 40 mg of elemental iron per period (Cohen and Gibor 1980). Blood loss may sometimes vary considerably from cycle to cycle in individuals but in most cases the variation is small (Hallberg and Nilsson 1964b).

The proportion of endometrium lost at menstruation has been a subject of much controversy over the years (Bohnen 1927; McLennan and Rydell 1965; Flowers and Willborn 1978; Nogales-Ortiz et al 1978), and it seems likely that there is wide individual variation. In some women there is relatively little tissue loss but a major restructuring of the tissue architecture, whereas in others there maybe a loss of most of the functional layer of the endometrium.

Modern understanding of the morphological events in the endometrium during menstruation is still based to a great extent on the elegant and meticulously described observations of Markee (1940) of intraocular endometrial transplants in rhesus monkeys. These observations are now being increasingly supplemented by corroborative morphological data and new biochemical and endocrine data in women. Spiral arterioles seem to be essential for the process of menstruation, and excessive growth and coiling of these vessels occurs during the secretory phase. The coiling is greatly accentuated during a period of endometrial regression which occurs immediately prior to endometrial breakdown (Markee 1940; 1950). The regression merges into a period of intense arteriolar constriction which is most obvious at the myometrial-endometrial junction and in the inner myometrium. This usually precedes bleeding by a few hours. It persists throughout menstruation but intermittent relaxation of individual vessels occurs with consequent bleeding.

The exact causes of the endometrial regression, intense vasoconstriction and vascular breakdown are unknown. In recent years evidence has accumulated to implicate the prostaglandins, especially PGE_2 and $PGF_{2\alpha}$, in the vasoconstriction and regulation of the volume of blood that is lost (Abel 1979). It is possible that these agents may also cause direct damage to the structure of the vessel walls and help to initiate bleeding. Since prostaglandins and related 'prostanoids' (cyclic endoperoxides, prostacyclin-PGI_2-and thromboxane A_2-TXA_2) have varying actions on blood vessels and platelets it seems likely that a balance between different prostaglandins is important. Interesting new information is compatible with the hypothesis that PGI_2 synthesised in the myometrium from endometrial precursors may have a role in the control of endometrial blood flow (Kelly 1981). It is possible that ischaemia increases free arachidonic acid concentration and that stasis prevents transfer of intermediates to the myometrium. This would lead to a marked increase in endometrial $PGF_{2\alpha}$ concentration and vasoconstriction limiting blood loss.

The sequence of events which links luteolysis and falling levels of oestradiol and progesterone with actual tissue breakdown is also far from clear. There is strongly suggestive evidence to implicate lysosomes in the initiation of tissue breakdown (Henzl et al 1972) but their role could be limited to remodelling of the tissue during and after breakdown (Wilson 1980). The elegant ultrastructural and histochemical study of Henzl et al (1972) demonstrated that acid phosphatase is released from lysosomes into the cytoplasm and intercellular spaces immediately prior to tissue breakdown. This release appears to be consequent upon a fall in plasma levels of oestradiol and progesterone. In most tissues, lysosome membrane stability is influenced by ambient steroid levels, but at the present time little information is available on the behaviour of lysosomes of endometrial origin. Additionally,

very little is known about the different hydrolase enzymes which could be present in endometrial lysosomes. Of particular interest is the observation that the arachidonic acid mobilising enzyme, phospholipase A_2, is sometimes present in lysosomes. This could provide the link between lysosome activity and prostaglandin release at the onset of menstruation.

Haemostatic mechanisms play a central role in control of the volume of blood lost (Aparicio et al 1979; Paton et al 1980; Sixma et al 1980; Christiaens et al 1982) and this role is complementary to arteriolar constriction. The haemostatic response in the endometrium is defective compared with other tissues such as skin. This defective response is almost certainly due partly to the highly active fibrinolytic system within the endometrium (Rybo 1966b) and possibly also to increased release of prostacyclin and heparin (Foley et al 1978; Paton et al 1980). Morphological studies indicate that bleeding begins when gaps appear in the blood vessel walls. Intravascular haemostatic plugs containing platelets and later some fibrin slowly lead to partial or complete occlusion of vessels, but these platelet and fibrin plugs are not seen around the outside of vessels as in other tissues. The haemostatic response is so defective that in the premenstrual phase gaps in vessel walls with exposed collagen may appear without any evidence of platelet plug formation. Platelet plugs only form in the vessel lumina during the first 12-24 hours of menstruation and fibrin is only detectable in small amounts during the first 48 hours. Haemostatic plugs are shed with the superficial tissue layers into menstrual fluid. Uterine contractility increases markedly at the onset of menstruation and encourages rapid drainage of the uterus. All these features seem to be aimed at preventing the deposition of significant amounts of fibrin and true blood clot within the cavity. This aim is obviously desirable since organisation of a true clot could result in the formation of intrauterine adhesions with serious consequences for

future reproduction.

Since haemostatic plugs and fibrin are only present in endometrium during the early stages of menstruation, the mechanism of haemostasis during the remainder of menstruation remains unexplained. It is possible that blood loss after the first 12 hours is limited mainly by vasoconstriction and after 48 hours by surface regeneration of the epithelium (Ferenczy 1976). The major quantity of blood loss occurs during the first two days and usually then tails off rapidly within the next 2-3 days (Haynes et al 1977).

The most 'normal' menstrual patterns appear to occur following oestrogen priming of the endometrium for 1-2 weeks followed by oestrogen and progesterone together for a further 2 weeks. Predictable menstruation then occurs following simultaneous withdrawal of oestrogen and progesterone. Any departure from this pattern may be accompanied by abnormalities of menstruation, as is well illustrated by the endocrine patterns with the progestogen-only minipill (Landgren and Diczfalusy 1980). Conversely, it should be recognised that disturbances of menstruation may sometimes occur when circulating hormone patterns are indistinguishable from normal.

The average menstrual period lasts 5 days with 90 per cent having a duration between 2 and 8 days. Cycle length is also very variable with the mean around 29.4 days (Gray 1980) and only 80 per cent of women in the mid-reproductive years exhibiting cycles between 25 and 35 days. There is a skewed distribution towards longer cycles, and irregular cycles are much more common in adolescence and the perimenopause.

DYSFUNCTIONAL UTERINE BLEEDING:

Dysfunctional uterine bleeding is one of the most poorly understood of common gynaecological conditions. This is partly due to great confusion over definitions and terminology, and few authorities can agree on consistent

criteria to delineate the condition. The definition which this author prefers is "excessively heavy, prolonged or frequent bleeding of uterine origin which is not due to recognisable pelvic or generalised medical disease, or to pregnancy". Therefore this is a diagnosis of exclusion - a useful "working diagnosis". The commonest complaint is of excessively heavy bleeding (menorrhagia) and it is this symptom which is mainly considered in the following review and the subsequent investigations.

There is little doubt that whatever definition is used the aetiology is multifactorial and currently only a few categories can be defined clearly. Perception of symptoms by the patient can be quite misleading but forms a crucial part of the initial presentation and the clinical assessment. It is convenient to group the women into those with acute or chronic symptoms and ideally also into those who are predominantly ovulatory or predominantly anovulatory. Few patients with dysfunctional bleeding are exclusively ovulatory or anovulatory (Fraser and Baird 1974).

INCIDENCE:

DUB is a common diagnosis, being made in up to 10% of cases attending a gynaecological outpatients clinic (Taylor 1965). It may occur at any time between menarche and menopause, but is particularly common in the ten years leading up to the menopause. It is said that a small peak also occurs in adolescence but this is difficult to demonstrate because most cases are treated without any contact with hospital clinics. This is illustrated by a very large pathological study of DUB where only 4% of patients undergoing curettage were under 20 years (Sutherland 1949). By contrast 40% were 40-50 years and 50% in the 20-40 year age group. Anovulatory DUB is certainly commoner in adolescence and perimenopause, whereas 80% of cases in the mid-reproductive years are ovulatory. Cystic glandular hyperplasia (CGH) of the endometrium, one extreme of the spectrum of anovulatory DUB, is mainly seen in the 40-50 year age group although a very small peak occurs in

adolescence (Schröder 1954; Fraser and Baird 1972). It used to be taught that almost all cases of DUB in young girls were associated with endometrial hyperplasia (Jeffcoate 1937) but this is erroneous and the true incidence is probably between 5 and 15% (Sutherland 1953; Fraser and Baird 1972). The population incidence of adolescent CGH is very low and is probably about 5 new cases per million per year (Fraser and Baird 1972).

Population studies of menstrual blood loss indicate that menorrhagia with measured loss over 80 mls per cycle is relatively infrequent (Hallberg et al 1966; Cole et al 1971). These studies are subject to many biases but give an indication that perhaps 5-10% of women in the reproductive age group in a general population will exhibit objective menorrhagia at any one time. This seems to be different from the subjective complaint of "menorrhagia" as made by the patient, which is probably much commoner (Fraser et al 1981). It is calculated that 10-25% of women will complain of menorrhagia at some time (Richards 1979; Wood et al 1979 ; NOP Survey 1980).

DIFFERENTIAL DIAGNOSIS:

Since dysfunctional uterine bleeding is a diagnosis of exclusion, recognition of other potential causes of excessive uterine bleeding is of central importance.

Pelvic disease causing excessive bleeding.

Leiomyomata (fibroids) are a common cause of menorrhagia (Buttram and Reiter 1981). Submucous and intramural lesions seem much more likely to cause menorrhagia than subserous ones, although no objective measurements of blood loss have been reported. The mechanism appears to be gross distortion of the venous architecture around the fibroid (Farrer-Brown et al 1970 b).

Endometriosis is a common clinical association with menorrhagia although again no objective measurements of blood loss have been reported (Sensky and Liu 1980). The mechanism is unknown but may be due to a disturbance of

local prostaglandin secretion.

Adenomyosis is another relatively common association with menorrhagia, although the diagnosis is usually not made until the time of hysterectomy (Vora et al 1981). Again prostaglandins are thought to be involved in the mechanism of disturbed bleeding.

Chronic pelvic inflammatory disease may cause heavy or irregular bleeding, presumably through uterine vascular congestion and dilatation.

Endometrial polyps are somewhat mysterious lesions of uncertain aetiology, which are found more frequently in women with anovulation. Use of the hysteroscope suggests that they are probably much commoner than is generally appreciated from routine curettage, and they are undoubtedly associated with a proportion of cases of clinically diagnosed DUB (Peterson & Novak 1956).

Endometrial adenocarcinoma usually presents with post menopausal bleeding but 30-40% present before the menopause with irregular, intermenstrual, post coital or excessive bleeding. Menorrhagia is uncommon as the sole presenting feature. Many of these cases give a preceding history of anovulatory DUB (Chamlian and Taylor 1970). This is undoubtedly the most serious of all the differential diagnoses of DUB.

Polycystic ovarian disease is often associated with infrequent episodes of menorrhagia due to anovulation with elevated circulating oestrogen levels (Goldzieher 1981).

Pelvic congestion syndrome is a rather nebulous concept which may or may not exist as a distinct entity capable of causing menorrhagia.

Bicornuate uterus is said to be associated with menorrhagia due to the increased endometrial surface area, but objective evidence is not available.

Rarities which may be associated with menorrhagia include functional ovarian tumours, endometrial

haemangiomata, uterine lymphangiomata and pelvic arterio-venous malformations.

Surface lesions of the genital tract may cause irregular or intermenstrual bleeding at any age, although in these cases regular menstruation usually continues normally.

Complications of pregnancy must necessarily be excluded in all cases of acute or subacute 'menorrhagia'.

Medical disease causing excessive bleeding.

Disorders of haemostasis may present as rare causes of menorrhagia (Quick 1966) but can occur in as many as 20 per cent of adolescents with menorrhagia in whom pelvic pathology has been excluded (Claessens and Cowell 1981). Any type of haemostatic disorder may cause excessive bleeding in some individuals, but in many, especially those on anticoagulants, menstruation may be completely normal. The disorders most commonly associated with menorrhagia are disturbances of platelet function including thrombocytopoenia, various thrombocytopathies, Von Willebrand's disease and possibly chronic excessive aspirin ingestion.

Hypothyroidism is classically said to cause anovulation and menorrhagia, although many will develop amenorrhoea or oligomenorrhoea instead (Scott and Mussey 1964).

Systemic lupus erythematosus may cause menorrhagia either by damage to vascular function or through the action of a circulating coagulation-inhibiting antibody.

Other medical causes of menorrhagia include congestive cardiac failure, chronic hepatic disease and chronic renal failure following adequate dialysis.

AETIOLOGY AND MECHANISMS:

It is convenient to divide these patients into those who are anovulatory and those who are ovulatory although it should be recognised that women with a history suggestive of anovulation will not infrequently have some ovulatory cycles (Fraser and Baird 1974).

Anovulatory cycles.

The classical studies of Brown, Kellar and Matthew (1959) have thoroughly documented the urinary oestrogen excretion in women with anovulatory cycles. They have shown that oestrogen levels usually fluctuate substantially but may occasionally be relatively constant for some weeks. In their studies heavy menstrual bleeding usually occurred when oestrogen levels were falling but occasionally would occur when levels were steady or even when they were rising. These authors investigated the oestrogen levels in terms of the degree of endometrial proliferation and clearly demonstrated that cystic glandular hyperplasia of the endometrium was associated with high fluctuating levels of oestrogen or with moderate and prolonged unopposed levels of oestrogen (Brown and Matthew 1962). It is now clear that the condition of 'metropathia haemorrhagica' with multi cystic ovaries, cystic glandular hyperplasia of the endometrium and erratic menorrhagia is merely the severe end of the spectrum of anovulatory dysfunctional uterine bleeding rather than a specific disease-entity (Schröder 1954; Brown, Kellar and Matthew 1959).

It appears that ovarian synthesis and subsequent metabolism of oestrogens is within normal limits in peri-menopausal women with anovulatory DUB but the cyclical dynamics of oestrogen secretion are disturbed (Baird and Fraser 1974; Fraser and Baird 1974). In anovulatory women in the late reproductive years significant amounts of oestradiol may be secreted by both ovaries. This may come from large and sometimes multiple active follicles suggesting a disturbance of intra ovarian follicular control mechanisms and/or abnormalities in gonadotrophin release (Baird and Fraser 1975).

Very little information is available on human ovarian blood flow but it appears that ovarian blood flow is similar in women with anovulatory DUB and normal cycles (Baird 1973; Baird and Fraser 1973).

Detailed studies of perimenopausal women with anovulatory DUB have reported results consistent with a variety of defects both in hypothalamic-pituitary and intra ovarian mechanisms (Aksel and Jones 1974; Van Look et al 1977). The perimenopausal findings are in striking contrast to the mechanism of anovulatory DUB in adolescents. Detailed investigation has demonstrated that the majority of adolescents with anovulation have a defect in the positive feedback response to oestrogen (Fraser et al 1973; Van Look et al 1978). It is suggested that these girls may be experiencing a delay in the maturation of hypothalamic control (Aksel and Jones 1974), although many continue to experience menstrual disturbance for many years (Fraser and Baird 1972).

Hyperprolactinaemia is occasionally recorded in women with anovulatory DUB. Since treatment with bromocriptine often does not cure the condition, it may just be a chance association or more likely may be a consequence of the elevated oestrogen levels.

The erratic, prolonged and excessively heavy bleeding which is typical of anovulatory DUB is clearly associated with prolonged unopposed stimulation of the endometrium by fluctuating and sometimes excessively high levels of oestradiol. However, the exact mechanism of abnormal breakdown of endometrium has not been elucidated. The endometrium is rarely found to be atrophic and generally varies from different degrees of proliferation to cystic glandular hyperplasia. Atypical forms of hyperplasia are associated with an increased risk of endometrial malignancy later (Chamblian and Taylor 1970). Endometrial polyps are sometimes found. The histological findings have been well described by Sutherland (1949). Orderly development of the endometrial vasculature does not occur and there is usually poor spiral arteriole development with exaggerated venous vascularity and the development of venous sinusoids (Beilby et al 1971).

It is usually stated that bleeding follows a fall in oestrogen levels but this is not necessarily so and the relative importance of different biochemical mechanisms has not been elucidated. There is increasing evidence to implicate the prostaglandins and two groups of investigators have demonstrated an increased endometrial concentration or synthetic capacity for prostaglandin E_2 in anovulatory DUB (Willman et al 1976; Smith et al 1981b). There may also be abnormal intrauterine haemostasis due to excessive fibrinolysis (Rybo 1966) or to excessive heparin production by endometrial mast cells (Foley et al 1978; Paton et al 1980). It is not known whether there is any abnormality of endometrial lysosome function although it could be expected that uterine lysosome membrane stability would be reduced in the presence of unopposed oestrogen (Szego 1971).

Unopposed oestrogen may also lead to an increased rate of endometrial blood flow (Killam et al 1973) which could be translated into excessive blood loss at the time of endometrial breakdown. In normal women endometrial blood flow may remain elevated until very shortly before the onset of menses (Fraser and McCarron 1984)*, but information is lacking on the changes occurring in women with anovulatory DUB.

Ovulatory cycles.

The great majority of women with DUB in the mid reproductive years will experience regular ovulatory cycles. Daily plasma measurements of LH, FSH, oestradiol and progesterone have indicated that these cycles are indistinguishable from normal women (Haynes et al 1980). This suggests that the disorder is due to a local functional abnormality within the uterus itself or to some unidentified circulating factor.

The majority of these women have secretory endometrium which is indistinguishable from normal (Nedoss 1971; Taw 1975). The literature is replete with descriptions of irregular

* unpublished data

ripening, irregular shedding and secretory hyperplasia of endometrium. These appearances are presumably due to an abnormal stromal and/or glandular response to progesterone and perhaps oestrogen. The underlying causes and relative importance of these appearances are quite unknown. It has also been suggested that increased uterine weight and increased uterine surface area may be important although a recent careful study has not confirmed this (Chimbira et al 1980).

Increasing evidence again implicates abnormalities of prostaglandin metabolism in the mechanism of bleeding. There is reasonable evidence to implicate a decreased production of prostaglandin $F_2\alpha$ (Smith et al 1982). There is also evidence to indicate that endometria from these patients have an increased capacity to synthesise prostacylin (Smith et al 1982). Prostacyclin is a potent vasodilator and inhibitor of platelet aggregation and hence could substantially influence degree and duration of menstrual bleeding. The factors responsible for the shift in endometrial conversion of prostaglandin endoperoxides from $PGF_2\alpha$ to PGE_2 and PGI_2 are unknown. It will be of interest to look for an abnormality of catechol oestrogen metabolism in these patients, since 2 hydroxy oestradiol is a potent stimulator of prostaglandin synthesis (Kelly 1981). Receptor binding for PGE may also be increased in women with abnormal menstrual bleeding (Hoffman et al 1983). It must be remembered that endometrium is a highly biochemically active tissue and there may well be abnormalities of fibrinolysis, heparin secretion, lysosome activity, prolactin secretion (Maslar and Riddick 1979) or even relaxin (Dallenbach-Hellweg, Dawson and Hisaw 1966; Yki-Jäninen et al 1983) or renin production (Johnson 1980).

One study has suggested that iron deficiency and anaemia are causative or exacerbating factors for DUB (Taymor et al 1964), whereas it seems much more probable that the majority of patients who develop iron deficiency anaemia trigger a compensatory mechanism which tends to

reduce the menstrual blood loss. Jacobs and Butler (1965) studied 15 women with iron deficiency anaemia and found that in the great majority the blood loss per menstrual period almost doubled following correction of anaemia. The increase in blood loss was greatest in those women whose initial blood loss was about average. Clearly the development of anaemia depends upon a number of factors including iron intake, efficiency of iron transport and storage, and the efficiency of the erythropoietic system as well as upon the amount of menstrual blood loss. This is clearly seen in women blood donors who can maintain a normal haemoglobin, and compensate for blood loss at a rate of up to 300 mls per month (Fowler and Barer 1942).

For some years it has been alleged that surgical tubal sterilisation may cause dysfunctional uterine bleeding with menorrhagia and the question is still unresolved. Some have even reported that sterilisation may ultimately lead to a high incidence of hysterectomy (Muldoon 1972). Most of the investigations indicating a relationship between tubal interruption and subsequent menorrhagia have been retrospective and based entirely on the woman's perception. However, some of these studies have been adequately controlled and are apparently convincing (Neil et al 1976; Lawson et al 1979). On the other hand there are now numerous studies in the literature which have found no evidence of an adverse relationship (Edgerton 1977; Lieberman et al 1978; Sapire and Davey 1980).

The only study which has attempted an objective and prospective evaluation of menstrual blood loss in sterilised women found no increase in measured menstrual loss up to one year following operation (Kasonde and Bonnar 1976). These investigators measured menstrual loss objectively over two to three cycles before sterilisation and at intervals up to twelve months in 25 women.

It has been suggested that ceasing oral contraceptive use at the time of sterilisation may be responsible for a

real increase in perceived menstrual bleeding in many of these women. Clearly this effect may be particularly disturbing for some women who have not experienced normal menstruation for many years because of pregnancies, lactation and prolonged pill use. However, this does not account for all cases and it seems likely that in some the problem is one of decreased tolerance of menstruation. For these women recurring menstruation is no longer a reminder of continuing fertility and in addition it may be associated with undesirable features such as pain and premenstrual tension. Nevertheless some women do develop genuine menorrhagia some years following sterilisation and it is impossible to say that this association is not causal in some cases.

If there is a genuine causal association between surgical sterilisation and menorrhagia in a small number of women the mechanism is quite unknown. Decreased corpus luteum secretion of progesterone has been reported in several studies (Donney et al 1981) but this type of endocrine disturbance is not noted as a cause of menorrhagia in other situations. The most likely explanation would be interruption of vascular transport of some unknown factor from the ovary along the utero-tubal arcade. However, further speculation awaits a convincing prospective demonstration that the post tubal ligation menorrhagia syndrome really exists. In the meantime it is reasonable to manage these patients as cases of ovulatory dysfunctional uterine bleeding, from which they are indistinguishable in other ways at the present time (Fraser et al 1981; Fraser 1985).

It is important to recognise that many cases diagnosed on clinical grounds as DUB will ultimately be found to have pelvic or systemic pathology of sufficient degree to cause the symptoms. This is clearly shown in a retrospective study from the Chelsea Hospital for Women, London where 40 per cent (105 out of 287) of women undergoing hysterectomy for 'dysfunctional uterine haemorrhage' were found to have

substantial pelvic pathology (Beazley 1970). Nowadays there is even the suggestion that some of these cases may be associated with minor degrees or even microscopic deposits of endometriosis.

PSYCHOLOGICAL ASPECTS:

These are of the utmost importance in the assessment of DUB, since the diagnosis is based almost entirely on the history presented by the patient.

Perception of menstrual bleeding is inaccurate and retrospective recall is unreliable (Gray 1980). Many women have little idea how their menstrual loss compares with the norm and their recorded perception may bear little relation to measured loss (Hallberg et al 1966; Chimbira et al 1980; Fraser et al 1981). Hallberg et al (1966) found that 40% of women with menstrual loss exceeding 80 mls considered their periods only moderate or scanty, while 14% of those with a loss of less than 20 mls judged their periods to be heavy. In a recent study of 69 women presenting with good clinical histories of menorrhagia (Fraser et al 1981) it was found that only 31% had a measured menstrual loss of greater than 80 mls and 22% actually had a loss of less than 35 mls - the mean for the normal population. A more detailed assessment demonstrated that some of these women had difficulty in perceiving major changes in volume from cycle to cycle and even from one day to the next (Fraser et al 1984a).

It is not generally appreciated that only a small proportion of the total menstrual flow (on average 36%) is made up of whole blood while the remainder appears to be mainly endometrial tissue fluid (Fraser et al 1984b). Abnormalities of this relationship may well influence perception of menstrual blood loss volume in some individuals.

It seems likely that women under different types of emotional strain may have decreased tolerance of disturbances of menstruation and this may be particularly so in women who

have been surgically sterilised. There is some evidence to suggest that women with a complaint of menorrhagia are more likely to exhibit features of depression (Greenberg 1983).

From time to time anecdotal reports are mentioned of women who have experienced sudden unheralded bleeding in the face of unexpected emotional trauma. The extent of this phenomenon and the possible mechanisms are speculative.

PROGNOSIS:

Very little accurate information is available about this aspect of DUB. None of this is based on objective measurements of blood loss.

Single episodes of DUB in adolescence probably have a good prognosis, whereas the adolescent with several episodes of DUB has a poor prognosis in terms of persistent menstrual disturbances (30-80%), repeated need for curettage (40-55%), anaemia (30%) and even endometrial carcinoma if inadequately treated (1-2%) (Southam 1959; Southam and Richart 1966). This prognosis is particularly bad when CGH is diagnosed (Fraser and Baird 1972), and is a good reason for diagnostic curettage in the adolescent with repeated episodes of DUB.

It is said that prognosis in ovulatory DUB in the mid reproductive years is good, but convincing evidence is not available. In some communities many of these women undergo hysterectomy and an accurate assessment of prognosis is no longer possible. From older literature it appears that long term prognosis for anovulatory DUB in the later reproductive years is poor and recurrence is frequent (Israel 1967).

It is often said that curettage leads to long term reduction in menstrual blood loss in many patients. However, published data do not confirm this. Objective measurements indicate a reduction in loss in the first cycle following curettage but a return to pretreatment levels thereafter (Nilsson and Rybo 1971; Haynes et al

1977). The persisting subjective benefit which many women experience is presumably related to reassurance about the absence of pathology and a change in tolerance of symptoms.

ASSESSMENT OF MENSTRUAL BLOOD LOSS:

There is only one reliable means of assessing volume of menstrual loss and that is direct measurement. This has been recommended by several investigators, but there is resistance by many doctors to asking patients to collect their sanitary towels for laboratory assessment. This is most unfortunate in view of the major errors which many women make in perception of their menstrual loss. In fact, menorrhagia must be one of the few common subjective medical complaints upon which treatment decisions of far reaching import are made without any attempt at objective measurement.

Many clinical means have been recommended for quantitating menstrual loss, but none of these are generally helpful. Even recognition of blood "clots" in the flow does not correlate well with volume. "Flooding" is probably the most reliable single symptom although women with measured loss between 60 and 80 mls per period may sometimes report unequivocal "flooding" (Fraser et al 1981). Tiredness and lassitude are unhelpful, whereas a previous history of objectively confirmed anaemia is strong supportive evidence. Some doctors still rely on a sanitary towel count to give a measure of volume of loss although several authors have demonstrated that this is unreliable (Chimbira et al 1980; Fraser et al 1981). A recent study of 276 menstrual periods in women complaining of menorrhagia found no significant correlation between objectively measured menstrual loss and number of pads or tampons used (Fraser et al 1981).

Several methods have been proposed for measurement of menstrual blood loss (Cohen and Gibor 1980) but the one which has gained most popularity because of relative simplicity and reproducibility is that of Hallberg and

Nilsson (1964a). This method relies on careful collection of all menstrual pads, tampons and "clots" followed by extraction of haemoglobin and conversion to alkaline haematin with 5% sodium hydroxide. Practical application of the method has been greatly increased by the use of a semi-automatic extractor (Newton et al 1977). More widespread clinical use of objective methods of assessment such as this must occur if progress is to be made in management of menorrhagia.

MANAGEMENT:

A more rational approach to correction of pituitary, ovarian and uterine dysfunction should be a logical sequel to recent pathophysiological research on DUB. Although studies of management are not directly reported in this thesis they are a critical end point of the clinical research picture. The aim of basic studies is ultimately to improve management and studies of therapy are therefore a logical extension of this approach.

Management of DUB has always involved a choice between medical and surgical measures, both of which have an important place in modern therapy. Medical therapy is usually preferred initially because it does not involve the hazards of anaesthesia and will potentially preserve fertility. However, it may need to be used for many years.

Over the past 20 years or so the mainstay of medical therapy has been the use of synthetic hormones (March 1979). These may be of great value, but increasing experience has indicated the importance of tailoring regimens to the requirement of each individual.

For anovulatory women oral progestogens alone are a logical first choice to replace missing luteal phase progesterone. They should usually be given for 7 - 10 days from day 15 - 18 of the cycle, eg. norethisterone (NET) 5 mg bd or tds. This will usually permit good secretory change in the endometrium and a predictable withdrawal

bleed. However, some women with excessive oestrogen secretion from early in the cycle may need a longer duration of progestogen exposure, for example from day 5 to 25 to reduce endometrial proliferation. This regimen has the further advantage that it is contraceptive and can be used in women who are ovulating. After 2-3 courses the dosage can often be reduced as low as 5 mg NET or 10 mg MPA daily.

If taken continuously oral progestogens may also be used to produce complete amenorrhoea, an acceptable scientific solution to the problem but one which may be psychologically unacceptable. There are many racial, religious and individual concerns about hormonally-induced amenorrhoea (Snowden and Christian 1983) and careful counselling of the patient is required. Injectable progestogens (eg. depot medroxyprogesterone acetate, DMPA) have also been used to produce amenorrhoea by inducing extreme degrees of endometrial suppression (Bonte 1978). Unfortunately the response is unpredictable with many women (30-40%) experiencing irregular scanty bleeding and 10-20% having episodes of prolonged, frequent or rarely heavy bleeding (Fraser 1981). A novel approach is the use of a progesterone-releasing intrauterine device which has proved valuable in a small number of women with menorrhagia following haemodialysis for chronic renal failure (Newton et al 1976). Although these patients obtain a substantial reduction in total volume of blood loss this is often at the expense of an increase in duration of scanty inter-menstrual bleeding.

Recently, the novel synthetic steroid, danazol, has been used in varying doses to induce amenorrhoea in women with DUB (Chimbira et al 1979). In lower doses of 200-400 mg daily menstruation is not always abolished but measured menstrual blood loss is still reduced. This steroid probably also acts by reducing cyclical endometrial growth through a combination of mechanisms at all levels in the reproductive system (Fraser 1979; Reyniak & Lauerson 1982).

Many women with ovulatory or anovulatory DUB will benefit from a combined oestrogen-progestogen oral contraceptive and theoretically this should have a relatively high progestogenic balance. This acts by suppressing both ovulation and endometrial proliferation. Women using combined oral contraceptives are much less likely to complain of menorrhagia, irregular bleeding or intermenstrual bleeding (Royal College of General Practitioners 1974). Combined oral contraceptives can also be shown to reduce substantially the measured menstrual blood loss in women complaining of menorrhagia (Nilsson and Rybo 1971). However, it appears that at least 20% of patients will not respond.

It must also be accepted that many women have risk factors which make them unsuitable for long-term hormonal therapy. These factors are well-known and include smoking, age over 40 years, strong family history of cardiovascular disease, diabetes mellitus, hyperlipidaemia and many others.

In women with anovulatory DUB and infertility induction of ovulation may treat both problems. The drug of first choice is usually clomiphene, which is thought to act mainly as an anti-oestrogen at the hypothalamic level. Bromocriptine may be appropriate in the occasional patient with mild to moderate hyperprolactinaemia. If these do not work the second line of treatment involves injections of FSH and LH with midcycle HCG or pulsatile infusion of gonadotrophin-releasing hormone (GnRH). In general, pregnancy rates with these treatments are lower in women with anovulatory DUB than in similarly treated patients who have amenorrhoea (Cox 1976). The reasons for this are not yet clear.

Other approaches to medical therapy are intended to correct biochemical abnormalities occurring within the uterus itself. One of the most promising recent developments has been the use of antiprostaglandin agents. These include several groups of drugs which act mainly

by inhibiting the cyclo-oxygenase enzyme system which converts arachidonic acid into cyclic endoperoxides. It has recently been shown that some of these agents may induce a dramatic reduction in menstrual blood loss in some women with menorrhagia. The most extensively investigated preparation is mefenamic acid administered as 500 mg tds during the days of menstruation only (Anderson et al 1976; Guillebaud et al 1978; Fraser et al 1981). This results in a mean reduction in blood loss of 28%, but is usually proportionately more effective in those with more excessive loss. The benefit is usually maintained with repeated treatment over long periods of time (Fraser et al 1983), and may also be seen in women with menorrhagia due to an IUCD, fibroids, or even Von Willebrand's disease (Guillebaud et al 1978; Fraser et al 1981). Very limited published information suggests that flufenamic acid, indomethacin and naproxen may have similar beneficial effects (Anderson et al 1976; Damarawy and Toppozada 1976; Davies et al 1981).

Since the antiprostaglandin agents inhibit synthesis of PGE_2, $PGF_2\alpha$, PGI_2 and TXA_2 it has been suggested that their beneficial effect on menorrhagia may be mediated by a relative increase in synthesis of a vasoconstrictive leukotriene. However, not all women will experience a reduction in blood loss with PG inhibitors, and therefore these drugs may act merely by altering the balance of prostanoid synthesis within the uterus. The fenamates may have an advantage because of a weak end-organ action inhibiting prostaglandins that have already been synthesised (Collier and Sweatman 1968; Smith et al 1975).

Fibrinolytic inhibitors administered orally also have a rational basis in therapy. Epsilon amino caproic acid (EACA) and tranexamic acid (AMCA) have been used by several investigators with striking benefit in some women with menorrhagia and intermenstrual bleeding (Kasonde and Bonnar 1975a; Weström and Bengtsson 1970). Unfortunately, the widespread use of these drugs has been limited by side

effects and concerns about long term safety. Intrauterine application may avoid these risks and appears to produce benefit for several cycles following one treatment (Tauber et al 1977). Several diamidines and guanidines which are potent inhibitors of fibrinolysis are under investigation as potentially valuable therapeutic or preventive agents for IUD-related bleeding problems (World Health Organization 1979).

Ethamsylate is a rather mysterious drug which is said to reduce menstrual blood loss by a reduction in capillary fragility (Harrison and Campbell 1976) acting perhaps as an antihyaluronidase and an antiprostaglandin agent. A 50% reduction in blood loss in women with spontaneous menorrhagia and 19% reduction in IUD users has been reported by one group (Harrison and Campbell 1976) and confirmed by another (Kovacs and Annus 1978). However, this benefit could not be demonstrated by Kasonde and Bonnar (1975b).

Other agents which may correct abnormalities of uterine biochemistry include vasopressin analogues, such as triglycyl lysine vasopressin (Pavlin et al 1978), antiheparin agents, including the rather non-specific toluidine blue and protamine sulphate (Rumbolz et al 1952) and antagonists of histamine, kinins and complement (World Health Organisation 1979). Distant possibilities include the use of specific thromboxane analogues or prostacyclin inhibitors delivered locally into the uterus. It has been suggested that micro-capsules placed in the vagina might provide a convenient route for the delivery of intrauterine medication. There is little doubt that greater understanding of the multiple mechanisms of DUB will assist in development of alternative logical approaches to medical therapy.

In the meantime surgery remains a frequent necessity in the therapy of menorrhagia, whether it be due to pelvic disease, medical disorders or DUB. Conservative surgery may include myomectomy for fibroids and bilateral ovarian wedge resection for polycystic ovarian disease. However, the

choices with DUB are virtually limited to therapeutic curettage and hysterectomy, although endometrial cryosurgery and laser ablation have been reported (Droegemuller et al 1971; Goldrath et al 1981).

Therapeutic curettage has often been advocated for DUB but the evidence for its efficacy is uncertain. Many women will experience a decrease in measured menstrual loss in the cycle following curettage (Haynes et al 1977) but this is not usually maintained. However, the subjective benefit may persist and is probably related to reassurance and increased tolerance.

Hysterectomy is probably the most widely used treatment for DUB and is undoubtedly curative. Unfortunately, it involves major abdominal or vaginal surgery and may occasionally be associated with a wide range of short and long term complications. It is clearly a highly satisfactory method of treatment for severe or refractory DUB but is increasingly used for less severe cases. A recent survey of health insurance statistics concluded that approximately 40 per cent of Australian women will undergo hysterectomy at some time in their lives (Selwood and Wood 1978). Although some of these cases would be for cancer or uterine prolapse it is obvious that many must have been for menstrual disturbances and particularly 'menorrhagia'. In which case it is highly probable that many women with menstrual blood loss in the normal range have undergone hysterectomy for a symptom which is perceived rather than real. It seems likely that the same situation pertains in many centres in North America and perhaps also in Europe.

One of the major concerns about hysterectomy is the occurrence of post-operative psychological disturbances (Menzer et al 1957). However, there is increasing evidence that full counselling and ample thinking time prior to finalising the decision for hysterectomy almost eliminate significant adverse reactions. Use of medical agents for control of abnormal bleeding may be a valuable adjunct during this decision-making period.

CONCLUSION:

In the past two decades numerous advances have been made in understanding the group of conditions currently known as dysfunctional uterine bleeding. There is now better agreement as to the conditions which this term includes although controversy still exists. Patient perception of menstrual disturbances, especially menorrhagia, is often inaccurate and greatly complicates management.

Mechanisms are still not clearly defined although it seems certain that there may be functional abnormalities of the hypothalamic-pituitary unit, the ovaries or the uterus itself. The condition occurs most frequently in the 5-10 years preceding the menopause and may represent one aspect of the ageing process in the reproductive system. However, it may also occur at any other time during the reproductive years.

Management now includes a range of therapies designed to correct hormonal, prostaglandin or fibrinolytic abnormalities. However, medical therapy may need to be continued for many years and there are still a substantial number of treatment failures. Therefore, surgery including particulary hysterectomy, is still a very important option in therapy. Ongoing research is aimed at a much better understanding of the many underlying pathophysiological mechanisms and, as a consequence, more precise therapy.

SECTION 2

OESTROGEN DYNAMICS DURING THE MENSTRUAL CYCLE

OESTROGEN DYNAMICS DURING THE MENSTRUAL CYCLE:

An experimental technique involving a combination of isotope dilution techniques and direct sampling of ovarian venous blood and follicular fluid was used to study the ovarian secretion and metabolism of oestradiol and oestrone in 11 women with normal menstrual function and 10 with dysfunctional uterine bleeding (DUB). The mean metabolic clearance rate of oestradiol in women with DUB (1198 ± 97 SEM, ℓ plasma/24 hours) was not different from that recorded in women with normal cycles (1202 ± 55 ℓ plasma/24 hours). Similarly, the conversion ratio of oestradiol to oestrone was not significantly different (DUB: 0.089 ± 0.010 compared with controls: 0.105 ± 0.010). In normal women the blood production rate of oestradiol (µg/24 hour) increased from 63 during menstruation to reach peaks of 394 immediately prior to ovulation and 337 during the mid-luteal phase. Women with DUB also demonstrated cyclical changes in oestradiol blood production rate. The highest production rate of 497 µg/24 hour was seen in a patient with cystic glandular hyperplasia of the endometrium. Two other women exhibited inappropriately high production rates at an early stage of the cycle. All three patients with DUB who ovulated in the cycle studied had a corpus luteum on each ovary and one further patient had bilateral large functional follicles. In the luteal phase oestrogen secretion and metabolism was normal.

In normal women over 95% of oestradiol entering the blood originated from either the pre-ovulatory follicle or corpus luteum. In 6 out of 10 patients with DUB significant amounts of oestradiol were secreted by both ovaries. The ovarian secretion of oestrone fluctuated in a similar manner to that of the blood production rate of oestradiol to reach a maximum of just over 100 µg/24 hour at mid-cycle, and no differences were seen in women with DUB. Ovarian blood flow was calculated in 10 women with a mean of 18.9 ± 5.2 SEM, ml/min in 6 normal women and 22.3 ± 4.0 ml/min in 4 women with DUB. Absolute blood flow was

calculated at approximately 2.3 ml/g/min. The highest rates of flow (36.4 and 33.1 ml/min) were recorded in the luteal phase and the lowest flow (4.6 ml/min) during luteolysis.

In follicular fluid the concentration of oestradiol was very much higher than that of oestrone. The concentrations of oestrogens were similar in fluid collected from small follicles at all phases of the cycle. The concentration of oestradiol in large follicles (>1 cm diameter) was much higher in the mid-late follicular phase (1520 ± 375 ng/ml; mean ± SEM) than in large or small follicles at other phases of the cycle. In the mid-late follicular phase large follicles were invariably associated with high concentrations of oestradiol in venous plasma draining the corresponding ovary (13.8 ± 3.1 ng/ml). The mean ratio of oestradiol to oestrone in fluid from large follicles in which the concentration of oestradiol was greater than 400 ng/ml (18.0 ± 1.0, n = 10) was significantly higher than the ratio in corresponding samples of ovarian venous plasma (10.7 ± 1.4, n = 11). These findings suggest that when the concentration of oestradiol in a Graafian follicle exceeds 1250 ng/ml it is likely to be functionally active (i.e. contributing to circulating oestradiol). In women with DUB the concentrations of oestradiol and oestrone were similar to the normal women, but sometimes several large active follicles were present. It is suggested that in women with DUB inappropriate oestrogen secretion may result from multiple follicular development due to abnormalities in gonadotrophin release and/or intra ovarian control mechanisms.

Chapter 2

BLOOD PRODUCTION AND OVARIAN SECRETION RATES OF OESTRADIOL-17β AND OESTRONE IN WOMEN WITH NORMAL MENSTRUAL CYCLES:

INTRODUCTION:

Ovarian secretion of "oestrogen" is abnormal in women with anovulatory dysfunctional uterine bleeding (Brown and Matthew 1962) but little is known about the details of direct secretion of different oestrogens and their subsequent metabolism in this condition. In addition details about ovarian secretion of oestrogens in normal women and women with anovulatory dysfunctional uterine bleeding were far from clear at the time of execution of this study.

An indirect index of ovarian secretion of oestrogens can be obtained by measurements of urinary excretion of oestrogen conjugates (Brown and Matthew 1962) or by measurements of peripheral blood concentrations of oestrone and oestradiol (Baird 1968). A more direct index can be obtained by measurement of the production and secretion rates of oestrogens in urine by isotope dilution techniques which require the injection of at least 2 radioactive tracers labelled with different isotopes (Barlow and Logan 1966). However, the interpretation of the secretion rates of oestradiol estimated by this method is complicated by the fact that at least two other known precursors of oestradiol conjugates (androstenedione and oestrone) are secreted by both ovary and adrenal glands (Vandewiele et al 1968).

The measurement of production and secretion rates of steroid hormones using the constant infusion technique and sampling in blood, overcomes many of the problems of the urinary technique (Baird et al 1969). The metabolic clearance rates (MCR) and transfer constants of oestrone and oestradiol have been measured in normal men and women (Longcope et al 1968; Longcope and Tait 1971) although it has been suggested that this technique is inapplicable to the study of oestrogens because of difficulty in achieving

the steady state (Hembree et al 1969). In spite of the possible sources of error in both methods estimates of the production rate of oestrone and oestradiol obtained from a knowledge of the mean MCR and plasma concentrations are similar to those based on urinary isotope dilution studies (Kirschner and Taylor 1972).

To determine the relative importance of secretion by the adrenals and each ovary to the total production rate of a steroid, direct sampling of glandular venous blood is necessary (Baird 1970). In the present study isotope dilution techniques involving continuous infusion techniques were combined for the first time with direct sampling of ovarian venous blood and follicular fluid, to investigate the site of secretion and production of oestrone and oestradiol in 11 subjects throughout the menstrual cycle.

The investigations in this section were developed from an idea of Dr. Baird's and were planned, executed and analysed jointly by Dr. Baird and myself. Mr Alan Galbraith measured most of the endogenous plasma oestrogen concentrations. I carried out all the extractions, purifications and estimations of radioactive oestrogens and on 2 occasions assayed the endogenous plasma oestrogens. I was also responsible for developing a celite chromatography separation and radioimmunoassay for oestrone and oestradiol for comparison with the double isotope derivative method and as a basis for future studies.

METHODOLOGY:

Radioactively-labelled reagents -

^{3}H-6,7-oestradiol (44 Ci/mmole), ^{14}C-4-oestrone (50 mCi/mmole) and ^{14}C-4-oestradiol (50 mCi mmole) were obtained from the Radiochemical Centre, Amersham and purified every 3 months by thin-layer chromatography in System 1 (see below) followed by paper chromatography in a Bush type system (stationary phase: methanol; water 3:1; mobile phase: toluene). The amount of radioactivity in samples was determined by adding 10 ml of scintillation

fluid (Baird 1968) and counting for at least 200 min. The settings on the counter were adjusted so that the efficiency for counting ^{3}H was 34% and for ^{14}C was 52% in their respective channels. The background in the ^{3}H and ^{14}C channels was 16 and 12 cpm respectively, and the efficiency of ^{14}C in the ^{3}H channel was 14% and negligible for ^{3}H in the ^{14}C channel.

Metabolic clearance rates -

Metabolic clearance rates were measured by the constant infusion technique (Longcope et al 1968; fig 1). The experiments were started between 7 and 8 am with the subjects fasting and supine for at least 10 hrs before and throughout the experiment. A priming dose of 4 μCi ^{3}H 6,7-oestradiol was injected into an arm vein at 0 min after 50 ml of blood had been withdrawn for estimation of the mass of endogenous oestrone and oestradiol-17β. At 30 min a constant infusion of ^{3}H-oestradiol was started at the rate of 2 μCi per hour and continued throughout the rest of the experiment (usually 260 min). The techniques of sampling the solution used for the priming dose and the rate of infusion were as described by Longcope, Layne and Tait (1968), a glass syringe and teflon tubing being used to prevent absorption of the steroid. Forty ml samples of blood were collected from a cannula in a vein in the contralateral arm at 150, 165, and 180 min. The infusion was continued during induction of anaesthesia with intravenous sodium thiopentone (0.5 g) between 180 and 195 min. Anaesthesia was maintained by the injection of 20-30 ml of bupivacaine into the epidural space and supplemented by inhalation of nitrous oxide and oxygen. Premedication was not given.

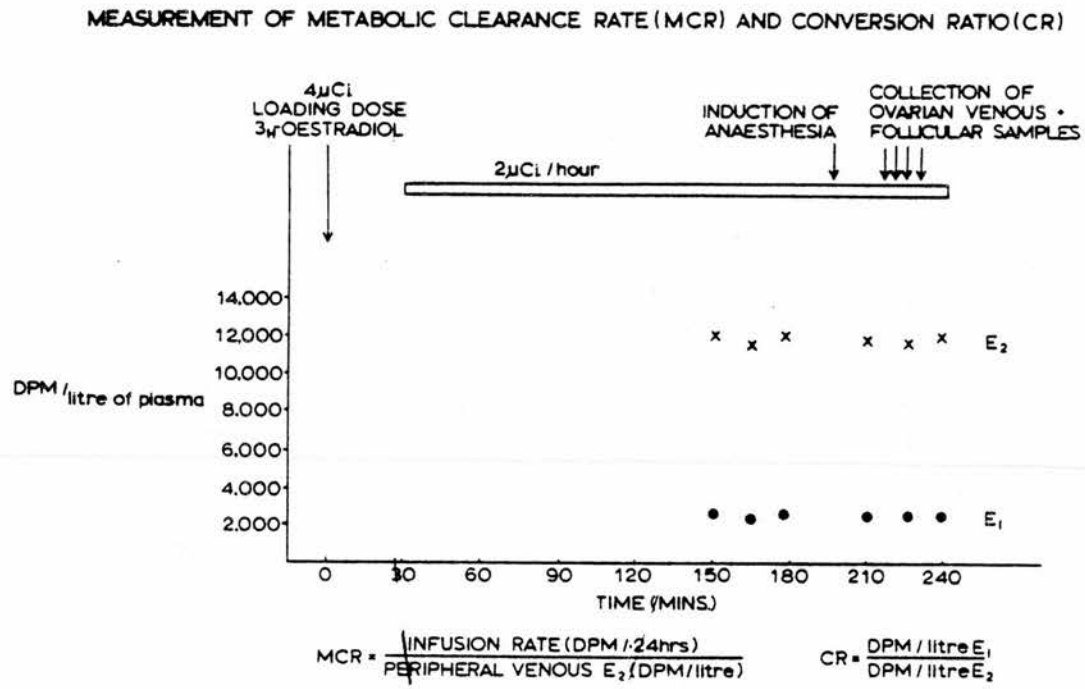


Figure 1. Layout of constant infusion technique for the measurement of MCR of oestradiol (E_2) and CR for oestradiol to oestrone (E_1). (DPM = disintegrations per minute)

Subjects -

Informed consent was obtained from all subjects who were admitted to hospital for hysterectomy for preinvasive (Stage 0) carcinoma of the cervix. They were otherwise healthy and had a history of regular menstrual cycles (24-31 days). Their mean age was 33 years (27-47) and all were parous except patient no.4.

Urine was collected for at least 4 days prior to the experiment for serial determination of total oestrogen and pregnanediol excretion (Brown 1971). The endometrium on the day of the experiment was examined histologically and dated according to the criteria of Noyes, Hertig and Rock (1950). The ovaries were examined by the naked eye at operation for the presence of follicles and/or corpora lutea, and biopsies were taken for microscopic examination

if there was doubt about the nature of any structure. This information together with the day of the cycle was then used to estimate the date of ovulation, and the subjects were grouped around this estimated point (Table 1).

Sampling during surgery -

When the abdomen was opened the state of the ovaries was noted, and clamps were placed on the ovarian ligaments on both sides to occlude the ovarian branch of the uterine vein and artery. Particular care was taken to ligate veins that drain the lateral pelvic wall and the uterus, and that run in the leaves of the broad ligament to join the ovarian venous plexus. In some initial studies a main trunk of the ovarian vein on each side of the pelvis was cannulated with a portex catheter allowing repeated sampling; in most cases blood was collected by needle aspiration of a vein in the infundibulopelvic ligament 2-3 cm from the ovary. Three samples of peripheral venous and sometimes brachial arterial blood were collected at approximately 15-min intervals between 210 and 275 min, together with samples of ovarian venous blood and follicular fluid. The heparinized samples were cooled on ice after collection, centrifuged at 2000 rpm for 20 min at 4 C and the plasma stored at -15 C until analysis.

Measurement of oestrogen concentration -

The concentration of oestrone and oestradiol-17β in peripheral and ovarian venous plasma and in follicular fluid was measured using a complex, time consuming, moderately sensitive, highly specific and precise double isotope derivative method (Baird 1968). This method involved addition of ^{3}H-oestrone or ^{3}H-oestradiol 17β (42.4 curie/mmol) as indicator to the biological sample before extraction to allow for the calculation of losses. The phenolic extract of blood was reacted with ^{35}S p-iodobenzene sulphonyl chloride (pipsyl chloride; 150-200 millicurie/mmol) and the derivatives purified by 4 chromatography and 2 further derivative steps. About 10% of both steroids was present in the final sample used for counting.

Table 1:

Clinical details of 11 regularly menstruating women studied by isotope dilution techniques combined with direct sampling of ovarian venous blood.

Subject	Age (lyr)	Surface area sq.m.	Day of cycle	Endo-metrium	State of ovary		Estimated ovulation ±
					Right	Left	
1	32	1.56	1	M			-14
2	34	1.53	4	EP	SF	SF	-10
3	29	1.61	11	MP	LF	SF	- 4
4	31	1.43	10	LP	LF		- 2
5	30	1.67	11	LP	LF	CH	0
6	36	1.81	14	ES	CH		+ 2
7	47	1.61	14	ES	CL		+ 2
8	30	1.52	20	MS		CL	+ 5
9	27	1.82	22	MS		CL	+ 8
10	33	1.62	25	MS	CL		+ 9
11	40	1.73	25	LS		CL	+12

M=menstruating
EP=early proliferative
MP=mid proliferative
LP=late proliferative
ES=early secretory
MS=mid secretory
LS=late secretory
SF=follicle<1cm
LF=follicle>1cm
CH=corpus haemorrhagicum
CL=corpus luteum

The volumes of peripheral and ovarian venous plasma extracted were approximately 20 and 5 ml, respectively. For follicular fluid the volume (0.2 ml) was made up to 5 ml with water. The blank (ng±SD) for 20 ml samples (oestrone: 0.19 ± 0.11 n = 27; oestradiol: 0.12 ± 0.12 n = 27) was subtracted from the value of the estimate before it was expressed in pg/ml. The blank for a 5 ml sample (oestrone and oestradiol <50 pg) was negligible in comparison to the mass of oestrogen in ovarian venous plasma or follicular fluid and was not subtracted from the estimate. The coefficient of variation of replicate estimates of a pool of nonpregnant female plasma assayed with each batch of samples was 8.6% and 14.2% for oestrone and oestradiol, respectively (n = 12).

Extraction and purification of radioactive oestrogens -

One hundred dpm of ^{14}C-4-oestrone and 150 dpm ^{14}C-4-oestradiol as indicators for estimation of losses and 50µg each of oestrone and oestradiol as carriers were thoroughly mixed with 10 ml of plasma to which 0.1 ml of N sodium hydroxide had been added. Extraction with ether and neutral phenolic partition was performed (Baird 1968). The following solvent systems were used for thin-layer chromatography (TLC) on silica gel (GF254) 0.250 mm thick:

I	chloroform:acetone	85:15
II	cyclohexane:ethylacetate	1: 1
III	benzene:ethanol	98: 2
IV	chloroform:acetone	97: 3
V	chloroform:acetone	92: 8

The phenolic extract of plasma was chromatographed in System I, and oestrone (R_f 0.68) and oestradiol (R_f 0.46) eluted separately. Both oestrogen fractions were reacted separately with pipsyl chloride and the monopipsylates further purified by chromatography in TLC Systems II and III. The amount of ^{3}H and ^{14}C were estimated in the purified sample and the ^{3}H dpm present in the plasma sample calculated after correcting for losses. The overall mean recovery was

68% (41-90) for oestrone and 71% (42-87) for oestradiol.

Validity of purification procedure -

In a preliminary experiment 20 ml samples of plasma were extracted. Following TLC in System I the oestrogen fractions were further divided. One half was purified in the usual way by forming the pipsyl derivative; the remaining half was acetylated (Longcope et al 1968), and the monoacetates of oestrone and oestradiol were purified by TLC in Systems IV and V, respectively. The overall recovery using this procedure was 72% (30-92) and 76% (69-86) for oestrone and oestradiol, respectively.

The mean concentration (dpm/10 ml plasma) of oestrone and oestradiol in 6 samples purified by forming the pipsylates (79 and 748, respectively) were not significantly different (paired Student's t test $p>0.2$) from those found with the procedure involving acetylation (90 and 759). As the pipsylates are more easily visualized by ultraviolet light this procedure was adopted as the standard procedure.

Calculation of results -

The metabolic clearance rate (MCR) in liters per day was calculated from the equation

$$MCR = \frac{R}{x^2} \qquad \text{(Tait 1963).}$$

When R = the rate of infusion of ^{3}H-oestradiol in dpm per day and x^2 = the concentration of ^{3}H-oestradiol in dpm/l obtained from the mean value of the samples at 150, 165 and 180 min. The Blood Production Rate of oestradiol ($P_B^{E_2}$) expressed in µg/24 hr was calculated from the product of the plasma concentration at 0 min and MCR^{E_2}. The conversion ratio of oestradiol and oestrone ($C_{BB}^{E_2E_2}$) was calculated from the ratio of the concentration of radioactive oestrone (x^1) to that of oestradiol (x^2).

The ovarian secretion rate of oestrone ($S_{ov}^{E_1}$) was calculated from the formula

$$S_{ov}^{E_1} = \frac{MCR^{E_2} \times (r^{E_1} - 1) \times i_A^{E_1}}{r^{E_2} - 1} \qquad \text{(Baird 1970)}$$

where

$$r^{E_2} = \frac{\text{Concentration of oestradiol in ovarian venous plasma } (i_{ov}^{E_2})}{\text{Concentration of oestradiol in arterial plasma } (i_A^{E_2})}$$

$$r^{E_1} = \frac{\text{Concentration of oestrone in ovarian venous plasma } (i_{ov}^{E_1})}{\text{Concentration of oestradiol in arterial plasma } (i_A^{E_1}}$$

RESULTS:

Metabolic clearance rates and conversion ratio -

There was no significant change in the concentration of either ^{3}H-oestrone or ^{3}H-oestradiol in the 3 samples obtained prior to operation (Tables 2 and 3). Espressed as a percentage of the mean, the values (±SEM) at 150, 165 and 180 min were 100.3 ± 1.2, 99.3 ± 1.1 and 100.4 ± 1.0 for oestradiol and 98.6 ± 1.7, 97.5 ± 2.0 and 104 ± 2.5 for oestrone, respectively. The regression equation of % of mean value (y) against time in minutes (x) for samples taken prior to operation between 150 and 180 min were: $y = -0.016x + 102.6$, $r = -0.085$ $p>0.1$ for oestradiol and $y = -0.005 + 101.3$, $r = -0.023$ $p>0.1$ for oestrone. The relatively high SEM of the mean MCR (1346 ± 152 l/24 hr) in the present series was due to one value (subject 9) which was well outside the 95% confidence limits of the mean. Omitting subject 9 the mean value was 1202 ± 55 l/24 hr. There appeared to be no change in MCR with respect to the stage of the menstrual cycle.

After induction of anaesthesia there was a significant increase in radioactivity in all subjects except subject 8. The mean values ± SEM (expressed as a percentage of the mean value 150-180) collected at approximately 220, 235 and 250 min were 116 ± 4, 121 ± 4 and 125 ± 4 for oestradiol and 130 ± 6, 118 ± 8 and 114 ± 13 for oestrone. The regression of mean % value against time was represented by the equation $y = 0.324x + 43.9$, $r = 0.417$ $p<0.05$ for oestradiol and $y = 0.176x + 82.1$, $r = 0.138$ $p>0.1$ for oestrone. Thus, during the operation the MCR of oestradiol fell to an average of 83% of that prior to surgery. The mean conversion ratio

Table 2:

Concentration of ^{3}H-oestradiol (x^2) in plasma following infusion of ^{3}H-oestradiol into 11 regularly menstruating women.

Subject	Rate of infusion dpm/24hr	dpm/10 ml plasma (x^2) after priming dose (min)					
		150	165	180	220 (210-260)	235 (225-260)	250 (240-275)
1	1.13×10^8	840	900	854	965	1147	1131
2	0.96×10^8	797	746	793	951	885	955
3	1.18×10^8	1063	1171	1247	1247	1204	1307
4	0.95×10^8	960	888	926	1005	1152	1180
5	1.23×10^8	850	841	863	1130	1142	1234
6	1.24×10^8	972	918	898	1039	1024	1053
7	1.19×10^8	864	-	924	-	1127	1112
8	1.21×10^8	1208	1135	1210	1040	1173	1220
9	0.93×10^8	338	347	321	381	373	394
10	1.23×10^8	1221	1372	1331	1618	1935	1836
11	1.12×10^8	943	881	865	1163	1137	1198

Table 3:

Concentration of ^{3}H-oestrone (x^1) in plasma following infusion of ^{3}H-oestradiol into 11 regularly menstruating women.

	dpm/10 ml plasma (x^1) after priming dose (min)								$C_{BB}E^2E_1$	
Subject	150	165	180	220 (210-240)	235 (225-260)	250 (240-275)	*MCR	MCR/ sq.m.	150-180	220-250
1	124	136	127	130	162	151	1361	872	0.161	0.134
2	61	66	86	102	69	77	1237	808	0.089	0.089
3	160	160	184	236	238	221	1073	666	0.153	0.185
4	126	116	116	150	141	153	1028	719	0.129	0.133
5	82	83	86	111	103	111	1447	866	0.099	0.092
6	102	81	106	119	99	84	1335	738	0.103	0.097
7	73	75	79	-	133	91	1325	823	0.085	0.072
8	143	136	152	153	155	155	1024	674	0.122	0.134
9	27	30	30	41	24	43	2788	1531	0.087	0.093
10	67	61	65	101	108	111	940	580	0.049	0.060
11	73	75	67	86	79	86	1250	722	0.080	0.072
mean ± SEM					**1202 ± 55	747 ± 30	0.105 ± 0.010		0.106 ± 0.0011	

*Calculated from mean value 150, 165 and 180 min.

**Subject no. 9 omitted from mean.

($C_{BB}^{E_2E_1}$) from 220-250 min (0.106 ± 0.011) was not different from that observed from 150-180 min (0.105 ± 0.010).

Blood production rates of oestradiol -

In 4 subjects (no.9 of this study and 3 subjects in another study) the concentration of oestrone and oestradiol in the sample taken at 0 time was compared to that of a sample obtained by pooling aliquots of collections from 150 to 180 min (Table 4). There was no change in the concentration of oestradiol and only minor fluctuations in the concentration of oestrone. In subject no.9 the concentration of both oestrogens were not markedly different at 235 min.

$P_B^{E_2}$ (blood production rates of oestradiol) was calculated from the product of MCR and plasma concentration (Table 5). The values increased from 63 µg/24 hr during menstruation in subject no.1 to reach a maximum value of 394 µg/24 hr at 2 days before ovulation (Subject no.4; figure 2).

Concentration of oestrone and oestradiol in ovarian venous plasma -

The concentration of oestrone and oestradiol was very similar when measured in consecutive samples of ovarian venous plasma collected in 3 subjects (Table 6). In subject no.9 the concentration increased 2-fold following ligation of a vein from the pelvic wall draining into the infundibulopelvic ligament.

The concentration of oestradiol was much higher in plasma from the ovary containing the preovulatory follicle or corpus luteum ("active" ovary) than from the contralateral side ("inactive" ovary) (Table 5). The difference in concentration of oestrone in plasma from the two ovaries was less marked than that of oestradiol. This is reflected by the significant difference in ratio of oestradiol to oestrone between the two sides (6.44 ± 0.97 vs 3.37 ± 0.61 for active vs inactive, respectively).

Table 4:

Concentration of oestrone and oestradiol (pg/ml) in peripheral venous plasma in 4 subjects at intervals during this experiment.

	Time (min)			
	0		150-180	
Subject	Oestrone	Oestradiol	Oestrone	Oestradiol
A (no.9)	111	121	115	122
			*87	*141
B	149	318	210	291
C	126	176	79	176
D	104	244	189	309

* At 235 min.

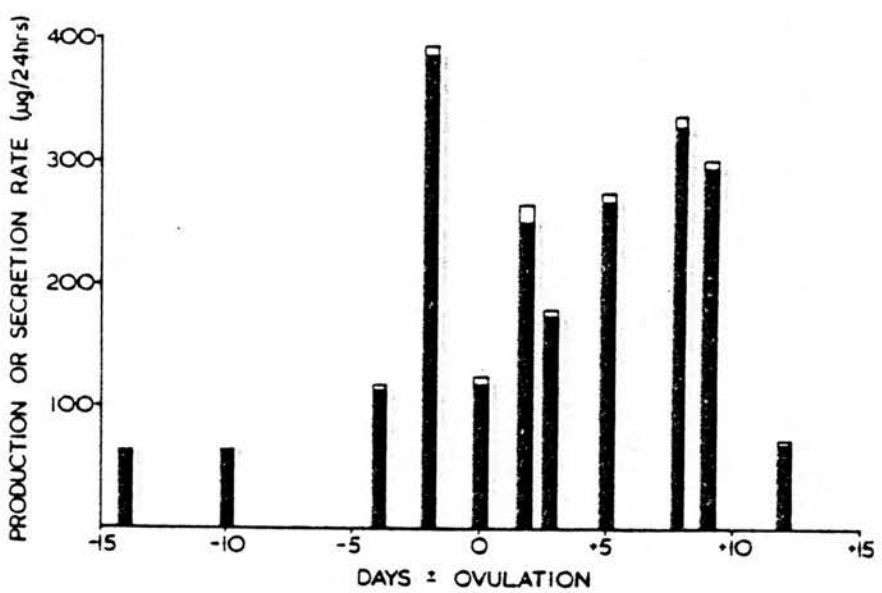


Figure 2. Blood production ($P_B^{E_2}$) and ovarian secretion rate (Sov^{E_2}) of oestradiol (E_2) throughout the menstrual cycle. The subjects have been grouped around the day of estimated ovulation (Table 1). The $P_B^{E_2}$ for each subject is indicated by the total height of each column and the individual components by the corresponding codes i.e., ■ Sov^{E_2}; □ E_2 derived from E_1.

Table 5:

The concentration of and calculations for $P_B{}^{E_2}$ and $S_{ov}E^1$ oestrogens in peripheral and ovarian venous plasma in 11 normal subjects.

	Concentration pg/mℓ plasma								
			Ovarian						
	Peripheral		Right		Left		$P_B{}^{E_2}$	$S_{ov}{}^{E_1}$ µg/24 hr	
Subject	E_1	E_2	E_1	E_2	E_1	E_2	µg/24 hr	Right	Left
1	26	46	42	140	70	324	63	-	9.9
2	<20	52	290	924	194	312	64	21.4	-
3	45	108	778	9360	84	280	116	9.1	-
4	97	383	8450	31679	800	671	394	104.9	-
5	68	85	220	1156	112	686	123	17.4	-
6	167	198	890	8233	78	358	264	23.8	-
7	54	134	370	3020	-	-	178	19.4	-
8	86	287	66	384	1871	10590	294	-	50.9
9	111	121	-	-	602	3903	337	-	43.7
10	75	320	1933	19803	276	654	301	28.7	-
11	29	57	130	306	2714	18328	71	-	10.4

Table 6:

Concentration of oestrone and oestradiol (pg/ml) in serial samples of ovarian venous plasma in 3 subjects.

Subject	Time (min)	Concentration pg/ml	
		Oestrone	Oestradiol
1	226	80	140
	231	80	166
3	224	778	9360
	232	648	8230
9	222	330	2640
	*226	595	4570
	238	880	4500

* Connection from lateral pelvic vein clamped after 222 min.

The calculated secretion rate of oestrone from the "active" ovary varied from 9.9 µg/24 hr in the early follicular phase of the cycle (Subject no.1) to a preovulatory maximum of 104.9 µg/24 hr (Subject no.4) (Table 5).

DISCUSSION:

The studies described in this section were designed to investigate the secretion and metabolism of oestradiol and oestrone in women with regular and normal menstrual cycles for comparison with women with dysfunctional uterine bleeding. The theoretical and practical aspects of these techniques have been thoroughly discussed by a number of authors (Tait 1963; Tait et al 1962; Gurpide et al 1963; Bardin and Lipsett 1967; Longcope et al 1968; Hembree et al 1969; Baird et al 1969).

In order to measure accurately the parameters described in this section, it is necessary to assume that steady state conditions apply. In this situation the peripheral plasma concentration of steroid is constant and the rate of entry of the steroid into the plasma is equal to its rate of exit. Unfortunately, in practice the system is not always in a steady state mainly because of circadian variations in secretion and metabolism, episodic secretion or postural variations which alter liver blood flow and metabolism. However, it has been shown that, if the rate of metabolism is high and the variations in secretion are relatively slow, the rates of entry and exit of the steroid from blood are approximately equal and the calculations reasonably accurate for the time of study. This appears to apply to oestradiol and oestrone although in some subjects the steady state is only reached very slowly (Hembree et al 1969).

The simplest means of measuring the metabolic clearance rate of oestradiol (the rate of irreversible removal from plasma; expressed as litres of plasma cleared per 24 hrs) is to perturb the system by a continuous constant rate

infusion of a radioactively labelled tracer (eg. ^{3}H-oestradiol) which simulates entry of steroid from a gland. The achievement of steady state is hastened by a priming dose. Once the steady state has been reached the concentration of ^{3}H-oestradiol in the blood is constant and the rate of clearance equals the rate of infusion of labelled tracer. The metabolic clearance rate then equals the rate of infusion divided by the radioactive steroid concentration (Tait et al 1962). Serial blood samples are required to confirm that the steady state has been achieved.

The blood production rate of a steroid is the total amount of steroid entering the blood from all sources in a given time. In the case of oestradiol in women in the reproductive years it now appears that almost the entire blood production rate is accounted for by secretion from the ovaries, specifically from the developing follicle or corpus luteum (see Chapters 3, 4 and 5). This means that secretion rate from the ovary equals blood production rate, which in the steady state can therefore be calculated as the product of metabolic clearance rate and peripheral arterial concentration. Since negligible clearance occurs in the peripheral tissues of the arm peripheral oestradiol concentration in the cubital vein is equal to the arterial concentration. Provided that the metabolic clearance rate is relatively constant changes in plasma levels of oestradiol should directly reflect changes in ovarian secretion.

Provided that ovarian secretion rate equals blood production rate for one steroid (eg. oestradiol) the ovarian secretion rate of a second steroid which enters the blood from more than one type of gland (eg. oestrone) can be calculated from the ratio of concentration of the second steroid in ovarian venous effluent and arterial afferent blood applied in the formula described earlier in the chapter (Baird 1970).

The assessment of all these parameters provides information on several aspects of ovarian oestrogen secretion and metabolism, and is therefore a good back-

ground for studying possible abnormalities in women with dysfunctional uterine bleeding (Chapter 3).

The results in the present study confirm the validity of the constant infusion technique for the measurement of MCR^{E_2} and $C_{BB}{}^{E_2E_1}$ for normal women as has been shown for men (Longcope and Tait 1971). The lack of any significant trend in values from 150 to 180 min indicates that the steady state for both precursor and product had been obtained. The maintenance of fasting and the strict standardization of position during the infusion may have minimized variations in hepatic blood flow which would be expected to alter MCR (Baird et al 1969). The fall in MCR after induction of anaesthesia was probably related to the fall in systolic blood pressure (to about 100 mm Hg) with the associated reduction in hepatic blood flow.

The concentrations of oestrogens in peripheral and ovarian venous plasma are similar to those previously published (Baird and Guevara 1969; Lloyd et al 1971; Korenman and Sherman 1973). Their concentration showed very little change during the course of the experiment although the considerable variations in the concentration of oestradiol which have been described in peripheral plasma of ambulant subjects (Korenman and Sherman 1973) may have been missed during the relatively short period of sampling. The secretion rates may therefore only apply to the period of measurements and to subjects in the supine position, although the calculated production rates are in the same range as those based on cumulative urinary specific activity (Vande Wiele 1965; Barlow and Logan 1966; Kirschner and Taylor 1972). It should be remembered however that considerable individual variation may occur in production rate of oestradiol (Brown 1971).

The mean values of MCR^{E_2} (1202 ± 55 l/24 hr) and $C_{BB}{}^{E_2E_1}$ (0.105 ± 0.010) are very similar to those reported for a comparable group of women at various stages of the menstrual cycle (Longcope et al 1968). $P_B{}^{E_2}$ illustrated

in Fig.2 was measured directly in each subject, and the amount of E^2 derived from E^1 was calculated assuming MCR^{E1} of 2210 ℓ plasma per day and C_{BB}^{E1E2} of 0.041 (Longcope et al 1968). P_B^{E2} exhibits a biphasic pattern with the maximal values occurring preovulatory and in the midluteal phase of the cycle (Fig.2). That these peaks of production represent maximal secretory activity of the preovulatory follicle and corpus luteum respectively is indicated by the relatively high concentration of oestradiol in the plasma draining the "active" as compared to the contralateral "inactive" ovary. Using the mean ratio of plasma concentrations from active/inactive ovary (26.1 ± 6.2 SE) and knowing that insignificant amounts of circulating oestradiol derive from conversion from oestrone (Longcope et al 1968) or adrenal secretion (Baird, Uno and Melby 1969), it can be calculated that about 95% of circulating oestradiol originates from secretion by the preovulatory follicle or the corpus luteum.

Having established that oestradiol is secreted almost exclusively from the active ovary, the ovarian secretion of oestrone and other steroids can be calculated. A similar method has been described for the testis and adrenal using testosterone and cortisol respectively as unique secretory products (Baird 1970). This latter technique was used by Kirschner and Jacobs (1971) to calculate the adrenal androgen secretion in hirsute women undergoing retrograde catheterization of adrenal and ovarian veins via the femoral vein. They commented that there was no known unique secretory product of the ovary which could be used to calculate ovarian androgen production. Although in normal women oestradiol appears to be such a unique secretory product, in pathological conditions e.g. polycystic ovarian disease, an appreciable proportion of plasma oestradiol may be derived by peripheral conversion of oestrone (Baird 1973).

In contrast to oestradiol, less than half P_B^{E1} is

derived by direct ovarian secretion (Fig.3). In constructing Fig.3 the total ovarian secretion $S_{ov}{}^{E1}$ was calculated by adding $S_{ov}{}^{E1}$ from the "active" ovary (which was measured directly) to the $S_{ov}{}^{E1}$ from the inactive ovary. This latter value was obtained by multiplying the $S_{ov}{}^{E1}$ from the active ovary by the ratio of concentration of oestrone in plasma draining inactive:active ovary. Although this calculation assumes that $S_{ov}{}^{E1}$ is proportional to $i_{ov}{}^{E1}$, i.e. the blood flow from both ovaries is similar, the $S_{ov}{}^{E1}$ is relatively small.

Significant amounts of oestrone are produced by extraglandular conversion of oestradiol (Fig.3) while the remainder of $P_B{}^{E1}$ originates either from adrenal secration (Baird, Uno and Melby 1969) or by peripheral conversion of androstenedione (Baird et al 1969). The extent of this latter source is likely to vary depending on the stage of the cycle, because the concentration of androstenedione in peripheral plasma rises significantly at midcycle (Judd and Yen 1973) corresponding to the growth of the preovulatory follicle (Baird et al 1973).

In subject no.5 a recently ovulated follicle in the left ovary (corpus hemorrhagicum) and a follicle 1-2 cm diameter on the point of rupture in the right ovary were observed at operation and confirmed by histological examination of biopsies. The preovulatory peak excretion of total oestrogen in urine had occurred 2 days prior to the day of experiment and the endometrial histology showed late proliferative changes. Taken in conjunction with the day of the cycle (day 11) there can be little doubt that ovulation had just occurred in this subject. In spite of the bilateral ovulation, the $P_B{}^{E2}$ and the $S_{ov}{}^{E1}$ are much lower than during the proliferative or luteal phase of the cycle. Thus the marked midcycle drop in plasma concentration of oestrogens, is due to virtual cessation of ovarian oestrogen secretion at ovulation (Baird and Guevara 1969).

It was considered important to ensure that undiluted glandular efferent blood was collected by clamping any veins anastomosing with the plexus of ovarian veins in the

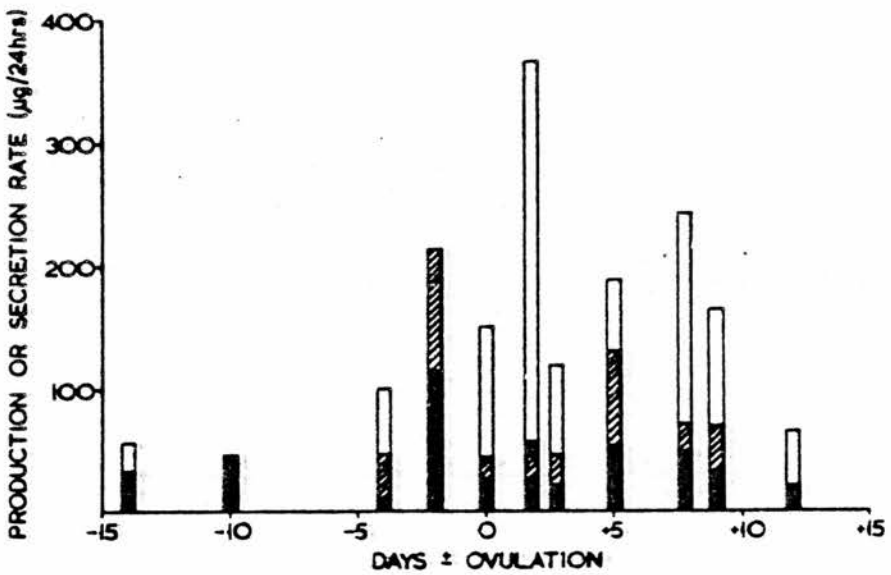


Figure 3. Blood production rate ($P_B^{E_1}$) and ovarian secretion rate ($S_{ov}^{E_1}$) of oestrone (E_1) throughout the menstrual cycle. The subjects have been grouped around the day of the estimated ovulation (Table 1). The $P_B^{E_1}$ for each subject is indicated by the total height of each column and the individual components by the corresponding codes i.e. ■ $S_{ov}^{E_1}$; ▨ E_1 derived from E_2; □ E_1 derived from other sources.

infundibulopelvic ligament. Particular care was taken to occlude a branch(es) from the uterine plexus which has been demonstrated by radiographic studies to anastomose with the contralateral ovarian veins (Jacobs 1969). If ovarian venous blood were diluted by blood draining the uterus and from the pelvic plexus, the concentration of steroids would be falsely low although the calculated ovarian secretion rates would remain unaltered (Baird 1971). The MCR^2 and plasma concentration ($i_A E^2$) used when calculating $S_{ov}E^1$ were measured prior to induction of anaesthesia. It is possible therefore that the $P_B E^2$ did not represent that at the time when the ovarian vein samples were obtained at laparotomy 30-60 min later. However, the change in peripheral plasma concentration of oestradiol was negligible (Table 4) and the observed fall in MCR^{E2} was less 20%. The error from this source therefore in calculating $S_{ov}E^1$ is unlikely to be large.

The secretion rate of oestrone from the "active" ovary changed from 9.1 µg/24 hr in subject no.1 to a maximum of 104.9 µg/24 hr in subject no.4 at 2 days prior to ovulation. The concentration of oestrone in plasma draining the "inactive" ovary was about 10% that draining the "active" ovary (mean ratio concentration in active/inactive = 10.3 ± 3.1 SEM). Assuming the blood flow to the ovaries is similar, the maximum ovarian secretion of oestrone is unlikely to exceed 120 µg/24 hr. These results compare with $S_u E^1$ based on simultaneous injection of oestrone and oestradiol labelled with different isotopes, of 0-76 µg/24 hr (Barlow and Logan 1966) and 4.4, 36.9 and 44.1 µg/24 hr (Vande Wiele 1965).

None of the subjects showed any evidence of endocrine abnormality. It has been reported that the $P_u E^2$ as measured by the injection of ^{3}H-oestradiol, was higher in premenopausal women with early carcinoma of the cervix (including some with Stage 0 lesions) than in control subjects (Fraser et al 1967). However, there was considerable overlap between the groups and the increased incidence of dysfunctional uterine bleeding in this age group may have contributed to the slightly higher $P_u E^2$.

Chapter 3

BLOOD PRODUCTION AND OVARIAN SECRETION RATES OF OESTRADIOL AND OESTRONE IN WOMEN WITH DYSFUNCTIONAL UTERINE BLEEDING:

INTRODUCTION:

Abnormalities of oestrogen excretion have been recognized in women with dysfunctional uterine bleeding for many years (Jeffcoate 1937; Schröder 1954; Brown et al 1959), but little is known of the underlying changes in oestrogen secretion and metabolism.

Production and secretion rate of oestrogens have been measured in normal women by urinary isotope dilution techniques (Barlow and Logan 1966; Vande Wiele et al 1968), and by constant isotope infusion and blood sampling techniques (Longcope et al 1968; Hembree et al 1969). Constant isotope infusion with direct sampling of ovarian venous blood and follicular fluid has been applied to the study of blood production and ovarian secretion of oestradiol-17β and oestrone in 11 normal women throughout the menstrual cycle (Chapter 2). In the current study this technique has been applied to the measurement of blood production and ovarian secretion of oestradiol-17β and oestrone in 10 women with dysfunctional uterine bleeding.

MATERIALS AND METHODS:

Subjects

Informed consent was obtained from 10 patients all of whom had a clinical history suggestive of anovulatory dysfunctional uterine bleeding sufficiently troublesome to necessitate hysterectomy. Their mean age was 43 yr (range 35-48 yr) and all were parous (1 to 10 pregnancies each: mean = 4.6). All patients except no.10 had a recent curettage of the uterus and 8 of the 10 (not no.1 and no.10) had measurements of the excretion of total oestrogen and pregnanediol in the urine at least once per week for up to 50 days prior to the study. The pattern of menstrual cycles based on endometrial histology and hormone measurements is

given in Table 1. Most had a history of anaemia but objective measurements of menstrual blood loss were not available.

TABLE 1:

Menstrual cycle patterns and endometrial histology for 1 yr prior to hysterectomy in 10 women with dysfunctional uterine bleeding.

Patient	Previous year	Previous cycle	Current cycle
1	A + O	A	A*
2	A + O	A	A*
3	A	A*	A
4	A*	A	A
5	A + O*	A	O
6	A + O*	?	O
7	O	?	O
8	A + O*	O	?
9	A + O	O	?
10	?	?	?

The pattern of menstrual cycles is classified on history, endometrial histology, hormone measurements and appearance of the ovary at operation as ovulatory (O), anovulatory (A) or mixed (A + O). Three time periods are current cycle, cycle prior to the one studied (previous cycle) and the 12 months prior to study (previous year). The finding of cystic glandular hyperplasia of the endometrium is indicated by the asterisk.*

Dynamic studies were carried out on the day of hysterectomy. The endometrium obtained at operation was examined histologically and dated according to the criteria of Noyes, Hertig and Rock (1950). The ovaries were examined by the naked eye at operation for the presence of follicles and/or corpora lutea, and biopsies taken for microscopic examination, if there was doubt about the nature of any structure. This information was taken together with the day of the cycle to estimate the stage of the cycle (Table 2).

TABLE 2:

Clinical details of 10 women with dysfunctional uterine bleeding.

Patient	Age	Surface area sqm.	Day of cycle	Endo-metrium	State of ovary (R)	State of ovary (L)
1	35	1.57	16	CGH		LF
2	46	1.59	16	CGH	LF x 3 SF x 2	LF
3	36	1.50	5	EP	SF	LF
4	46	1.74	24	MP		SF
5	44	1.70	21	MS	CL	CL
6	48	1.32	23	LS	CL	CL
7	44	1.66	19	ES	CH	CH
8	48	1.69	7	MP	LF	LC
9	39	1.67	10	MP	LF	LF
10	46	1.74	12	LP	LF	

CGH=cystic glandular hyperplasia
EP=early proliferative
MP=midproliferative
LP=late proliferative
ES=early secretory
MS=midsecretory
LS=late secretory
SF=small follicle<1 cm.
LF=large follicle>1 cm.
CL=corpus luteum
CH=corpus hemorrhagicum
LC=luteal cyst

The infusion and assay methods and the calculations have been described and discussed in detail in chapter 2.

RESULTS:

Seven of the 10 patients showed evidence of cystic glandular hyperplasia of the endometrium either in the cycle under study or within the previous 12 months (Table 1). Only 2 patients (no.3 and no.4) had shown evidence of ovulation at any time in the year prior to study. Three patients (no's 8, 9 and 10) were studied too soon after the onset of the last episode of vaginal bleeding to determine whether the current cycle was ovulatory or not.

TABLE 3:

Concentration of ^{3}H-oestrone (x^{1}) in plasma following infusion of ^{3}H-oestradiol.

Patient no.	Rate of infusion dpm/24hr	dpm/10 ml plasma (x^{1}) after priming dose (min)					
		150	165	180	215	230	245
1	1.04×10^{8}	56	65	64	55	59	59
2	1.15×10^{8}	75	85	79	95	80	142
3	1.13×10^{8}	113	92	129	151	142	133
4	0.93×10^{8}	61	69	80	88	85	88
5	1.08×10^{8}	114	114	114	140	160	144
6	1.03×10^{8}	92	85	101	95	99	100
7	0.98×10^{8}	49	46	47	46	37	50
8	1.08×10^{8}	69	77	60	84	96	78
9	1.15×10^{8}	76	78	78	87	94	86
10	1.03×10^{8}	97	97	102	108	110	132

TABLE 4:

Concentration of ^{3}H-oestradiol (x^2) in plasma following infusion of ^{3}H-oestradiol.

Patient no.	dpm/10 ml (x^2) after priming dose (minutes) 150	165	180	(200-255) 215	(215-270) 230	(230-285) 245	*MCR	MCR/sqm	$C_{BB}E^2E^1$ 150-180	215-245
1	667	749	772	782	736	724	1,427	909	0.085	0.079
2	749	786	834	714	853	668	1,456	916	0.101	0.142
3	874	880	925	1,364	1,137	1,433	1,265	843	0.124	0.108
4	902	908	980	992	1,062	1,114	998	574	0.075	0.082
5	1,026	1,061	1,050	1,048	1,025	1,043	1,033	608	0.109	0.142
6	1,421	1,458	1,510	1,739	1,624	1,710	714	533	0.064	0.058
7	693	701	726	818	835	893	1,386	835	0.067	0.052
8	746	659	812	756	634	498	1,461	864	0.093	0.137
9	771	722	789	919	953	1,033	1,509	904	0.101	0.092
10	1,463	1,286	1,344	1,505	1,527	1,722	745	428	0.072	0.073
							1,198±97	741±58	0.089±0.005	0.097±0.10

mean ± SEM

*Calculated from mean value 150, 165 and 180 min.

Metabolic clearance rates (MCR) and conversion ratio ($C_{BB}^{E_2E_1}$).

There was a small but significant rise in the concentration of ^{3}H-oestradiol and ^{3}H-oestrone in three samples obtained prior to the operation (Tables 3 and 4). Expressed as a percentage of the mean values (±SEM) at 150, 165 and 180 min were 98.4 ± 1.3, 97.7 ± 1.2 and 103.9 ± 1.0 for oestradiol and 97.3 ± 1.7, 99.9 ± 2.1 and 103.4 ± 2.5 for oestrone, respectively. The regression equations of the percentage of the mean value (y) against time in minutes (x) for samples taken prior to operation between 150 and 180 minutes were $y = 0.183x + 69.8$ ($r = 0.495$ $p<0.01$) for oestradiol and $y = 0.205x + 66.4$ ($r = 0.366$ $p<0.05$) for oestrone. In 6 out of the 10 patients, there was a rise in ^{3}H-oestradiol between 150 and 180 min indicating that a completely steady state had not been achieved. The mean MCR for the whole group was 1198 ± 971 plasma/24 hr and was not significantly different from those patients in whom the current cycle was anovulatory (cases 1 to 4 = 1286 ± 105/24 hr), ovulatory (cases 5 to 7 = 1041 ± 197 l/24 hr) or ?ovulatory (cases 8 to 10 = 1238 ± 247 l/24 hr).

In 6 patients there was a small increase in plasma levels of radioactivity after induction of anaesthesia. The mean values ± SEM (expressed as a percentage of the mean value between 150 and 180 min) collected at approximately 220, 235 and 250 min were 112.5 ± 5.3, 110.1 ± 4.0 and 113.6 ± 8.5 for oestradiol and 113.6 ± 4.5, 114.2 ± 6.2 and 121.6 ± 7.2 for oestrone. The regressions of mean percentage value against time were represented by the equations $y = 0.037x + 103.5$ ($r = 0.307$ $p>0.05$) for oestradiol and $y = 0.248x + 59.5$ ($r = 0.256$ $p>0.1$) for oestrone, respectively. Thus during the operation the MCR of oestradiol fell to an average of 93% of that prior to surgery, but a steady state appeared to have been reached. This intra-operative MCR was not significantly different from the pre-operative value ($p>0.1$).

The mean $C_{BB}^{E_2E_1}$ from 220 to 250 minutes (0.097 ± 0.010) was not different from that observed from 150 to 180

minutes (0.089 ± 0.005; $p>0.2$), and there was no difference between the anovulatory and ovulatory groups.

Blood production rates of oestradiol ($P_B^{E_2}$)

$P_B^{E_2}$ was calculated from the product of MCR and peripheral plasma oestradiol concentration (Table 5). In 2 patients (no.3 and no.8) the blood production rate was high (245 and 403 µg/24 hr, respectively) relative to the values in normal subjects at a similar time after the onset of the last menstrual period (5 and 7 days). The patient with the highest $P_B^{E_2}$ (no.2; 497 µg/24 hr) had classical cystic glandular hyperplasia of the endometrium, while the patient (no.1) with a $P_B^{E_2}$ of 251 µg/24 hr showed mild hyperplastic endometrial changes on day 16. The values obtained from those patients who had ovulated in the current cycle (no's. 5, 6 and 7) were within the range found in normal subjects in the luteal phase. These findings are graphically illustrated in figure 1, which also includes $P_B^{E_2}$ in women with normal cycles. The amount of oestradiol derived from oestrone was calculated assuming MCR^{E_1} of 2210 ℓ/24 hr and $C_{BB}^{E_1E_2}$ of 0.041 (Longcope et al 1968).

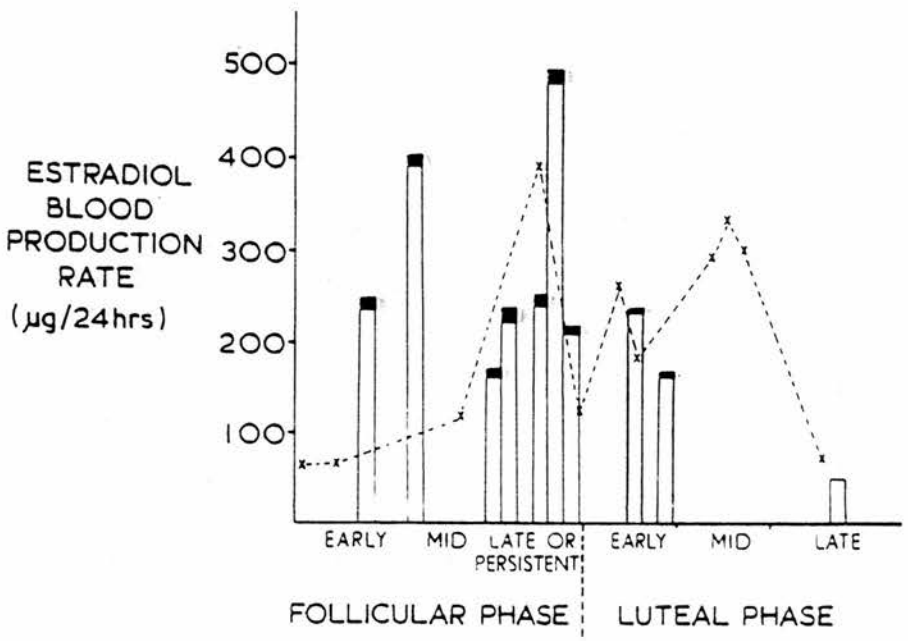


Figure 1. Oestradiol blood production rates throughout the menstrual cycle in women with normal cycles (dotted line) or dysfunctional uterine bleeding (vertical bars). Open bars indicate portion derived from ovarian secretion; black bars indicate portion derived from peripheral conversion.

Concentration of oestrone and oestradiol in ovarian venous plasma

The plasma concentrations of oestradiol and oestrone are shown in Table 5. In 5 cases (no's. 1, 2, 4, 8 and 10) the oestradiol concentration from one ovary (the "active" ovary) was 4.5 to 33 times greater than the other (the "inactive" ovary). In these 5 cases the difference in plasma concentrations of oestrone from the two ovaries was less marked than the oestradiol difference. All 3 patients studied during the luteal phase (no's 5, 6 and 7) had a corpus luteum on each ovary and one patient during the late follicular phase on day 10 (no.9) had a large follicle of preovulatory size on each ovary. In these 4 cases significant plasma concentrations of oestrone and oestradiol were present in venous blood from both ovaries. In patient no.3, concentrations from each ovary were similar, and the values were higher than one would expect on day 5. The overall mean ratio of oestradiol/oestrone in plasma from the more "active" ovary (9.67 ± 1.20) was significantly greater than from the less active ovary (6.32 ± 1.59; $p<0.02$). The overall mean ratio in ovarian venous plasma (9.67 ± 1.20) was significantly higher than the ratio in peripheral venous plasma (3.33 ± 0.87; $p<0.005$).

The calculated secretion rates of oestrone from the "active" ovary in the 4 cases where more than 90% of the circulating oestradiol was derived from one ovary were all in the range 29.4 to 46.1 µg/24 hr. Meaningful oestrone secretion rates could not be derived in the other 6 cases since a large proportion of oestradiol was being secreted by the less active ovary.

DISCUSSION:

The validity of the techniques used in this investigation has been discussed at length (Chapter 2). In the study of normal women the steady state for both precursor and product had been obtained between 150 and 180 min, whereas most of the women with dysfunctional uterine bleeding showed a small rise in peripheral venous plasma concentration of

^{3}H-oestradiol and ^{3}H-oestrone between 150 and 180 min. The reason for this difference between the two groups is uncertain, but it is possible that the ^{3}H-oestradiol infusion was being made into a larger or more complex endogenous oestrogen "pool" in the women with dysfunctional bleeding. Although the steady state had not been reached in the majority of these women in the preoperative period, it was achieved in the intra-operative period and the mean intra-operative MCR was not significantly different from the preoperative MCR. There was no difference in the overall MCR and $C_{BB}^{E_2E_1}$ of the present group with dysfunctional uterine bleeding and the group of normal women studied using identical techniques. This suggests that the metabolism of oestrogen in the women with dysfunctional uterine bleeding is similar to that in normal women, although the inability to reach an early steady state for the precursor during infusion may indicate that there are some differences in oestradiol distribution. In both groups a fall in MCR occurred after induction of anaesthesia and an intra-operative steady state for precursor was achieved.

The $P_B^{E_2}$ in those patients in whom the current cycle was anovulatory (no's. 1 to 4) was greater than 200 µg/24 hr, even as early as day 5. Cystic glandular hyperplasia of the endometrium was present in 2 of these patients, one of whom (no.2) had the highest $P_B^{E_2}$ recorded in this group and in the group of normal women. In this patient the oestradiol was demonstrated to be secreted from the right ovary in which there were five cysts the fluid of which contained high concentrations of oestradiol (44.1 to 166.0 µg/100 ml). These were presumably functionally active. These findings are summarized in Figure 2 and are in agreement with the reports of elevated urinary excretion of oestrogens over prolonged periods of time in some women with cystic glandular hyperplasia of the endometrium (Brown et al 1959) and with the observation that cystic glandular hyperplasia can be produced by treatment with large doses of oestrogens (Schröder 1954). The explanation for this early and persistently high production of oestradiol is uncertain, but may be due to a disturbance of the intra-ovarian follicular control

mechanisms (Vande Wiele et al 1970), resulting in large active follicles which fail to become atretic prior to the onset of uterine bleeding. It appears either that the follicles are responding abnormally to pituitary gonadotrophins or that the pattern of pituitary gonadotrophin secretion is abnormal such as occurs in adolescent anovulatory dysfunctional uterine bleeding (Chapter 6).

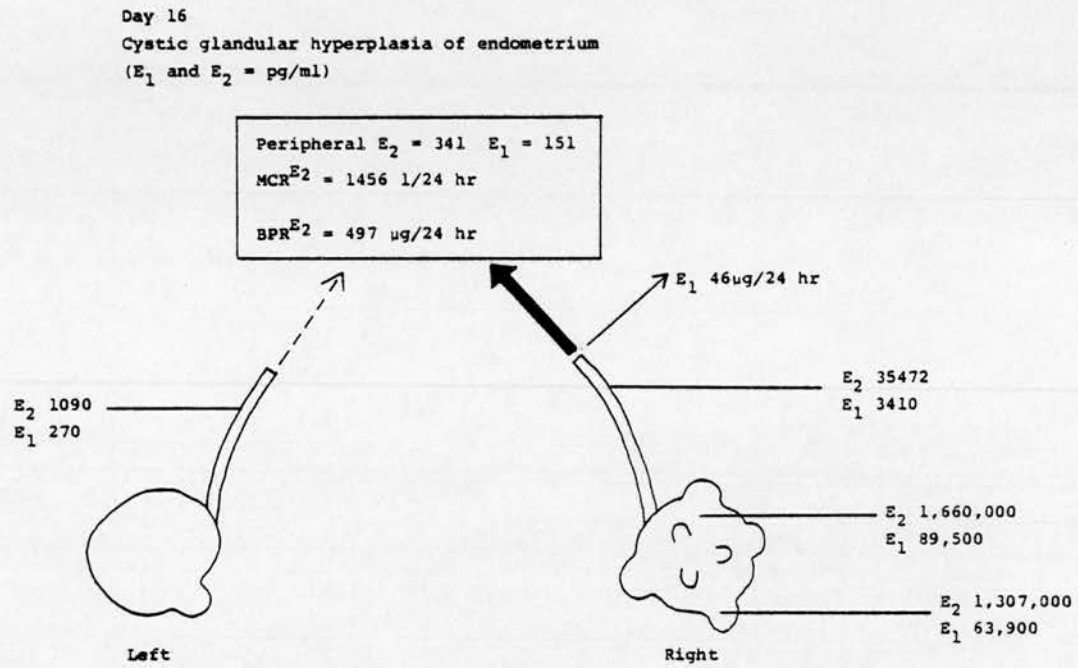


Figure 2. Oestradiol and oestrone concentrations in different bodyfluids in a woman with endometrial cystic glandular hyperplasia (Subject No 2).

The 3 women who ovulated showed bilateral ovulations, and 2 other patients (no.3 and no.9) appeared to have bilateral functioning follicles. This is in contrast to normal women where only one preovulatory follicle usually develops and in whom over 95% of circulating oestradiol is secreted by one ovary. This again suggests a disturbance of intra ovarian follicle control.

The current investigation has confirmed the intermittent nature of the disorder in dysfunctional uterine bleeding (Brown et al 1959). Most of the patients had recent evidence of both ovulatory and anovulatory cycles and it was impossible to predict the status of the current cycle on the basis of

recent history (Table 1). Two out of the 3 patients who ovulated had recent histological confirmation of cystic glandular hyperplasia of the endometrium while in contrast both patients who were found to have hyperplastic endometrium at hysterectomy had not shown this previously at curettage of the uterus.

The concentrations of oestrone in ovarian venous plasma of all 10 women were within the same range as the normal group of women. There appeared to be a slightly greater relative production of oestradiol than oestrone by both ovaries compared to normal women, the ratios of oestradiol/oestrone in venous plasma from the more active ovaries being 9.67 ± 1.20 in the dysfunctional bleeding group and 6.44 ± 0.97 in the normals; the figures for the less active ovaries being 6.32 ± 1.59 and 3.37 ± 0.97, respectively. This may reflect the overall higher secretion of oestrogen in the group with dysfunctional uterine bleeding.

Chapter 4

OVARIAN BLOOD FLOW IN WOMEN WITH NORMAL MENSTRUAL CYCLES OR WITH ANOVULATORY DYSFUNCTIONAL UTERINE BLEEDING:

INTRODUCTION:

Virtually nothing is known about blood flow in the human ovary but some information is available in animals (Bruce and Moor 1975). Measurements of changes in total ovarian flow mainly come from direct ovarian venous cannulation at operation (Stormshak et al 1963; Mattner and Thorburn 1969), from ovarian autotransplants (Baird et al 1968; Goding et al 1967; Baird et al 1973) or Doppler ultrasonic transducers (Brown et al 1980) while capillary flow has been measured by the Sapirstein technique (Setchell 1969) or more recently by the use of radioactively labelled microspheres (Abdul-Karim and Bruce 1973; Brown et al 1974; Mattner et al 1981). It appears that the ovary has a relatively high rate of blood flow which is mainly due to high flow around follicles and through the corpus luteum (Bruce and Moor 1975).

This chapter describes the calculation of ovarian blood flow in 10 women using data obtained from isotope dilution techniques described in the previous 2 chapters.

Materials and methods

Ovarian blood flow can be calculated from knowledge of the secretion rate of a substance uniquely produced by that ovary and from the arteriovenous concentration difference of that substance across the ovary (Baird 1970). Oestradiol (E_2) is a substance almost uniquely produced by the ovary and during a major part of each normal menstrual cycle over 95% of the oestradiol is produced by the one ovary containing the developing follicle or corpus luteum (hereafter referred to as the "active" ovary). For practical purposes it can thus be said that the secretion rate of oestradiol from the active ovary is approximately equal to the total blood production rate of oestradiol. A formula for calculating the blood flow

of the active ovary can therefore be derived.

(1) $SR^{E_2} = OPF \times (i_{ov}^{E_2} - i_a^{E_2})$

where SR^{E_2} = secretion rate of E_2 from active ovary

OPF = active ovarian plasma flow

$i_{ov}^{E_2}$ = active ovarian venous plasma concentration of E_2

$i_a^{E_2}$ = arterial plasma concentration of E_2

(2) $P_B^{E_2} = MCR^{E_2} \times i_a^{E_2}$

where $P_B^{E_2}$ = blood production rate of E_2

MCR^{E_2} = metabolic clearance rate of E_2

Therefore, if $SR^{E_2} = P_B^{E_2}$

and $i_a^{E_2} = i_v^{E_2}$, where $i_v^{E_2}$ = peripheral venous plasma E_2 concentration (Baird 1970)

(3) $OPF \times i_{ov}^{E_2} - i_v^{E_2} = MCR^{E_2} \times i_v^{E_2}$

(4) $$OPF = \frac{MCR^{E_2} \times i_v^{E_2}}{(i_{ov}^{E_2} - i_v^{E_2})}$$

[this must be multiplied by $\frac{10^3}{1440}$ to convert from ℓ/24 hr to ml/min]

(5) Ovarian blood flow = OPF $\div$ (1-haematocrit)

This assumes good mixing of ovarian effluent at the point of sampling, and no contamination with non-ovarian blood. It also assumes insignificant transport of oestradiol into the peripheral circulation via the ovarian lymphatics. In this study all non-ovarian blood was excluded by clamping and dividing the ovarian ligament and all tubal and pelvic wall connections before sampling. Samples were collected by direct needle puncture of the largest vein in the ovarian plexus and slow withdrawal of the blood through a 21 gauge needle into a 20 ml syringe. Preparation and assay of samples has been described in chapter 2. The isotope dilution technique and calculation of metabolic clearance rate have

also been thoroughly described in chapter 2.

SUBJECTS:

Six women who gave a history of regular menstrual cycles (subjects no. 3, 4, 6, 8, 10 and 11 in chapter 2, N1-N6 respectively) and 4 women with anovulatory dysfunctional uterine bleeding (subjects no. 1, 2, 8 and 10 in chapter 3, D3, D4, D1 and D2 respectively) were studied at the time of hysterectomy. Clinical details have been described in chapters 2 and 3, and relevant details of cycle day, estimated day of ovulation and haematocrit are summarised in table 1. Only those subjects in whom 95% or more of the secreted oestradiol originated from the active ovary have been included in the investigation described in this chapter. This is illustrated by the ratio $\frac{\text{active } E_2}{\text{inactive } E_2}$ (table 1). This ratio is calculated from: active E_2 = plasma concentration of oestradiol in vein draining the active ovary minus peripheral venous plasma oestradiol concentration; inactive E_2 = plasma concentration of oestradiol in vein draining the inactive ovary minus peripheral venous plasma oestradiol concentration.

RESULTS:

Active ovarian blood flow and necessary data required for the calculations are summarised in table 1. The ratio of $\frac{\text{active } E_2}{\text{inactive } E_2}$ indicates that 95% or over of secreted oestradiol came from a single ovary in each instance.

Mean ovarian blood flow in 6 women with regular cycles was 18.9 ± 5.2 ml/min, (mean±SEM) and in 4 women with anovulatory dysfunctional uterine bleeding was 22.3 ± 4.0 ml/min. The highest blood flow (36.4 and 33.1 ml/min) was recorded in 2 women in the luteal phase and the lowest flow (4.6 ml/min) was recorded from the late luteal phase, presumably during luteolysis. There was no obvious difference between ovarian blood flow in the women with regular cycles and those with anovulatory dysfunctional uterine bleeding, although numbers in each group were small. Therefore, it seems reasonable to calculate mean blood flow for the active ovary for the whole group of 10 women at 20.2 ± 3.4 ml/min.

TABLE 1:

Data required for calculation of 'active' ovarian blood flow in 6 women with regular menstrual cycles (N1-N6) and 4 women with anovulatory dysfunctional uterine bleeding (D1-D4).

Subject no.	Cycle day	HCT	Intra op. MCR ℓ/24 hr	Peripheral E_2 conc. (pg/ml)	Active ovarian E_2 conc. (pg/ml)	Inactive ovarian E_2 conc. (pg/ml)	Ratio of $\frac{\text{Active } E_2}{\text{Inactive } E_2}$	AOP flow ml/min	AOB flow ml/min	Stage
N1	11	41.7	942	108	9360	280	54:1	7.8	13.4	- 4
N2	10	38.8	1028	383	31679	671	108:1	8.8	14.4	- 2
N3	13	42.8	1193	198	8233	358	50:1	20.8	36.4	+ 2
N4	20	37.5	1060	287	10590	384	106:1	20.7	33.1	+ 5
N5	24	40.4	580	320	19803	654	58:1	6.7	11.2	+ 9
N6	25	38.6	1250	57	18328	306	77:1	2.8	4.6	+12
									18.9 ± 5.2	
D1	7	40.1	1717	244	17790	765	34:1	17.1	28.5	MF
D2	12	41.8	635	319	19600	824	37:1	7.5	12.9	LF
D3	16	36.8	1387	176	9895	715	18:1	18.5	29.3	CGH
D4	16	43.5	1544	341	35472	384	47:1	10.4	18.4	CGH
									22.3 ± 4.0	
								TOTAL	20.2 ± 3.4	

(Footnote) HCT=haematocrit
"Stage"=± estimated day of ovulation or stage of cycle
AOP=active ovarian plasma
AOB=active ovarian blood
MF=mid follicular phase
LF=late follicular phase
CGH=cystic glandular hyperplasia of endometrium
Intraop=intraoperation

DISCUSSION:

This study was the first investigation of ovarian blood flow in women although limited data were available on ovarian blood flow in animals (Bruce and Moor 1975).

It had been noticed that ovarian venous blood was often a brighter red colour than peripheral venous blood (Fraser, Baird and Cockburn 1973) suggesting a high rate of ovarian blood flow or perhaps a reduced rate of tissue oxygen consumption. It seems unlikely that overall ovarian tissue oxygen consumption is low since the corpus luteum at least is a highly metabolically active tissue and individual ovarian cells have a high metabolic rate invitro (Ahren and Hamberger 1969). Ovarian oxygen consumption has been calculated at 0.014 ml/g/min in women (Fraser, Baird and Cockburn 1973) and 0.03-0.05 ml/g/min in sheep (Baird et al 1973).

Ovarian weight varies considerably with degree of development of follicles and corpus luteum and with age of the woman (Wehefritz 1923). In the age group 20-50 years the weight of each ovary in a non-pregnant woman is generally between 7 and 11 g with a mean of 8.8 g. With a blood flow of approximately 20 ml per ovary per min this gives a tissue blood flow of approximately 2.3 ml/g/min. This can be compared with thyroid and adrenal blood flow of about 5 ml/g/min, heart and brain blood flow of 0.5 ml/g/min or kidney blood flow of 7.5 ml/g/min (Handbook of Biological Data 1956).

The ovary is a complex organ and purely on morphological and functional grounds it could be expected that blood flow to metabolically active areas like the corpus luteum or theca interna would be much higher than to the stroma, as demonstrated in animals (Bruce and Moor 1975). On the other hand, follicular (or cyst) fluid and granulosa cells are components of the ovary with zero blood flow. For example; the three large ovarian follicles in subject D4 will have contributed substantially to the non-perfused portion of that ovary which overall had a blood flow of 18.4 ml/min.

The technique described in this chapter for measuring

human ovarian blood flow should be reasonably accurate allowing for the above limitations and the assumption that oestradiol is continuously secreted. It is now known that oestradiol may be secreted in an episodic fashion (Alford et al 1973) but this is unlikely to introduce a major source of error.

Ovarian blood flow has been extensively studied in sheep. Much of the early work was carried out by direct cannulation of the main ovarian vein at operation (Stormshak et al 1963; Mattner and Thorburn 1969). These studies give a value of up to 8 ml per ovary (with corpus luteum) per min in the anaesthetised animal which is much lower than the value of 14 ml/min in non-anaesthetised ewes in whom the ovary has been transplanted to a skin loop in the neck (Baird et al 1968; Goding et al 1972; Baird et al 1973). This difference may be partly related to compensatory hypertophy in the transplanted ovary (Sundaram and Stob 1967). The tissue blood flow calculated from above studies of 3-4 ml/g/min (up to 10 ml/g/min for luteal tissue (Abdul-Karim and Bruce 1973) was several times higher than capillary flows measured using indicator techniques for the sheep ovary (0.2-0.3 ml/g/min; Setchell 1969) or the sow ovary (0.53 ml/g/min; Rattmacher and Anderson 1968). Although, it now appears that soluble indicator techniques may give a falsely low measure of corpus luteum flow (Brown et al 1974), the difference in total organ blood flow and capillary flow in non-luteal ovaries (Brown et al 1974) and the relatively high PO_2 and oxygen content of ovarian venous blood in women (Fraser, Baird and Cockburn 1973) and sheep (Baird et al 1973) suggest that a significant proportion of ovarian arterial blood by-passes the capillary bed. Arterio-venous shunts which have been described within the substance of the human ovary (Clara 1956) could explain this. Some recent studies in sheep support this concept (Mattner et al 1981), although experiments in rabbits do not (Ahren et al 1974).

Chapter 5

OESTROGENS IN OVARIAN VENOUS BLOOD AND FOLLICULAR WITH NORMAL MENSTRUAL CYCLES OR DYSFUNCTIONAL UTERINE BLEEDING:

INTRODUCTION:

It has long been known that oestrone and oestradiol are present in high concentration in fluid collected from human Grafian follicles in both the follicular and luteal phase of the cycle (Smith 1960; Short and London 1961). Both oestrogens are secreted into the ovarian veins draining the ovary containing the preovulatory follicle or the corpus luteum (Schild 1966; Mikhail 1970; Lloyd et al 1971; Chapters 2 and 3). It is generally assumed that a follicle containing high concentrations of oestradiol also secretes oestradiol into ovarian venous blood. However, some studies have suggested that not all such follicles are functionally active (Gorgi 1965; Edwards et al 1972).

Many women with perimenopausal dysfunctional uterine bleeding, especially those who are anovulatory, are found to have several large follicles (or "cysts") on one or both ovaries (Schröder 1954; Chapter 3). This suggests that there may be abnormalities of pituitary gonadotrophin secretion or disturbances of intraovarian control mechanisms, or perhaps both, in these patients (Chapter 3). However, it is not known whether these multiple follicles are functionally active.

At the time this study was completed there was very sparse information on the steroid content of follicular fluid and virtually none on the relationship between follicular fluid, and ovarian and peripheral venous plasma concentrations of oestrone and oestradiol. This study was designed to assess:

1. details of the changes in oestrone and oestradiol fluid from follicles of different size through the menstrual cycle in women with normal cycles.

2. the relationship between follicular fluid, ovarian venous and peripheral venous concentrations of oestrone and oestradiol in women with normal cycles.

3. variations in follicular fluid, ovarian venous and peripheral venous concentrations of oestrone and oestradiol in women with dysfunctional uterine bleeding.

This study was designed, executed and analysed jointly by Dr. Baird and myself. Mr A. Galbraith measured the oestrogen concentrations.

MATERIALS AND METHODS:

Subjects

Samples of follicular fluid and ovarian and peripheral venous blood were obtained at laparotomy from thirty-four patients undergoing hysterectomy for various gynaecological conditions. The indications for surgery were either Stage 0 carcinoma of the cervix (fifteen) or menorrhagia due to fibroids or dysfunctional uterine bleeding (nineteen). The former group had regular menstrual cycles and were considered to be endocrinologically normal (Eleven were studied in Chapter 2). Of the group with menorrhagia, eleven ovulated in the cycle under study as indicated by the presence of a secretory endometrium and of at least one corpus luteum at the time of surgery. The remaining eight were considered to be in the mid-late or persistent proliferative phase of the cycle. Ten of the menorrhagia group were studied in Chapter 3.

The endometrium was examined histologically and dated according to the criteria of Noyes et al (1950). The ovaries were then examined macroscopically at the time of surgery and the presence of corpora lutea and follicles visible on the surface noted: follicle size was calculated by an approximate measurement of diameter across the exposed surface. This information, together with the number of days since the onset of the last menstrual period, was used to place the subjects into one of five groups: (a) early follicular, (b) mid to late follicular, (c) ovulatory, (d) early luteal, and (e) mid to late luteal.

Collection of samples

Samples of peripheral (50 ml) and ovarian venous

blood (9-20 ml) were collected within 10 min of one another into heparinized containers. Ovarian venous blood was sampled by needle puncture of the ovarian vein 2-3 cm from the ovary after clamping the ovarian and pelvic wall branches of the uterine vein (Chapter 2). Follicular fluid was aspirated through a 23G needle into a syringe after the collection of blood samples had been completed. Plasma and follicular fluid were stored at - 15° until assay.

Measurement of oestrogens

Oestrone and oestradiol were measured by a double isotope derivative method (Baird 1968). Details of precision and blanks for human plasma and follicular fluid have been discussed in Chapter 2.

RESULTS:

The concentrations of oestrone and oestradiol in the thirty-four patients are illustrated in Tables 1 and 2. In each group there was no difference between the values obtained for those subjects with Stage 0 carcinoma of the cervix (0) and those with menorrhagia (M). The results from all patients have therefore been pooled and the mean values illustrated in Figs. 1 and 2.

The concentration of oestradiol was similar in venous plasma draining both the right and left ovaries in the early follicular phase of the cycle (1.2 ± 0.34 vs. 0.96 ± 0.42 ng/ml ± SEM). In the mid-late follicular phase the concentration of oestradiol was much higher in venous plasma draining the ovary containing at least one large follicle (>1 cm) than in that draining ovaries containing only small follicles (13.8 ± 3.1 vs. 0.65 ± 0.14 ng/ml). In subject 8, in whom the concentrations of oestrogens were similar, both ovaries contained a large follicle. In both the early and mid-late luteal phase the concentration of oestrogens was much higher in venous plasma draining the ovary containing a corpus luteum (4.5 ± 0.85 and 7.9 ± 2.1 ng/ml). The concentration of both oestrone and oestradiol in venous plasma draining the ovary without a large pre-ovulatory follicle or corpus

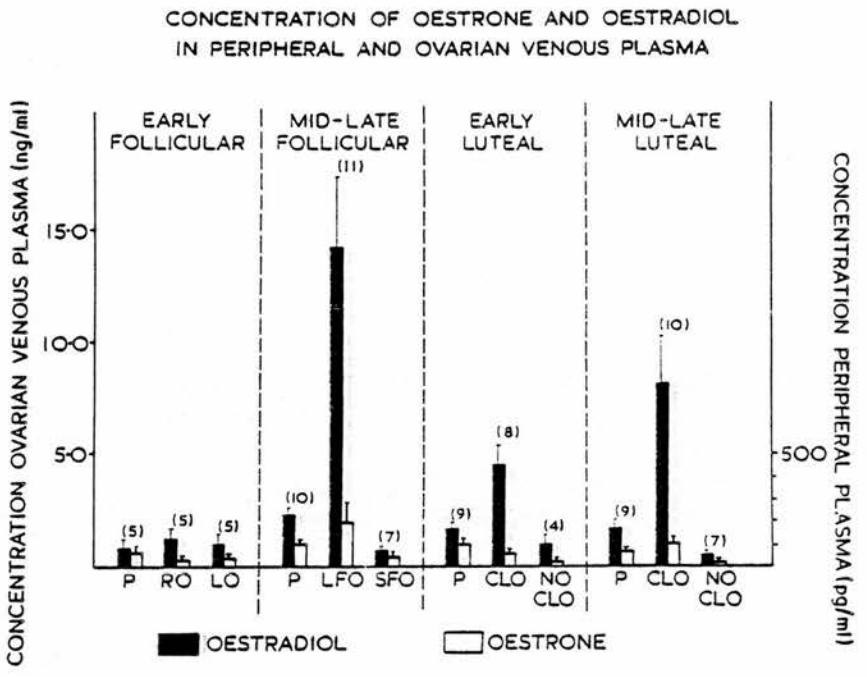


Figure 1. Concentration of oestradiol (solid columns) and oestrone (open columns) in ovarian and peripheral venous plasma throughout the menstrual cycle. The height of each bar represents the mean ±SEM of the number of observations indicated within the parentheses. (RO = right ovarian; LO = left ovarian; LFO = draining ovary with large follicle (>1 cm diameter); SFO = draining ovary with small follicles (<1 cm diameter); CLO = draining ovary with corpus luteum; no CLO = draining ovary with no corpus luteum.)

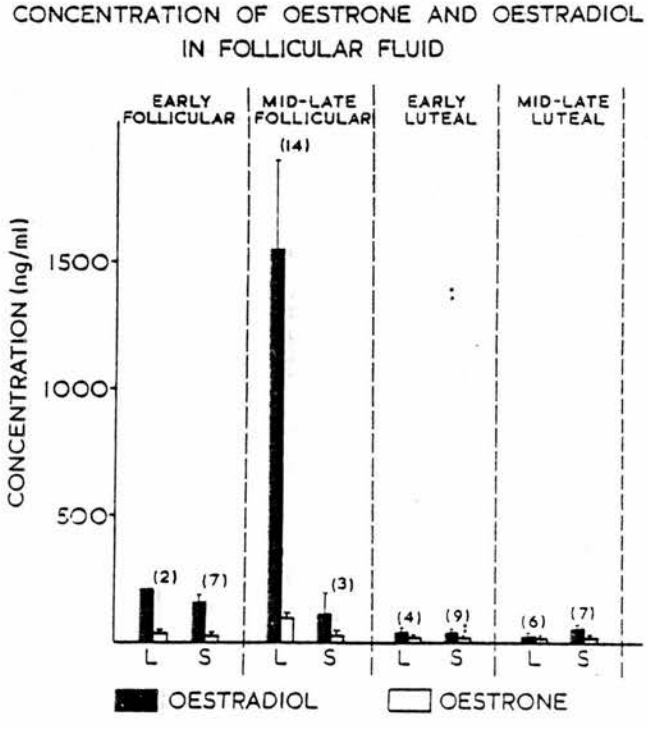


Figure 2. Concentration of oestradiol (solid columns) and oestrone (open columns) in follicular fluid of women at different stages of the menstrual cycle. The height of each bar represents the mean ±SEM of the number of observations indicated within the parentheses. L = follicles >1 cm diameter; S = follicles <1 cm diameter. The values for two small follicles from subject 18 have been omitted from the overall mean for that group and are indicated individually.

TABLE 1.

Concentration of oestrone (E_1) and oestradiol (E_2) in ovarian and peripheral venous plasma and follicular fluid of women in the follicular stage of the cycle. The numbers of large (diameter>1 cm) and small follicles (diameter<1 cm) in which oestrogens were not measured are indicated by + in the appropriate column.

Subject	Diagnosis	Day of cycle	Peripheral (pg/ml)		Ovarian (ng/ml)				Follicular fluid (ng/ml)							
					Right		Left		Right				Left			
									Small		Large		Small		Large	
			E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2
Early follicular																
1	0	1	26.3	45.1	0.04	0.14	0.07	0.32	7.0	113					28.3	161
2	0	1	20.2	18.3	0.59	1.56	0.16	0.46			27.2	228				
3	0	1	151	158	0.41	2.15	0.36	2.55	6.2	22			12.0	85		
4	0	4	20.2	51.6	0.29	0.91	0.20	0.30	19.0	296			9.0	86		
5	0	7	75.6	149	0.35	1.40	0.44	1.07	22.1	210			11.0	188		
Mid-late follicular																
6	M	6	61.6	141	0.59	9.2	0.07	1.17			73.4	1323				
											9.0	38				
7	M	7	105	241	1.56	17.6	1.40	0.76			246	3755				
8	M	10	75.6	111	0.48	7.4	0.32	6.11			+				+	
9	0	10	97.7	378	8.52	31.3	0.81	0.66			110	2360	48	271		
											229	3456				
10	0	11	45.4	107	0.79	9.2	0.08	0.28	3.0	21	+		3.1	11		
11	M	12	163	315	3.14	19.1	0.20	0.81			166	3701				
12	M	16	127	174	0.24	0.71	1.58	9.80							28.3	113
13	M	15	112	291	0.86	6.4	——	——			153	2347				
14	M	16	151	341	3.41	35.5	0.27	1.09			24.1	435				
											63.9	1307				
											89.5	1660				
											64.4	820				
											2.0	12				
15	M	18	79.5	113	0.29	2.51	——	——			3.9	4				

TABLE 2: Concentration of oestrone (E_1) and oestradiol (E_2) in ovarian and peripheral venous plasma and follicular fluid of women in the luteal phase of the cycle. The numbers of large (diameter 1 cm) and small follicles (diameter 1 cm) present in which oestrogen was not measured are indicated by + in the appropriate column. The presence of the corpus luteum is indicated by an asterisk.

Subject	Diagnosis	Day of Cycle	Peripheral (pg/ml)		Ovarian (ng/ml)				Follicular fluid (ng/ml)							
					Right		Left		Right				Left			
									Small		Large		Small		Large	
			E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2
Ovulation																
16	0	11	68.6	84.2	*0.22	1.14	*0.11	0.67								
Early luteal																
17	M	14	54.3	132	*0.37	2.99							8.1	79		
18	M	14	127	155	0.31	1.95	*0.48	2.92	39	1374			47	1333		
19	0	14	168	195	*0.90	8.12	0.08	0.36	1.1	13			17	3.1		
													8.1	42		
20	0	14		123									11	41	24	55
21	M	17	161	83	0.07	0.40	*0.30	2.89	1.1	8			5.0	32		
22	M	17	93.8	174									11	32		
													14	9.4		
23	M	19	31.4	171	*1.27	7.92	*0.58	3.45								
24	M	20	111	286	0.39	0.95									3.1	22
25	M	21	20.2	158	*0.32	5.15	*0.19	1.98			3.1	22			8.1	
Mid-late luteal																
26	0	20	86.8	283	0.07	0.38	*1.88	10.5	1.1		1.1	18	15	19	2.0	24
															5.0	11
27	M	20	69.4	135	*0.45	5.48	0.11	0.63	1.1	7						
28	M	22	48.4	156	0.06	0.21	*0.27	3.50					16	21		
29	0	22	112	119	*0.60	3.85	+									
30	M	23	<20.2	67.1	*0.27	2.00	*0.31	2.87				15	+			
31	0	25	75.6	316	*1.95	19.5	0.28	0.64	3.1	14			1.1	11		
32	0	25	29.1	56.4	0.13	0.30	*2.93	18.1								
33	0	25		34.7	*0.08	1.61	0.06	0.94			30	182			6.2	10
34	M	28	68.6	282	0.32	2.87	*1.61	12.7							1.1	32

luteum was similar at all stages of the cycle.

The concentration of oestradiol in follicular fluid was approximately 100 times greater than in ovarian venous plasma while that of oestrone was about 50-fold greater (note difference in scale in Figs. 1 and 2). The concentration of oestradiol from both large and small follicles was similar (20-300 ng/ml) at all stages except during the mid-late proliferative phase of the cycle. In eleven of the fourteen large follicles sampled in this latter group, the concentration of oestradiol exceeded 400 ng/ml with a mean value of 1520 ± 375 ng/ml.

The concentration of oestradiol in follicular fluid, ovarian venous blood (from 'active' and 'inactive' separately) and peripheral venous blood from the same women in mid-late follicular phase is graphically illustrated in Figure 3. Oestradiol to oestrone ratios in the same fluids are illustrated in Figure 4.

DISCUSSION:

This study has confirmed that the largest follicle of diameter 1 cm or greater in the mid to late follicular, and the corpus luteum in the luteal phase of the cycle, are the major source of ovarian oestradiol. The concentration of oestradiol in venous plasma draining the ovary containing these structures was 20-fold greater than that draining the contralateral ovary. The concentration of oestrone was also higher in plasma from the 'active' ovary but the difference was not as marked as that of oestradiol (Fig. 1).

The very high concentration of oestradiol in follicular fluid (up to 3750 ng/ml) is within the same range as found by other workers in normal (Smith 1960; Sanyal et al 1974) and gonadotrophin stimulated ovaries (Edwards et al 1972) and must be near the limit of its solubility. A specific binding protein of high affinity for oestradiol in follicular fluid would help to concentrate oestradiol (Takikawa 1966; Giorgi et al 1969). Detailed work by several groups of workers, and especially Dr. K. McNatty, has since confirmed

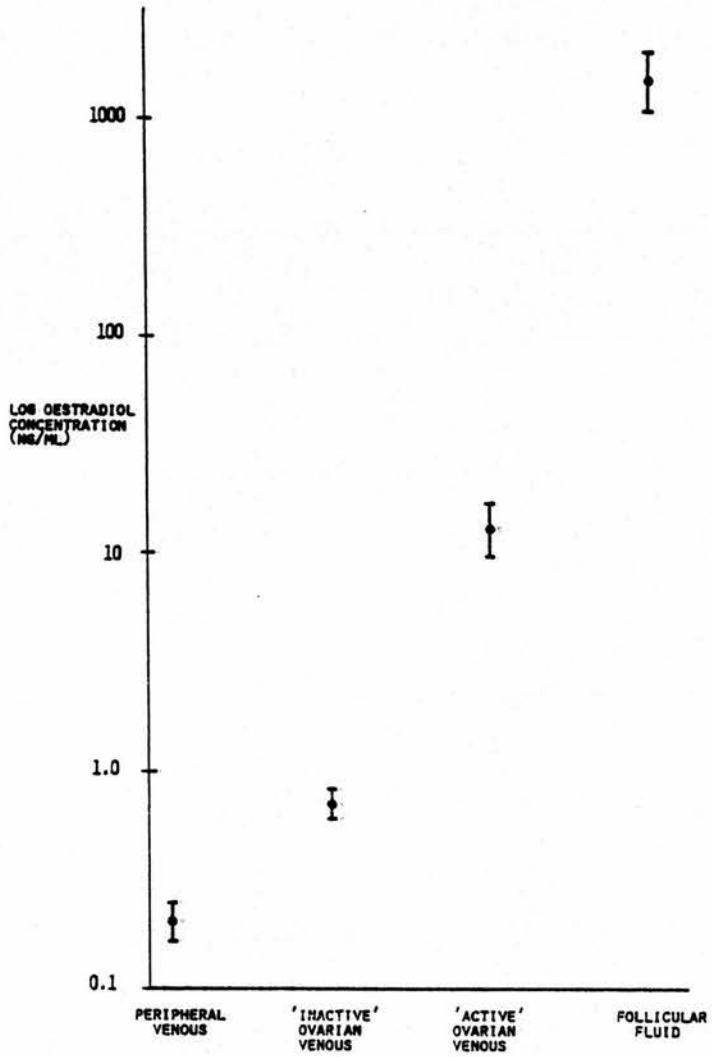


Figure 3. Comparison of oestradiol concentrations (log scale) in peripheral and ovarian venous blood and follicular fluid. Samples of each fluid were collected at approximately the same time from each woman.

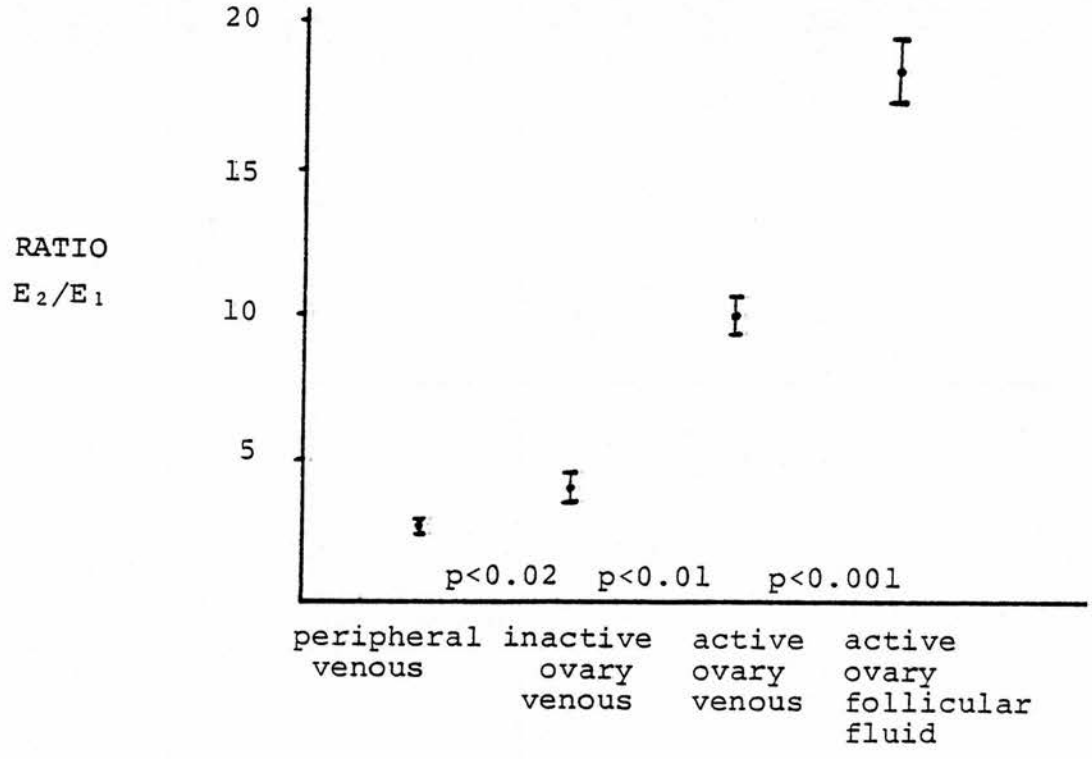


Figure 4. Comparison of the ratios of the concentration of oestradiol to oestrone in peripheral and ovarian venous blood and follicular fluid. Samples of each fluid were collected at approximately the same time from each woman.

and extended these findings described in this study (McNatty et al 1975; McNatty et al 1976; Channing and Louvert 1976; McNatty and Baird 1978; McNatty et al 1979; Hodgen 1982). The function of the high concentration of oestradiol within the follicle was unknown at the time of this study but was thought to act locally by increasing the rate of granulosa cell mitosis (Payne and Hellbaum 1955) and by increasing the sensitivity of the granulosa cells of the developing follicle to gonadotrophins (Goldenberg et al 1972). It is now known that oestradiol also has an important role in stimulating the synthesis of FSH receptors in granulosa cells (Louvert and Vaitukaitis 1976).

In the mid-late follicular phase large follicles were invariably associated with high concentrations of oestrogens in the corresponding ovarian venous plasma, indicating that they were functionally active. In six of the ten subjects in this group the concentration of oestradiol in at least one of the follicles exceeded 1250 ng/ml. In contrast large follicles at other stages of the cycle were never associated with high concentrations of oestradiol and were presumably atretic. Unfortunately histology was not carried out to assess the viability of follicles or oocytes. These findings suggest that when the concentration of oestradiol in a follicle is greater than 1250 ng/ml it is likely to be functionally highly active, (i.e. contributing significantly to circulating oestradiol) and in the preovulatory phase. The only other follicles in which the concentration of oestradiol exceeded 1250 ng/ml were found shortly after ovulation in subject 18 in whom the concentration of oestradiol in both ovarian veins was relatively high. It seems that any follicle containing a concentration of oestradiol greater than 1250 ng/ml is likely to be contributing to the peripheral circulating levels.

The concentration of oestradiol exceeded that of oestrone in follicular fluid, ovarian and peripheral venous plasma at all stages of the cycle (figure 4). The higher ratio of oestradiol/oestrone in ovarian venous plasma from the 'active'

ovary (9.73 ± 0.79, η = 29) relative to peripheral plasma (2.43 ± 0.27 η = 31) reflects the extra-ovarian production of oestrone (Baird 1971). The ratio of oestradiol/oestrone in follicular fluid of large follicles in the mid-late phase of the cycle in which the concentration of oestradiol exceeded 400 ng/ml (18.0 ± 1.0 η = 10) was significantly higher ($P<0.001$) than the ratio in corresponding samples of ovarian venous plasma (10.7 ± 1.4 η = 11). This supports the presence of a specific binding protein in follicular fluid with a relatively higher affinity for oestradiol than oestrone (Giorgi et al 1969). Although the concentration of oestrone in large follicles in the mid-late follicular phase rose significantly as compared to the concentration in follicles at other stages of the cycle, the mean maximum value (90 ± 20 ng/ml η = 14) was lower than that reported by Sanyal et al (1974) using radioimmunoassay (mean value of large follicle 200 ng/ml). The reason for these differences is not apparent although the range in both studies was considerable.

Although the numbers in this study are small there does not appear to be any major difference between oestradiol and oestrone concentrations in follicular fluid and ovarian venous blood in women with normal menstrual cycles or dysfunctional uterine bleeding. However, it seems likely that the intra-ovarian control mechanisms can be disturbed in some women with anovulatory dysfunctional bleeding. For example, Subject no.14 had 4 large follicles in one ovary. All of these were functionally active and contained greater than 430 ng/ml of oestradiol. The inter-relationships of oestrone and oestradiol in different fluids in this patient have been pictorially illustrated in Chapter 3. Three or four of the women with menorrhagia (Numbers 12-15) presumably had a persistent follicular phase with anovulation (days 15-18 of the cycle) and two of these had cystic glandular hyperplasia of the endometrium (Subjects no's. 12 and 14). These women had presumably embarked on the prolonged follicular oestradiol secretion seen in adolescents with anovulatory dysfunctional bleeding (Chapter 8)

and all had moderate to high peripheral plasma levels. Although very high ovarian venous and peripheral levels were seen in the subject with multiple active follicles the correlation between follicular fluid, ovarian venous and peripheral venous levels was not close in the other 3 subjects. However, it can be seen that when high concentrations of oestradiol are present in follicular fluid there are almost always high concentrations of oestradiol in ipsilateral ovarian venous blood.

SECTION 3

DYSFUNCTIONAL UTERINE BLEEDING IN ADOLESCENTS

DYSFUNCTIONAL UTERINE BLEEDING IN ADOLESCENTS:

Most adolescents with DUB are anovulatory and in the majority of these the episodes of excessive bleeding are isolated. Girls with persistent DUB are uncommon and those in whom cystic glandular hyperplasia of the endometrium (CGH) is found are rare. In our study which covered the whole population (1,100,000) of the South-Eastern Region of Scotland only 5.3 new cases were diagnosed per year over a 10 year period. This represented 4.0 percent of all cases of abnormal uterine bleeding in girls under 21 years in the Region requiring curettage, and 1.9% of cases of CGH in all age groups. Mild hyperplastic changes were found almost as frequently as the more classical "Swiss-cheese" CGH. All 17 of the cases diagnosed at the Royal Infirmary of Edinburgh between 1955 and 1964 were followed up in 1971. Patients showing more marked hyperplastic changes had a worse prognosis than those who only showed mild changes, and this condition appears to be the severe end of the spectrum of anovulatory DUB. These girls exhibited a high incidence of persistent menstrual abnormalities, repeated curettage, repeated requirement for hormone therapy, primary infertility, gynaecological laparotomies and possible polycystic ovarian disease.

Pituitary and ovarian relationships were assessed over a 3 month period in 4 adolescents with a history of confirmed CGH using daily measurements of oestrogen and pregnanediol excretion in urine and FSH and LH concentration in plasma. Three of the 4 patients showed 2 successive spontaneous anovulatory cycles with marked increases in oestrogen excretion but with a failure of the normal mid-cycle surge of LH. Clomiphene initiated a rise in plasma FSH and LH with subsequent follicular development suggesting that the negative feedback relationship between oestrogens and gonadotrophins was intact. The concentrations of oestradiol and oestrone in peripheral venous plasma were within normal limits for women in the reproductive age group.

These results suggested that the absence of ovulation in these adolescent women was associated with a failure to discharge LH in response to increasing levels of oestrogen - a failure of positive feedback - and this was tested in more detail in a further 9 young women with persistent anovulatory DUB. Ovarian activity was studied by twice weekly urinary excretion of oestrogen and pregnanediol over a 3-4 month period, and results were compared with 6 regularly menstruating women. All control women had ovulatory cycles, but 7 of the 9 with DUB failed to ovulate during at least 3 consecutive cycles. The profiles of urinary oestrogen excretion in these 7 women were consistent with regular follicle development, but the follicular phase was prolonged and the amount of oestrogen excretion increased greatly compared with controls. In 4 of these 7 patients the endometrium had previously shown CGH.

After injection of 50 μg of gonadotrophin - releasing hormone, the release of LH and FSH was normal in all subjects. However, the surge of LH induced in response to exogenous oestrogen (ethinyl oestradiol 200 μg per day for 3 days) was significantly lower in patients than controls (16.2 ± 3.7 mu/ml compared with 35.0 ± 5.5 mu/ml; $p<0.005$). It is concluded that the failure to ovulation in young women with anovulatory DUB is due to inadequate release of LH in response to oestrogen, supporting the hypothesis that the basic defect is a decrease of hypothalamic sensitivity to positive feedback.

Chapter 6

LONG TERM FOLLOW-UP OF ADOLESCENT GIRLS PRESENTING WITH ENDOMETRIAL CYSTIC GLANDULAR HYPERPLASIA:

INTRODUCTION:

Menstrual disorders in adolescence are common and the great majority of these comprise irregularities of menstruation associated with anovulation or irregular ovulation. Oligomenorrhoea with scanty menstrual loss is a very common problem during the first few years after the menarche, but is not usually associated with any serious health hazard. It is much less usual to see adolescent girls who experience persistent, heavy dysfunctional uterine bleeding, and there is no information on the true incidence in any population. The majority of these are also associated with anovulation and may sometimes present a difficult management problem.

A proportion of adolescents with menorrhagia and anovulation have high circulating levels of endogenous oestrogens which cause excessive proliferation of the endometrium resulting in the histological appearance of cystic glandular hyperplasia (Schröder 1954). This condition of irregular heavy menstrual bleeding associated with cystic glandular hyperplasia of the endometrium in an adolescent girl is at the severe end of the spectrum of anovulation and is sometimes known as juvenile metropathia haemorrhagica.

There is suggestive evidence that the small group of girls with this type of serious menstrual disorder have a high risk of continued gynaecological abnormalities over many years (Southam 1960; Southam and Richart 1966), but adolescents with proven cystic glandular hyperplasia have not been followed specifically.

Jeffcoate (1937) at one stage indicated that all or almost all cases of adolescent dysfunctional uterine bleeding were associated with endometrial hyperplasia, while Southam (1960) found hyperplasia in almost 50% but other studies have found it much less frequently (Sutherland 1953). It is often taught that endometrial hyperplasia shows peak incidence at

both ends of the reproductive life span in the perimenopausal and immediate postmenarchal age groups (Cope 1971), but the evidence for a peak in adolescence is inconclusive (Sutherland 1949; Schröder 1954).

This study was designed to record clinical details of all new cases of endometrial cystic glandular hyperplasia in adolescent girls presenting over a ten-year period at the gynaecological clinics of the Royal Infirmary of Edinburgh. In this study we have limited the term "adolescent" to include girls aged 20 or under. These cases were then followed-up over a period of 8 to 18 years. The incidence of adolescent CGH was compared with cases of cystic glandular hyperplasia of the endometrium in all age groups presenting in the whole South-Eastern Region of Scotland over the same period. Adolescent hyperplasia was also compared with other types of endometrial pathology in all cases of abnormal uterine bleeding in adolescents which required curettage during the same time period.

This study was jointly designed and analysed by Dr. D. Baird and myself, and executed predominantly by myself.

Clinical material and methods

Full details were obtained from the gynaecological pathology records for the South-Eastern Region of Scotland for the years 1955 to 1964 for all patients aged 20 years or less with endometrial curettings showing cystic glandular hyperplasia. Details of the histology of endometrial curettings were also obtained for all girls aged 20 years or less who had undergone curettage for abnormal bleeding but in whom cystic glandular hyperplasia was not found. Details of patients in other age groups with a diagnosis of endometrial cystic glandular hyperplasia were also recorded. The indication for curettage, the year of curettage and the age of the patient were recorded. The fullest possible details, including clinical case records, were obtained for the 17 adolescent patients with cystic glandular hyperplasia who had attended the Gynaecological Outpatient Department of

the Royal Infirmary of Edinburgh between 1955 to 1964. These patients were contacted by letter in 1971. Twelve were later interviewed personally and up to date clinical details were obtained from the other 5 by telephone, letter and questionaire. A full gynaecological, obstetrical and medical history was obtained, with particular emphasis on the years since the initial curettage.

The histological sections from all curettings from the 17 patients were critically reassessed and graded according to the degree of hyperplasia present. As a consequence of the small numbers the cases were only divided into two groups according to whether "mild"/"early" or "classical"/ "moderate" (hereafter referred to as "marked") changes of cystic glandular hyperplasia were present. The degree of hyperplasia was assessed mainly on the number and diameter of the dilated glands, but stromal hyperplasia was also taken into account.

RESULTS:

Table I details the number of cases of adolescent cystic glandular hyperplasia diagnosed in the South-Eastern Region of Scotland as a whole and at the Royal Infirmary of Edinburgh in each of the years 1955 to 1964. Thirty-two percent of the cases were seen at the Royal Infirmary. The incidence by age of adolescent cystic glandular hyperplasia in the South-Eastern Region of Scotland in the years 1955-1964 is illustrated in table 2. The peak incidence was seen at age 17, but the youngest patient in whom the diagnosis was made was aged 9 years and 4 months.

TABLE 1:

Number of cases of adolescent cystic glandular hyperplasia occurring in the South-Eastern Region of Scotland in the years 1955 - 1964.

YEAR	1955	'56	'57	'58	'59	'60	'61	'62	'63	'64	TOTAL
Total number of cases	6	8	8	3	4	4	1	8	3	8	53
Royal Infirmary cases	2	2	4	0	0	1	1	4	1	2	17

TABLE 2:

Incidence of adolescent cystic glandular hyperplasia by age group in the South-Eastern Region of Scotland in the years 1955-1964.

Age (years)	9	10	11	12	13	14	15	16	17	18	19	20	Total
Number of years	1	0	0	2	3	3	2	9	12	6	8	7	53

The endometrial histology in all girls aged 20 or under who underwent curettage for disorders of menstruation and from whom curettings were obtained in the South-Eastern Region of Scotland during the year 1955 is detailed in table 3.

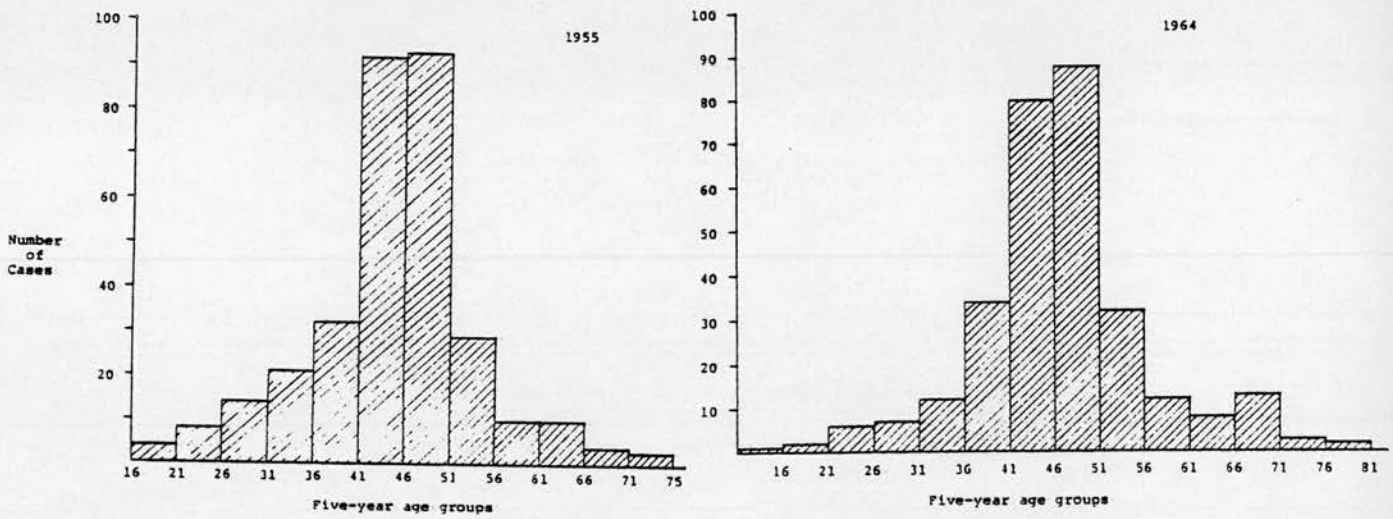
TABLE 3:

Histology of endometrial curettings from 151 girls aged 20 or under who underwent curettage for disorders of menstruation in the South-Eastern Region of Scotland during 1955.

Age	Total	Atrophic	Proliferative	Secretory	Menstrual	Adenomatous hyperplasia	Cystic glandular hyperplasia	Retained products of conception	Endometrial polypi	Chronic endometritis	Tuberculous endometritis
14	2		1	1							
15	0										
16	9		4	2			1		1	1	
17	17		8	5	1		1	2			
18	20		11	8			1				
19	54	1	19	24	2		1	6			1
20	49		16	17	4	1	2	6	1	1	1
Total	151	1	59	57	7	1	6	14	2	2	2

Only 6 patients out of 151 (4%) were found to have cystic glandular hyperplasia, and one additional patient had typical adenomatous hyperplasia. Normal proliferative changes were noted in 39%, while secretory changes were found in 37%. Other histological diagnoses were noted in 19%.

The population incidence of cystic glandular hyperplasia in the South-Eastern Region of Scotland in 1955 and 1964 by five-year age groups is shown in figure 1:



The peak incidence was seen in the 41 to 50 year age group and only a very small proportion of cases (1.9%) were seen in the 20 year and under group.

Table 4 summarises clinical findings in the 17 patients with adolescent cystic glandular hyperplasia who were diagnosed at the Royal Infirmary of Edinburgh during the years 1955 to 1964, and followed up in 1971. The mean age at menarche was 13.2 years and the mean age at the time of first curettage was 17.8 years. Of the ten girls who had an established normal menstrual pattern prior to the onset of symptoms leading to curettage only three have had persistently abnormal cycles since. By contrast, six out of the remaining seven girls have had abnormal cycles at all times. Several girls were given repeated hormone therapy with oestrogens, progestogens, androgens or a combination

TABLE 4:

Clinical findings and prognosis in adolescent girls with cystic glandular hyperplasia of the endometrium.

				Menstrual function							
Patient number	Age at menarche	Age at first curettage	Number of curettages	1-2 years following menarche	For at least 3 years following first curettage	Current	Dysmenorrhoea	Hormone therapy over a period of 3 years or more	Current parity	Number of years of involuntary primary infertility	Number of years of follow-up
1	11	19	7	N	A	A	No	Yes	0+0	15	16
2	12	20	2	N	N	N	Yes	No	4+2	0	16
3	11	19	2	A	A	A	No	Yes	0+0	13	13
4	14	20	2	N	A	A	No	Yes	2+1	0	15
5	14	17	1	N	N	N	No	No	4+0	0	14
6	14	16	4	N	A	N	No	No	1+0	3	14
7	12	18	1	N	N	N	No	No	3+0	0	14
8	15	19	2	N	A	N	No	No	0+0	No exposure	13
9	14	16	3	A	A	A	No	Yes	0+0	No exposure	12
10	14	19	5	A	A	N	No	No	2+1	0	12
11	17	20	1	A	A	A	No	No	2+0	3	11
12	13	18	1	N	A	N	No	No	2+0	0	10
13	12	15	1	A	N	N	Yes	No	0+0	7	10
14	12	16	2	N	A	A	Yes	Yes	1+0	0	9
15	13	16	2	A	A	A	No	Yes	1+1	0	8
16	14	18	1	A	A	A	No	Yes	3+0	0	7
17	13	17	1	N	N	N	No	No	0+0	No exposure	7

A = Abnormal N = Normal Parity is abbreviated as "viable + previable" pregnancies.

over a period of at least 3 years. It was surprising to find that seven of the girls had been treated with a course of 'oestrogen' alone on at least one occasion, usually without significant benefit. Only six had been specifically treated with progestogens alone or in combination although six others had tried the combined pill for contraception. One girl (no.4) had received a course of androstalone, a weak androgen.

In addition to the five patients with at least three years of involuntary primary infertility one further patient has had at least two years of secondary infertility. Thus 43% of the girls exposed to the risk of pregnancy have shown significant infertility. Only one patient (no.10) had had a normal full term pregnancy before curettage.

The clinical and histological findings at curettage are shown in table 5. At the initial curettage the utero-cervical canal length was greater in the group of patients with "marked" cystic glandular hyperplasia (mean 8.3 cms) than in those showing "mild" changes (mean 7.2 cms). The appearance of the curettings at the time of curettage was recorded as profuse in all patients who were found to have marked cystic glandular hyperplasia, while in all those showing mild changes the curettings were described as moderate or scanty. One or both ovaries were found to be palpably enlarged at the time of curettage in four patients. Two of these have since required laparotomies: one (no.7) for removal of a benign ovarian dermoid cyst and the other (no.10) for bilateral ovarian wedge resection of typical polycystic ovaries. Of the ten patients who required repeat curettage, six showed marked cystic glandular hyperplasia initially, and only three never showed more than mild changes. Patient no.1 showed markedly hyperplastic endometrium in all seven curettages.

In five patients the haemoglobin concentration at first curettage was less than 11.0 g percent, which suggests a significant amount of haemorrhage. Three patients required

TABLE 5:

Findings at the time of curettage in adolescent girls with cystic glandular hyperplasia of the endometrium

Patient number	Uterocervical canal length (cm).	Amount of curettings	Enlarged ovary	1st	2nd	3rd	4th	Haemoglobin level at curettage (g.per 100 ml.)
1	7	Profuse	———	CGH++	CGH++	CGH++	CGH++	14.1
2	9	Scanty	———	CGH	———	———	———	———
3	7	Mod.	———	CGH	CGH	———	———	11.4
4	7.5	Profuse	———	CGH++	CGH++	———	———	———
5	7	Scanty	Yes	CGH	———	———	———	13.3
6	7.5	Profuse	———	CGH++	Prol.	Sec.	Sec. and polyp.	11.3
7	6.5	Mod.	Yes	CGH	———	———	———	12.0
8	8.5	Profuse	———	CGH++	CGH	———	———	10.6
9	7.5	Mod.	———	CGH	CGH++	Prol.	———	7.4
10	9	Profuse	Yes	CGH++	Sec.	Prol.	CGH	12.9
11	7.5	Profuse	Yes	CGH++	———	———	———	———
12	11.5	Profuse	———	CGH++	———	———	———	12.9
13	7.5	Profuse	———	CGH++	———	———	———	8.6
14	7.5	Mod.	———	CGH	Prol.	———	———	11.7
15	7.5	Profuse	———	CGH++	Prol.	———	———	7.8
16	6.5	Scanty	———	CGH	———	———	———	9.2
17	6.5	Scanty	———	CGH	———	———	———	13.3

CGH++ = Marked cystic glandular hyperplasia
CGH = Mild cystic glandular hyperplasia
Prol. = proliferative
Sec. = Secretory

N.B: Case No 1 had 7 curettages
Case No 10 had 5 curettages

transfusion with two or more bottles of blood.

Table 6 summarises the difference in the clinical findings and prognosis between the group of girls who showed cystic glandular hyperplasia on at least one occasion and the group who never showed more than mild changes. There was a higher incidence of repeat curettage, primary infertility and menstrual abnormalities from the menarche until at least 3 years after curettage, and a smaller number of pregnancies, in the group showing marked hyperplasia. However, the other group showed a higher incidence of gynaecological abnormalities than expected in a normal population.

TABLE 6:

Comparison of clinical findings and prognosis in adolescent girls showing marked or mild changes of cystic glandular hyperplasia of the endometrium.

Number requiring curettage.	Mean number of curettages.	Mean number of pregnancies.	Number of cases of primary infertility	Abnormal menstrual cycles since menarche.	Abnormal cycles for at least 2 years.	Persistently abnormal cycles.	Mean haemoglobin at first curettage (g. per 100 ml.)	Repeated hormone therapy	Enlarged ovaries	Number undergoing laparotomy or laparoscopy
Group showing marked hyperplasia:										
7	2.8	1.6	4	5	9	5	10.8	4	2	2
(10)			(8)	(10)	(10)	(10)		(10)	(10)	(10)
Group showing mild hyperplasia:										
3	1.4	2.8	1	2	3	3	11.1	3	2	1
(7)			(6)	(7)	(7)	(7)		(7)	(7)	(7)

(total numbers in each group are shown in brackets).

Only one patient (no.10) was confirmed to have polycystic ovaries, but four others (no's 1, 9, 11 and 13) had several clinical features suggestive of the polycystic ovary syndrome or one of its variants. All five were overweight with persistently abnormal menstrual cycles and marked endometrial hyperplasia; four showed mild hirsutism; four have had repeated curettages: three out of four showed

significant primary infertility and two had palpably enlarged ovaries. Patient no.1 had normal sized, white, apparently "sclerocystic" ovaries seen at laparoscopy. Unfortunately, none of the modern endocrine investigations were available at the time to investigate this diagnosis more accurately.

Three patients had urinary total oestrogen measurements carried out (in the laboratory of Dr. J. B. Brown and R. J. Kellar) immediately following the curettage at which cystic glandular hyperplasia was originally diagnosed, and the results are illustrated in fig. 2. In no case was total oestrogen excretion elevated above 20 µg/24 hours during the few days after the curettage, in spite of the histological diagnosis of cystic glandular hyperplasia. One patient (no.6) was studied with urinary oestrogen estimations on three separate occasions over a period of 5 years. On two occasions she was clearly anovulatory while on the third occasion normal ovulation had occurred without assistance from ovulation - inducing drugs. This case illustrates the variable nature of the endocrine defect which occurs in many of these patients.

DISCUSSION:

Cystic glandular hyperplasia of the endometrium is a histological diagnosis which was first studied extensively by Schröder (1954), but many other groups of workers have also contributed to knowledge of the pathology, histopathology and variability of this condition. In most cases it is probably due to the prolonged or excessive and unopposed action of endogenous or exogenous oestrogens on the endometrium (Paschkiss and Rakoff 1950; Schröder 1954; Brown et al 1959).

Brown and colleagues (1959) carried out a very extensive and thorough study of the changes in urinary oestrogen excretion associated with different types of endometrial pathology, including cystic glandular hyperplasia. They found that cystic glandular hyperplasia occurred when urinary

total oestrogen excretion was constantly elevated above 30 µg/24 hours for several weeks or when oestrogen excretion rose to much higher levels (eg. 80-100 µg/24 hours) for shorter periods of time. These studies were mainly conducted on women with perimenopausal bleeding abnormalities. The limited oestrogen measurments conducted by Dr. J. B. Brown and his colleagues on the three girls reported in this study (fig.2) contrast markedly with their findings in perimenopausal women. In all three adolescents with cystic glandular hyperplasia the oestrogen excretion was low after curettage, in spite of prolonged bleeding before curettage. Brown et al (1959) carefully emphasised the dangers of trying to analyse urinary oestrogen data collected over short periods of time. However, the adolescent data did suggest that in their cases cystic glandular hyperplasia was probably associated with fluctuating rather than constant oestrogen levels but that much more detailed endocrine studies were required.

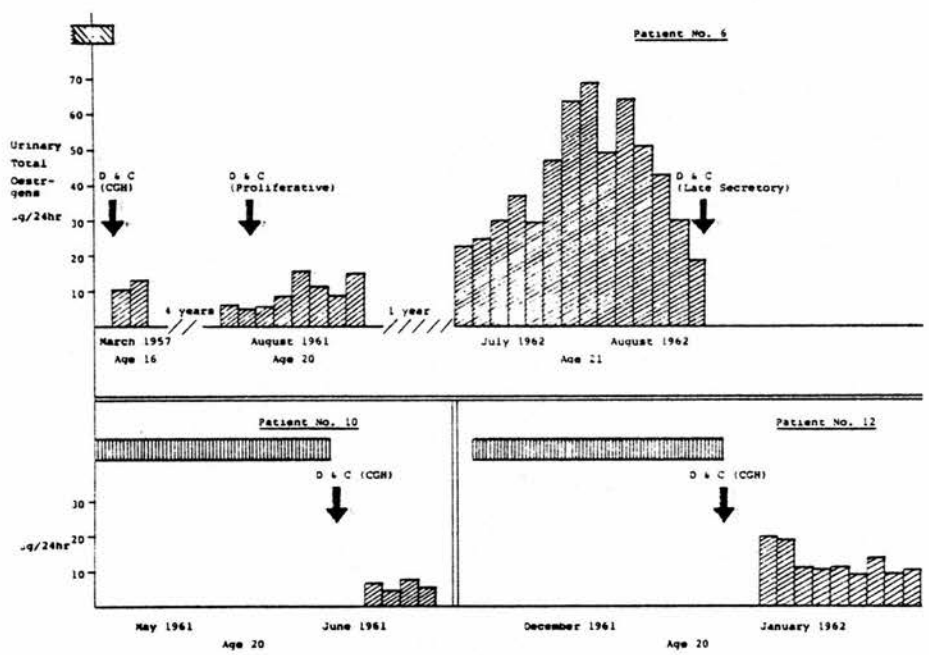


Figure 2. Urinary oestrogen excretion in 3 adolescent patients following D and C at which endometrial cystic glandular hyperplasia was diagnosed. Horizontal bars indicate bleeding.

It is well recognized that menstrual irregularities are common in adolescent girls (Arey, 1939; Dewhurst et al 1971) and are associated with a high incidence of anovulatory cycles.

Excessively heavy menstrual bleeding is much less common in adolescence than irregularities, and may occur from any

type of endometrium (Southam 1959).

Dysfunctional uterine bleeding and cystic glandular hyperplasia are much less common in adolescence than near the menopause. Sutherland (1953) found that only 3.9% out of a group of 1000 successive patients with abnormal uterine bleeding requiring curettage were aged 20 or under, and out of 265 cases of cystic glandular hyperplasia only 6(2.3%) were in this age group. In this study only 6 out of 322 cases (1.9%) of cystic glandular hyperplasia seen in 1955 were aged 20 or under. These two reports agree closely with the incidence of 2.7% calculated from the extensive data of Schröder (1954) for German women over a 40 year period. The age distribution of cystic glandular hyperplasia shown in the present study (fig.1) is also in agreement with the data of Schröder (1954), except that he showed a small peak in late adolescence. However, by combining fig.1 and table 2 it does appear that there may be a small peak incidence around the age of 17, but it would require very many cases to confirm this.

Out of 151 cases of abnormal uterine bleeding in adolescents in the South-Eastern Region of Scotland in 1955 only six (four per cent) showed cystic glandular hyperplasia. This finding is in direct contrast to the reports of many previous authors who found endometrial hyperplasia in almost all adolescent girls with dysfunctional uterine bleeding (Jeffcoate, 1937). However, Sutherland (1949) found an incidence of cystic glandular hyperplasia of only 15.5 per cent in 200 girls with dysfunctional bleeding. Part of this difference in incidence may be explained by the variation in definitions of "dysfunctional" or "functional" uterine bleeding, by the histological interpretation of the curettings, and by the reluctance or otherwise of the gynaecologist to carry out a curettage in an adolescent girl.

During the ten-year period 1955-64 only 53 cases of adolescent cystic glandular hyperplasia were diagnosed at the gynaecological pathology laboratory for the South-Eastern Region of Scotland, covering a population of about

1,100,000 people. The incidence of adolescent cystic glandular hyperplasia in this region can therefore be estimated as approximately one case per 208,000 of the population per year.

The number of adolescent girls (aged 10-19) in the Region was approximately 78,000 (Population census 1961), permitting the calculation that at that date approximately 1 adolescent per 14,700 was presenting with cystic glandular hyperplasia per year. Overall, a girl in this Region would have had approximately one chance in 1470 of curettage for cystic glandular hyperplasia during adolescence. It seems reasonable to assume that this calculation still gives a fair estimate of the incidence of this uncommon condition in the community. The present series includes all the cases having a curettage in a geographically defined area and may reflect the true incidence in the population more accurately than selected populations referred to a special clinic (Southam 1960).

This study indicates that there is a high incidence of persisting reproductive pathology in adolescents with a proven diagnosis of cystic glandular hyperplasia of the endometrium. This risk may also apply to certain other persistent or recurrent menstrual disorders in adolescence.

In a large group of adolescent girls referred to a specialist endocrine clinic after recurrent menstrual abnormalities of all types, and followed over many years, similar findings to those in the present series have been reported with regard to continuing menstrual abnormalities, anaemia, blood transfusion and probable polycystic ovarian disease (Southam 1960; Southam and Richart 1966). In the earlier series (Southam 1960) nearly 50 per cent of the cases showed hyperplastic endometrium with a lower incidence of repeated curettage than the present series (37.3 per cent against 56 per cent) but a higher incidence of laparotomy for ovarian cysts or polycystic ovarian disease (29.2 per cent compared with 11 per cent). Their series showed a high incidence of infertility; 75 per cent of those married women

with continuing heavy menstrual bleeding had persistent primary infertility, and 20 per cent of those who regained normal cycles still showed primary infertility. However, patients with oligomenorrhoea alone appeared to have relatively normal fertility in spite of persistent irregularities. Chamlian and Taylor (1970) have reported a rather similar incidence of persistent gynaecological abnormalities in a group of 97 women aged less than 35 years with adenomatous endometrial hyperplasia, except that their series includes a 14 per cent incidence of carcinoma of the endometrium occurring within one to 14 years of the diagnosis of endometrial hyperplasia. Southam and Richart 1966) reported four cases of carcinoma at ages of 20 to 33 years following persistent adenomatous hyperplasia (two of which also showed cystic glandular hyperplasia) out of a total of 291 patients with excessive adolescent dysfunctional bleeding. No cases of endometrial carcinoma occurred in the present small series, but patient No.1 must be a high risk and has been kept under close review and treatment with progestogens.

The small numbers in this series suggest that there is a trend for the prognosis to be less favourable in the patients who show the most marked changes of cystic glandular hyperplasia on at least one occasion during adolescence. It seems probable that the endocrine conditions necessary for the development of marked cystic glandular hyperplasia in adolescence are generally produced by a more persistent defect of the hypothalamo-pituitary-ovarian axis than in those patients who never show more than mild changes.

Chapter 7

PLASMA CONCENTRATIONS OF PITUITARY AND OVARIAN HORMONES OVER 3 CYCLES IN 4 ADOLESCENTS WITH DYSFUNCTIONAL UTERINE BLEEDING:

INTRODUCTION:

Following the demonstration of a high incidence of subsequent clinical problems in adolescent girls with dysfunctional uterine bleeding associated with endometrial cystic glandular hyperplasia (Chapter 6) and the evidence that this is probably the severe end of a spectrum of anovulatory dysfunctional uterine bleeding (Brown et al 1959) it was decided to investigate the mechanism of this anovulatory phenomenon in more detail. It was known that cystic glandular hyperplasia is almost always associated with anovulation (Schröder 1954) and relatively high oestrogen levels (Brown et al 1959; Brown and Matthew 1962). However, different patterns of urinary oestrogen excretion (reflecting different patterns of ovarian follicle development) had been described varying from high intermittent levels to moderate, more or less constant levels. Virtually no information was available on plasma levels of oestrogens or the simultaneous changes in pituitary gonadotrophins and the 'feedback' relationships in patients with this type of disorder, although abnormal hormonal feedback mechanisms at a hypothalamic-pituitary level had been suspected in young women with amenorrhoea and oligomenorrhoea (Dignam et al 1969; Newton 1972; Boon et al 1972).

In this study the daily concentration in plasma of luteinising hormone (LH) and follicle stimulating hormone (FSH) and the daily excretion in urine of 'total' oestrogen and pregnanediol were measured over 3 month periods in 4 adolescent girls with histories of persistent dysfunctional uterine bleeding associated with cystic glandular hyperplasia of the endometrium. The functional integrity of the hypothalamo-pituitary-ovarian axis was tested further by the administration of the anti-oestrogen clomiphene.

This study was planned and analysed by Dr. D. T. Baird

and myself and organised and executed predominantly by myself. Dr. L. Wide and Dr. E. A. Michie gave valuable assistance in planning the hormone assays and arranged for these assays to be carried out in their laboratories.

MATERIALS AND METHODS:

Urinary 'total oestrogens' were measured by fluorimetry using a semi-automatic extractor and an Aminco-Bowman Spectrophotofluorimeter (Brown et al 1968). Urinary pregnanediol was measured by the method of Klopper, Michie and Brown (1955) incorporating an Allen correction factor. Plasma follicle stimulating hormone and luteinizing hormone levels were measured by a radioimmunosorbent technique (Wide and Porath 1966; Wide 1969), FSH was measured by utilizing highly purified human pituitary FSH (Wide 1969) labelled with ^{125}I and guinea-pig anti-human pituitary FSH. The results were expressed in ng/ml using the FSH preparation which had a biological activity of 12,000 IU (2nd IRP-HMG) as a standard. One ng of the FSH preparation was equivalent to 369 ng and 0.14 ng of LER-907 in the FSH-immunoassay and LH-immunoassay respectively. LH was measured by utilizing highly purified human pituitary LH (Roos 1968) and rabbit anti-human pituitary LH. The results were expressed in ng/ml using the LH preparation which had a biological activity of 14,000 IU (2nd IRP-HMG) per mg as a standard. One ng of the LH preparation was equivalent to 84 ng and 0.096 ng of LER-907 in the LH-immunoassay and FSH-immunoassay respectively. All assays were made on 0.1 ml plasma in duplicate with the standard diluted in a serum with a very low gonadotrophin concentration. The limit of sensitivity was 0.2 - 0.3 ng/ml and the coefficient of variation for a mean of duplicate falling between 20% and 80% inhibition was about 5% for both FSH and LH assays. Plasma oestradiol and oestrone were assayed by a complex but sensitive double isotope method using S^{35}-pipsyl chloride (Baird 1968 See Chapter 2).

Each patient was studied over a three month period

with daily blood samples collected between 7 and 9 a.m. and continuous, accurate 24 hour urine collections. Informed consent was obtained from each patient, to whom the nature of the study was explained personally. An information sheet was given to each subject. Blood samples (5-10 mls) were collected in the patient's home within one hour of rising and placed in a heparinized container on ice. Plasma was separated within one to two hours of collection following centrifugation at 2,000 rpm for 20 minutes at 4°C, and was stored at -20°C until assayed. Urine samples were usually assayed on the day that each collection was completed or were stored at 4°C over the weekend.

Towards the end of the second month of the study the hypothalamic-pituitary axis was stimulated by giving clomiphene in a dose of 50 mg per day by mouth for 5 days, from day 5-9 of the cycle. Collections of blood and urine were continued for a further four weeks after the administration of the clomiphene (Figure 1-4).

Endometrial biopsies were collected without anaesthetic using a Novak curettage in the outpatient clinic at the start of each investigation, and were repeated in three of the patients at a later stage (Table 1). All patients were given iron supplements (ferrous sulphate 200 mg three times daily) for treatment of the heavy vaginal bleeding as well as for replacement of iron loss due to daily venepunctures. Haemoglobin concentrations remained within the normal range throughout the study except in one instance after one month of sampling (Table 2).

TABLE 1:

Endometrial biopsies in 4 adolescent girls with a history of anovulatory dysfunctional uterine bleeding and endometrial cystic glandular hyperplasia.

	Initial visit		Subsequent visit	
	menstrual cycle day	Biopsy features	menstrual cycle day	
1. J.M.	18	early to mid-proliferative	1	disintegrating secretory
2. L.R.	94	atrophic	40	proliferative; no hyperplasia
3. I.H.	9	mid-proliferative	-	-
4. L.S.	24	mixed picture: multi-layening of glandular epithelial cells with some basal glycogen vacuoles.	4	early proliferative

TABLE 2:

Haemoglobin levels (g/100 ml) during daily blood sampling of 4 adolescent girls with anovulatory dysfunctional uterine bleeding.

		Initial visit	Months after start of daily sampling 1	2	3
1.	J.M.	13.1	10.7	12.2	12.7
2.	L.R.	13.4	13.1	14.2	12.6
3.	I.H.	16.2	14.2	12.7	-
4.	L.S.	14.6	12.0	12.8	12.1

Patient No.1 (J.M.)

Menarche occurred at 14 years with a six week cycle and a light menstrual flow lasting two to three days. Menses became more regular until 19 years when suddenly, very heavy bleeding occurred three weeks after a normal period and lasted six days. Heavy and persistent bleeding occurred in the following month and curettage showed marked endometrial cystic glandular hyperplasia. Her cycle has remained irregular and sometimes very heavy. She was 20 years during this study.

Patient No.2 (L.R.)

Menarche occurred at 12 years with a regular 28 day cycle and seven day menstrual flow for two years. She then had three months amenorrhoea followed by continuous, often very heavy, vaginal bleeding for two months. Curettage showed classical changes of cystic glandular hyperplasia. Haemoglobin at this time was 8.8 g per 100 ml but returned to normal following therapy with intravenous iron. Two very heavy periods then occurred at eight and six weeks intervals, and at the start of this study at 15 years she had had amenorrhoea for three months. Since the study she has had several periods of amenorrhoea followed by very heavy bleeding, and is now symptomatically controlled on norethisterone 5 mg three times daily from day 18 to 23 of each cycle.

Patient No.3 (I.H.)

Menarche occurred at 10 years with regular 28 to 30 day cycle and five day menstrual flow for three years. At 13 years she had persistent vaginal bleeding for four months. Curettage showed classical changes of cystic glandular hyperplasia. Haemoglobin was 6.4 g per cent and a blood transfusion was given. Symptoms were controlled with a cyclical oestrogen-progestogen combination for 18 months, but menses became very heavy, although regular, when hormone therapy was stopped. Bleeding became increasingly irregular and heavy, but at 17 years after six months of marriage she became pregnant without artificial induction of ovulation.

She later aborted triplets at 24 weeks gestation. Following this she had several episodes of irregular, heavy and prolonged bleeding. Further curettage showed only fragments of menstrual endometrium without secretory changes. Irregular, heavy periods continued until the onset of this investigation at 19 years. Mild hirsutism developed after the age of 15 years.

Patient No.4 (L.S.)

Menarche occurred at 13 years with a regular 28 day cycle and light seven day flow. The length of flow increased markedly from 17 years onwards for up to four weeks at a time and often became very heavy. Curettage at 17 years showed marked endometrial cystic glandular hyperplasia. The subsequent menstrual pattern has been persistently irregular and heavy, with temporary improvements while taking norethisterone 5 mg twice daily or a combined oestrogen-progestogen pill. Laparoscopy has been carried out since this study and bilateral slightly enlarged sclerocystic ovaries were found. She was aged 19 years during this study.

All four girls had normal development of secondary sex characteristics, and were of normal stature and body build. They have all shown persistent menstrual abnormalities over 12 months since the conclusion of this investigation.

RESULTS:

The daily excretion of total oestrogen and pregnanediol in the urine and the concentration in peripheral venous plasma of LH and FSH are illustrated in Figure 1 to 4.

The hormonal patterns demonstrated by Patient No. 1 (J.M., Figure 1) were compatible with two ovulatory cycles. These were characterized by a progressive rise in oestrogen excretion indicating follicular development. Around the time of maximum oestrogen excretion there was a sharp increase in the concentration of LH in plasma followed by a fall in oestrogen excretion. The rise in oestrogen and pregnanediol

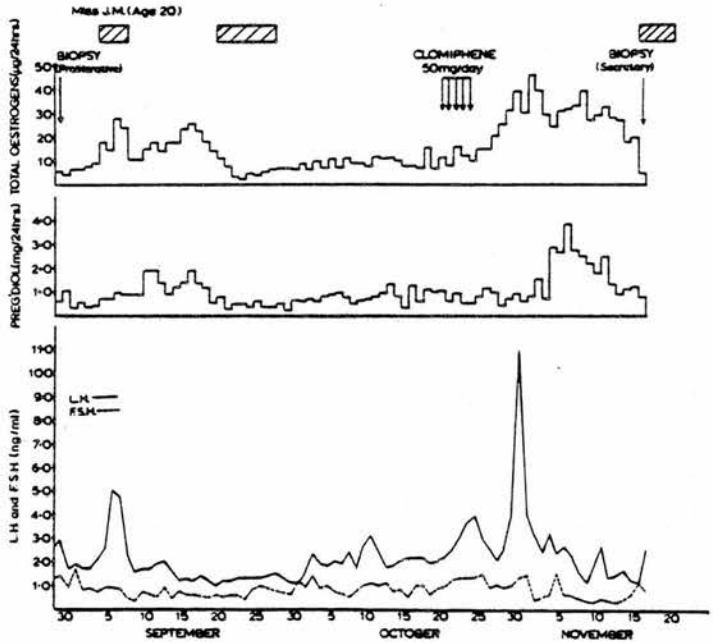


Figure 1. (Miss J.M.). The excretion of total oestrogen (µg/24 hr) and pregnanediol (mg/24 hr) in the urine, and concentration in plasma of FSH and LH (ng/ml) in Patient no. 1. This demonstrates a spontaneous cycle of ovulatory pattern and a normal cycle in response to clomiphene. ▨ = uterine bleeding.

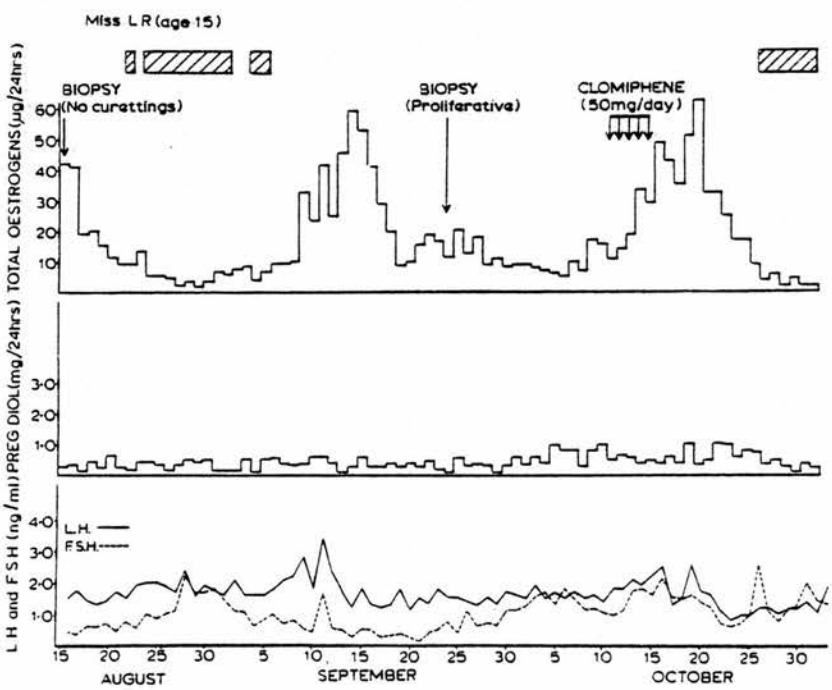


Figure 2. Patient No 2 (Miss L.R.). Legend as for figure 1. This shows no evidence of a midcycle LH surge or ovulation even in response to clomiphene.

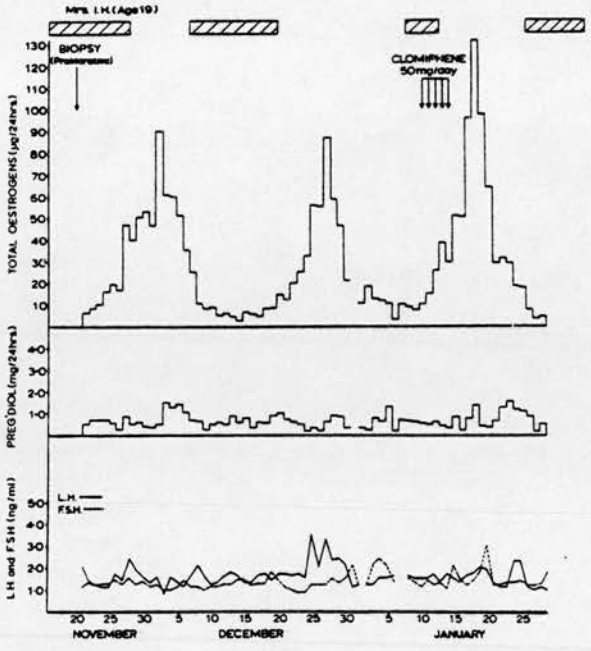


Figure 3. Patient No 3 (Mrs I.H.). Legend as for figure 1. This shows no evidence of a midcycle LH surge or ovulation, although excessively high estrogen excretion peaks have occurred on three occasions.

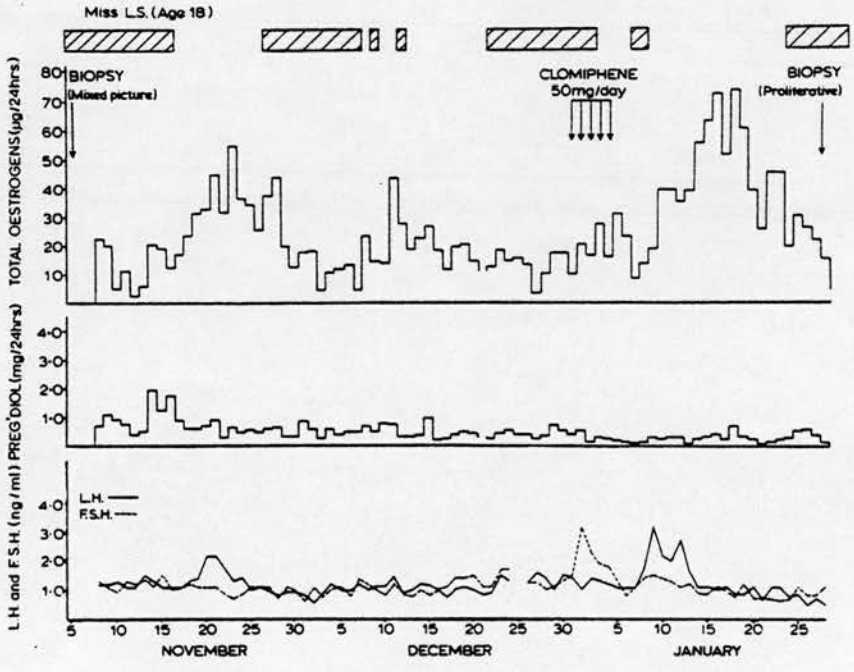


Figure 4. Patient No 4 (Miss L.S.). Legend as for figure 1. There is no evidence of a midcycle LH surge or ovulation even after clomiphene. Estrogen excretion is high and irregular and is associated with heavy, irregular bleeding.

excretion following the LH peak reflects the production of oestradiol and progesterone from the corpus luteum. Menstruation with average blood loss occurred following regression of the corpus luteum as indicated by the falling excretion of oestrogen and pregnanediol. During the first spontaneous cycle heavy vaginal bleeding suddenly occurred for four days in the follicular phase of the cycle at a time when the oestrogen levels were rising. Following a prolonged period of low oestrogen excretion and a small irregular rise in LH, clomiphene was given. This was associated with an immediate rise in LH and FSH levels with a subsequent ovulatory cycle confirmed by pregnanediol levels and an endometrial biopsy.

In contrast, the hormonal patterns throughout all cycles in the remaining three patients were distinctly abnormal. The failure of pregnanediol excretion to rise consistently above 1.5 mg per day and the lack of mid-cycle peaks of LH in plasma both indicate a failure of ovulation.

Patient No.2 (L.R., Figure 2) showed two spontaneous high oestrogen peaks neither of which were associated with ovulation. The second spontaneous cycle started at about 28th August with a rise in concentration of FSH following the onset of heavy prolonged menstrual bleeding. Twelve days after this the oestrogen excretion began to rise rapidly, the two minor peaks on 9th and 11th September being associated with small peaks of LH. Follicular activity continued as indicated by a further rise in excretion of oestrogen. The main oestrogen peak on 14th September was not followed by a pre-ovulatory discharge of LH. Urinary pregnanediol did not rise above 1 mg per day and an endometrial biopsy showed proliferative endometrium. Menstrual bleeding did not occur even after the sharp fall in oestrogens. After three weeks of low excretion of oestrogen a five day course of clomiphene was started. A very rapid rise in oestrogen excretion

occurred with peaks on 16th October and 20th October. Although there was a small rise in the concentration of LH, there was no pre-ovulatory surge of LH or subsequent rise in the excretion of pregnanediol. Heavy menstrual bleeding began on 27th October (day 18 after starting clomiphene).

Patient No.3 (I.H., Figure 3) showed a similar pattern to Patient No.2, but with even higher oestrogen peaks. Small rises in the concentration of LH were associated with the rises in excretion of oestrogen but there were no pre-ovulatory discharges of LH. The very high levels of oestrogen following clomiphene were associated with very little change in levels of gonadotrophin apart from a small discharge of FSH. Heavy prolonged menstrual bleeding occurred following the first cycle and was even heavier after the clomiphene cycle. The second spontaneous cycle, which showed a smaller total excretion of oestrogens, was only associated with moderate bleeding for four days. The excretion of pregnanediol fluctuated slightly but was never above 1.5 mg per 24 hours.

Patient No.4 (L.S., Figure 4) showed the same type of pattern as Patients No.2 and No.3, although the base-line excretion of oestrogen was higher. The spontaneous oestrogen peak in November was associated with a small LH peak of the same type as seen in the other two patients. During clomiphene there was a rise in FSH concentration followed by a marked increase in oestrogen excretion. There was a small increase in the concentration of LH followed by a further increase in excretion of oestrogen. Urinary pregnanediol remained very low throughout the investigation. Heavy irregular vaginal bleeding occurred throughout the study. The initial endometrial biopsy on 5th November showed some cells with basal vacuolation (positive for glycogen staining) compatible with a small increase in progesterone production as may occur with luteinization of an unruptured follicle. There was also multilayering

of glandular epithelial cells suggestive of increased unopposed oestrogen secretion. The biopsy following clomiphene showed early proliferative endometrium.

The significance of the rise in plasma FSH and LH levels during clomiphene administration was assessed by comparing the FSH and LH levels for five days before and for the five days during clomiphene administration by Student t test (Table 3). In three of the four subjects there was a statistically significant rise in the concentration of FSH during clomiphene therapy.

Oestrone and oestradiol-17β were measured in samples of peripheral plasma (14-20 ml) obtained by pooling collections over five to six consecutive mornings at two stages during the first cycle in each patient (Table 4). The absolute values and the ratio of oestrone to oestradiol were similar to those of normal women in the same age group during the follicular phase of the menstrual cycle.

DISCUSSION:

Three of the four patients failed to show evidence of ovulation throughout the duration of the study. Follicular development, as indicated by progressive rise in the excretion of oestrogen, occurred spontaneously and following clomiphene. However, although there was sometimes a small rise in the concentration of LH in plasma, the rising oestrogen levels were not followed by a massive surge of LH such as occurs at midcycle in normal menstrual cycles (Henzl and Segre 1970; Ross et al 1970), except in patient No.1 who demonstrated a defective spontaneous surge. In the other 3 patients, this indicates a major failure of the positive feedback mechanism by which oestrogen evokes an ovulatory discharge of LH from the pituitary (Vande Wiele et al 1970; Nillius and Wide 1971). The continued rise in oestrogen excretion may represent the stimulation of further follicular secretion of oestrogen by the small rise in basal levels of LH, or the failure of inhibition of ovarian oestradiol secretion in the absence of the massive normal midcycle LH surge.

TABLE 3:

Concentration of FSH and LH in the plasma of four patients with adolescent dysfunctional uterine bleeding. The significance of the difference in values between the five days prior to and the five days during clomiphene administration (50 mg daily) was assessed by Student 't' test.

		Mean plasma gonadotrophin concentration before clomiphene ng/ml (± SEM)	Mean plasma gonadotrophin concentration during clomiphene ng/ml (± SEM)	
Patient No.1	FSH	0.77 ± 0.09	1.21 ± 0.07	<0.02
Miss J.M.	LH	2.04 ± 0.04	3.04 ± 0.32	<0.05
Patient No.2	FSH	1.15 ± 0.09	1.70 ± 0.17	<0.05
Miss L.R.	LH	1.60 ± 0.07	2.12 ± 0.13	<0.025
Patient No.3	FSH	1.46 ± 0.17	1.40 ± 0.19	N.S.
Miss I.H.	LH	1.54 ± 0.02	1.54 ± 0.07	N.S.
Patient No.4	FSH	1.20 ± 0.06	2.14 ± 0.29	<0.05
Miss L.S.	LH	1.36 ± 0.10	1.26 ± 0.07	N.S.

N.S. = not statistically significant

TABLE 4:

Concentration of oestrone and oestradiol-17β in pooled samples of peripheral venous plasma in Patients No. 1 to 4.

Patient No.	Date	Oestrone pg/ml	Oestradiol-17β pg/ml	Ratio of Oestrone to oestradiol
1	28 Aug.-2 Sept	100	194	0.52
	3-7 Sept.	125	138	0.91
2	16-20 Aug.	128	222	0.58
	21-25 Aug.	71	162	0.44
3	21-25 Nov.	42	82	0.51
	26-30 Nov.	108	287	0.38
4	8-12 Nov.	167	345	0.48
	19-24 Nov.	146	362	0.40

Administration of clomiphene in all the patients was followed by a marked increase in excretion of oestrogen. Clomiphene is thought to work mainly by an antioestrogenic action at the hypothalamic level (Newton and Dixon 1971) and normally causes increased secretion of pituitary FSH and LH which stimulate ovarian follicle development. In Patients No.2 and No.3 follicular development had possibly already been initiated by the spontaneous release of FSH a few days prior to the administration of clomiphene. The rise in plasma concentration of FSH during the treatment with clomiphene in Patients No.1, No.2 and No.4 suggests that the negative feedback effect of oestrogen may be operating in these patients.

The excretion of oestrogen in Patient No.3 was high enough to suggest the development of more than one follicle. Eighteen months previously evidence of multiple ovulation had been obtained when she aborted triplets. Dysfunctional uterine bleeding in the perimenopausal age group is frequently associated with multiple follicular cysts in the ovaries (Schröder 1954) which are functionally active (See Chapter 5). In the absence of any obvious abnormality in the concentration of gonadotrophins during the follicular phase, it is possible that multiple follicular development may be due to a failure of the intra ovarian mechanism which normally inhibits development of all but the pre-ovulatory follicle (Vande Wiele et al 1970). However, minor abnormalities in the pattern of FSH and LH secretion could be responsible.

Cystic glandular hyperplasia of the endometrium is rare in adolescent girls in the geographical region involved in this study but is associated with a very high incidence of persistent menstrual disturbance, infertility, ovarian cysts and variants of the polycystic ovary syndrome (Chapter 6). None of the girls in this study were found to have recurrent changes of cystic glandular hyperplasia during the period of investigation, but this may have been related to the infrequency with which endometrial

biopsies could be carried out. It is often difficult to correlate endometrial patterns with absolute endocrine changes and patients with a history of anovulatory dysfunctional bleeding show proliferative changes in one cycle, cystic glandular hyperplasia in another, and occasionally even ovulation with secretory endometrium (Brown, Kellar and Matthew 1959). This suggests that the basic defect, presumably in the hypothalamic control mechanisms, may vary from cycle to cycle. The significance of the finding of sclerocystic ovaries in Patient No.4 in relation to the endocrine findings is uncertain, although this appearance may occur in any state of persistent anovulation.

The concentrations of oestrone and oestradiol and the oestrone to oestradiol ration in peripheral venous plasma were within the range found in normal women during the menstrual cycle (Baird and Guevara 1969). This would suggest that the bulk of circulating oestrogen is derived from secreted oestradiol rather than by peripheral aromatization of androstenedione as in postmenopausal women (MacDonald, Rombaut and Siitteri 1967) and in women with polycystic ovarian disease in whom cystic glandular hyperplasia of the endometrium may develop (MacDonald, Grodin and Siitteri 1971). Anovulatory dysfunctional uterine bleeding of this adolescent type appears to differ from patients with typical polycystic ovarian disease although there may be some overlap. Patients with typical polycystic ovaries usually have a markedly elevated plasma LH to FSH ratio (Yen and Vela and Rankin 1970) and an elevated plasma oestrone to oestradiol ratio (MacDonald, Grodin and Siitteri 1969) whereas the women in this study had normal ratios of all four hormones. Women with adolescent dysfunctional bleeding differ from women with the commoner "hypothalamic" type of anovulation with oligomenorrhoea or amenorrhoea in that these women typically have low plasma levels of FSH and LH and a defect of the negative oestrogen feedback mechanism (Jacobs 1976).

In a few cases there may also be a defect of the positive feedback mechanism but this is unusual.

This investigation has demonstrated a major defect in the positive oestrogen feedback mechanism which clearly requires more precise definition (See Chapter 8). Additional information about the pathophysiological mechanisms of adolescent dysfunctional uterine bleeding could be provided by serial ultrasound studies of follicle growth compared with circulating endocrine patterns, as well as study of local changes in intra ovarian regulatory peptides.

Chapter 8

DYNAMIC REPRODUCTIVE ENDOCRINE STUDIES IN YOUNG WOMEN WITH ANOVULATORY DYSFUNCTIONAL UTERINE BLEEDING:

INTRODUCTION:

The previous study of daily measurements of plasma gonadotrophins and urinary oestrogen and pregnanediol excretion demonstrated a complete absence of the mid-cycle LH peak in three adolescent girls with persistent anovulation and a history of cystic glandular hyperplasia of the endometrium (Chapter 7). The fourth individual with a previous history of cystic glandular hyperplasia demonstrated a small midcycle LH peak and reduced progesterone levels following spontaneous ovulation.

The present study was undertaken to test the hypothesis that the failure to ovulate may be due to a failure of the positive feedback mechanism whereby oestrogen evokes an LH discharge from the pituitary at midcycle. I was jointly involved in the planning, execution and analysis of the study with Dr. Van Look and Dr. Baird, and Dr. Van Look was responsible for performing the plasma oestrogen assays and for most of the statistical analyses.

MATERIALS AND METHODS:

Nine young women (designated aDUB 01-09) with ages ranging from 16-27 yr, were recruited from patients attending the Gynaecology Department, Royal Infirmary, Edinburgh, because of a history of DUB since adolescence, the latter being defined as the first 10 yr after menarche (Southam and Richart 1966). In all nine patients, irregular and heavy vaginal bleeding had been present for at least 2 yr (range, 2-8 yr) before the date of entry to the study and in six of them, DUB had started soon after menarche. One patient (DUB 02) had previously aborted triplets and had had one full term pregnancy. She was also studied in the previous investigation (Chapter 7, subject I.H.). The remaining two married

women were nonparous. Before entering the study, all of the subjects had undergone at least one uterine curettage in order to control the excessive bleeding, which in four of the patients was associated with cystic glandular hyperplasia of the endometrium. In the other five subjects, a proliferative endometrium was found.

Six regularly menstruating women (designated Con 01-06), aged 23-45 yr, served as controls. Control subjects were of proven fertility (parity range, 2-5), had regular menstrual cycles of 26-30 days' duration, and were in good health.

Design of study

Basal measurements and dynamic tests. Menstrual records were kept by all participants. Spontaneous ovarian activity was monitored throughout the entire duration of the study (3-5 months) by serial measurements of total oestrogen and pregnanediol excretion in 24-hour urine samples collected three times a week on Sundays, Tuesdays, and Thursdays. These measurements enabled us to determine the most appropriate time for performing the dynamic tests (see below) which were started during the early follicular phase of the cycle, i.e. when urinary total oestrogen and pregnanediol excretion were less than 12 μg and 1 mg/24 hour, respectively. The general layout of the study is illustrated in Figure 1.

Oestrogen provocation test. To assess hypothalamic-pituitary sensitivity to oestrogen plasma levels of FSH, LH, 17β-oestradiol, and ethinyl oestradiol were estimated daily before (days 1-4), during (days 5-7), and after (days 8-10) oral administration of ethinyl oestradiol (4x50 μg/day for 3 days).

Gonadotrophin-releasing hormone (GnRH) test. Pituitary sensitivity to GnRH was assessed by measuring plasma gonadotrophin levels before and after the rapid intravenous injection of 50 μg synthetic GnRH (Hoechst Pharmaceuticals). Blood samples were collected daily for 6 consecutive days during the early follicular phase of

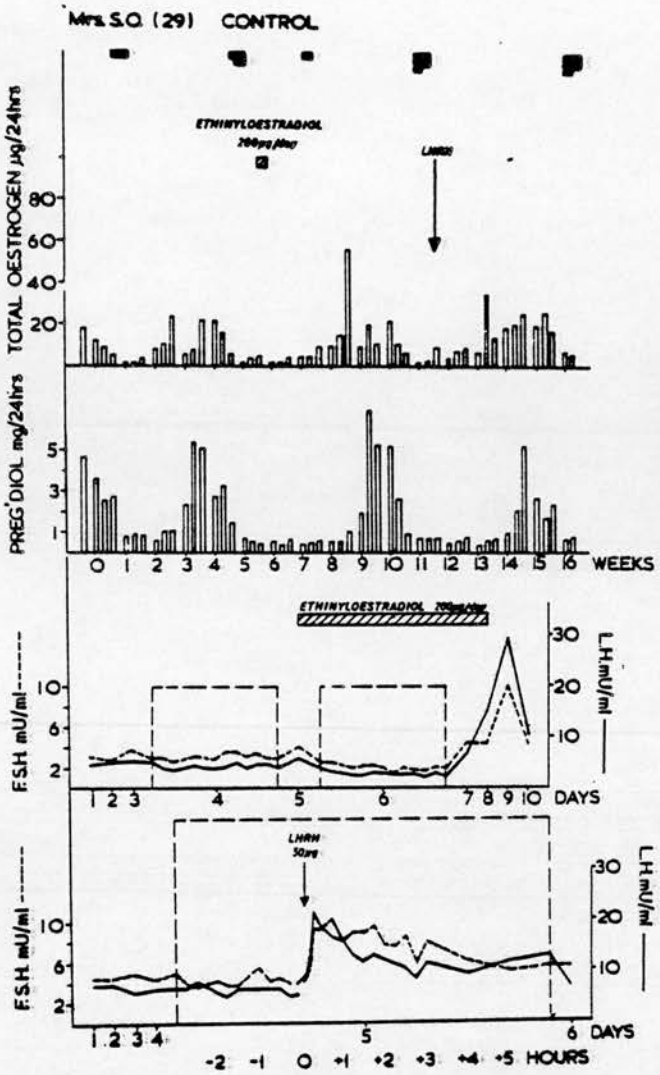


Figure 1:
Profile of urinary total oestrogen and pregnanediol excretion in a control patient during the 16 weeks of study. The format of the oestrogen provocation and gonadotrophin releasing hormone (LHRH) tests is shown in the lower two panels.

the cycle. On the morning of day 5, the GnRH was injected at time 0 and blood samples were collected at 15-min intervals from -180 to -30 min, at 5-min intervals from -30 to +30 min, at 15-min intervals from +30 to +180 min, and hourly thereafter until +360 min. All samples were assayed for FSH and LH, and hourly samples also were assayed for 17β-oestradiol. All blood samples were centrifuged at 2,000 rpm for 20-mins, and plasma stored at -20°C until assayed.

Hormone analyses

Urinary excretion of total oestrogen was measured fluorimetrically (Brown et al 1968) and urinary pregnanediol was measured by a gas-liquid chromatographic procedure (Chamberlain and Contractor 1968).

Plasma gonadotrophins were measured by specific double antibody radioimmunoassay (Hunter et al 1974). Preparations 68/39 (assuming a potency of 32.8 U/amp), both from the National Institute of Biological Standards and Control, were used as standard in FSH and LH assay, respectively. Concentrations of both gonadotrophins are expressed in milliunits per ml plasma where 1 mU FSH = 44.6 ng LER 907, and 1 mU LH = 11.6 ng LER 907. Within-assay precision (expressed as coefficient of variation) was 11.8% for FSH and 15.4% for LH. All samples from one subject were measured in duplicate in the same assay.

17β-oestradiol and ethinyl oestradiol concentrations in duplicate aliquots of plasma were estimated by using established, specific radioimmunoassays (Van Look et al 1977). Antisera were provided by Dr. H. Lindner, Dr. P Dean and Dr. R. Nieuweboer and exhibited no significant cross-reactions with relevant steroids. The assay procedures were virtually identical and involved diethyl-ether extraction followed by direct assay of the dried extracts without preparatory chromatography. Sensitivity of the method, defined as the amount of unlabelled 17β-or ethinyl-oestradiol required to displace 10% of the traces bound in buffer control tubes, was 5.0 pg ± 1.0 (S.D.) for 17β-oestradiol and 8.5 pg ± 2.5 for ethinyl oestradiol. Within-assay and between-assay precisions were 6.1 to 13.3% and 9.3% respectively for 17β oestradiol and 8.8% respectively for ethinyl oestradiol.

Statistical analyses

Results are given as arithmetic means ± SEM, unless indicated otherwise. Differences between mean values were analysed for statistical significance using Student t test or paired t test and were considered significant if P was less than 0.05.

RESULTS:

Urinary measurements

Normal women. The pattern of urinary total oestrogen and pregnanediol excretion in all six control women confirmed

the presence of normal follicular development and ovulation. The mean length of the first (control) and third (post - GnRH) cycle was 29.0 ± 0.4 and 32.0 ± 3.0 days, respectively ($P>0.05$). In contrast, the second cycle (i.e. the cycle after the oestrogen provocation test) was significantly longer (39.5 ± 3.7 days, $P<0.05$). The profiles of urinary total oestrogen and pregnanediol excretion during this cycle indicated that the delay in the onset of menstruation could be attributed to a prolongation of the follicular phase (from 16.3 ± 1.5 to 27.0 ± 3.5 days, $P<0.05$) and the development of a new crop of follicles. There were no significant differences between the three cycles in either the height of the total oestrogen peak at midcycle (30.7 ± 3.1, 45.7 ± 6.7, and 40.1 ± 4.3 µg/24 hour for first, second, and third cycles, respectively), the length of the luteal phase (13.0 ± 1.1, 12.5 ± 1.1, and 14.5 ± 1.2 days), or the maximal luteal pregnanediol excretion (4.1 ± 0.7, 4.3 ± 0.9, and 4.1 ± 0.3 mg/24 hour).

Women with DUB. Although the profiles of urinary oestrogen excretion were consistent with co-ordinated follicular growth in all nine women with DUB, seven patients consistently failed to ovulate during the study period, as evidenced by urinary pregnanediol excretion which remained below 1.5 mg/24 hour. Two representative examples are illustrated in Fig.2. The midcycle oestrogen peak in the remaining two patients (DUB 08 and 09) was followed by an increase in urinary excretion of pregnanediol, but the rise was small and short-lived and menstrual bleeding started prematurely 9 or 10 days later. Both patients, therefore, were considered to represent cases of defective corpus luteum function and their results were excluded from further analysis.

As anovulatory cycles were not always followed by vaginal bleeding and, conversely, menstrual bleeding could be present despite rising oestrogen levels (Fig.2), cycle length, as judged from the menstrual record charts of the patients, was highly variable and no valid comparison with the control group could be made on this basis. The profiles of urinary

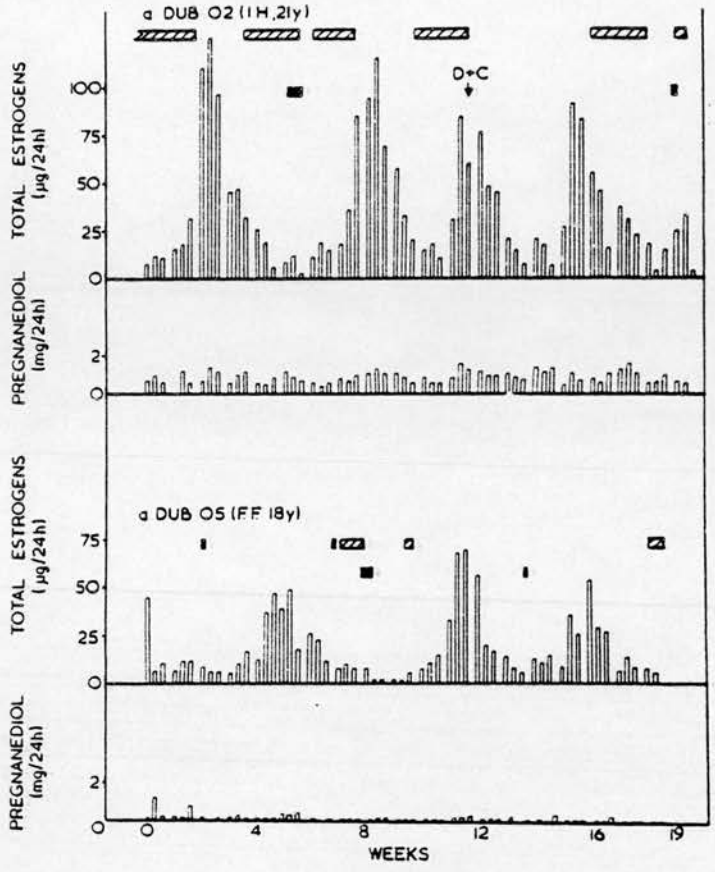


Figure 2:
Profiles of urinary total oestrogen and pregnanediol excretion in two young women with anovulatory DUB. Each open vertical bar represents a 24-h urine specimen. ■, Days of ethinyl oestradiol (left) or GnRH (right) administration; ▨, days of menstrual bleeding. D + C, Dilatation and curettage showing cystic glandular hyperplasia of the endometrium.

oestrogen excretion, however, indicated that the length of the cycle, when calculated from one oestrogen nadir to the next, was on the average about 5-6 days longer in patients than in controls, and that this was due to a prolongation of the follicular phase. The oestrogen excretion during the initial stages of follicular development was comparable in both groups and reached a level of 30-40 µg/24 hours after approximately 2 weeks (Fig.3). In control women, this concentration appeared to represent the threshold required for inducing LH release and ovulation, as evidenced by the subsequent rise in urinary pregnanediol. In the patients' group, however, ovulation did not occur and oestrogen levels continued to rise for an additional period of 5-6 days. As a result peak levels of oestrogen were significantly

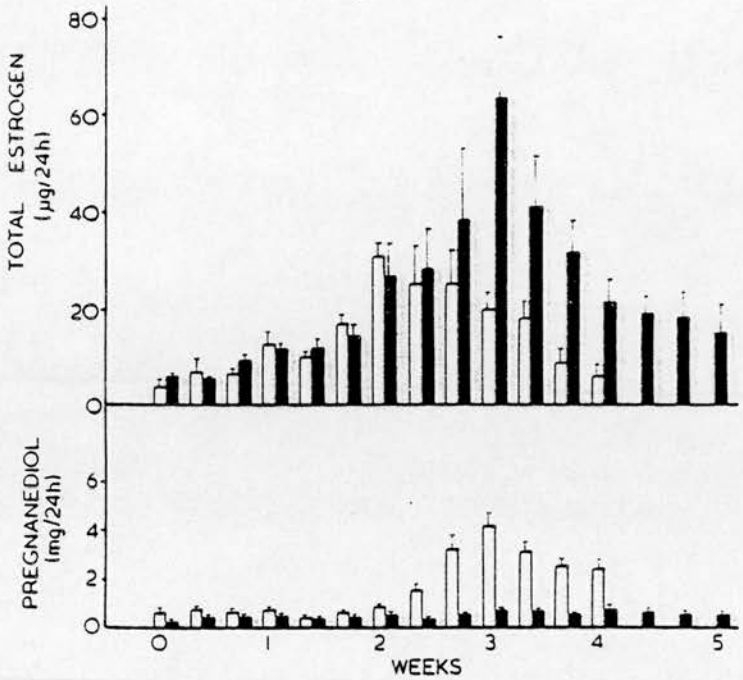


Figure 3. Mean (±SEM) urinary total oestrogen and pregnanediol excretion in normal women (open bars, n = 6) and patients with anovulatory DUB (black bars, n = 7). Each bar represents a 24-h urine collection. Collections were made three times a week and results in each group were centred around the midcycle oestrogen peak which occurred on the average 5-6 days later in patients than in controls.

($P<0.05$) greater in patients (62.8 ± 12.6 µg/24 hour) than in controls (30.7 ± 3.1 µg/24 hour). Within the patients' group, increased oestrogen excretion was not always related to the severity of bleeding or the endometrial histology at previous curettage, although the two highest oestrogen peaks (125.8 and 88.0 µg/24 hour) were found in patients with a history of cystic glandular hyperplasia of the endometrium.

Urinary pregnanediol remained low in all seven anovulatory women throughout the study. On several occasions, a short-lived rise in pregnanediol excretion, coincident with the midcycle oestrogen peak, could be observed, particularly in those patients who had high urinary oestrogen levels (e.g. DUB 02 in Fig.2), but normal luteal function never developed.

Oestrogen provocation test

The results are graphically presented in Fig.4.

Normal women

The concentration of FSH was rapidly suppressed to 60% of the mean initial level within 24 hours after the start of ethinyl oestradiol treatment. FSH levels remained low during the remainder of the treatment period, after which they gradually returned to pretreatment values. Under our experimental conditions, no evidence for a positive feedback effect of ethinyl oestradiol on FSH release could be obtained. There was, however, a significant inverse correlation between peripheral concentrations of FSH and ethinyl oestradiol ($y = 107.2 - 62.7 \log x$, where y = the percentage of change in FSH from mean pretreatment value and x = the peripheral ethinyl oestradiol concentration; $r = -0.5575$; $n = 27$; $P<0.01$), suggesting that FSH secretion under these conditions was regulated by negative feedback only.

Plasma levels of LH, on the other hand, showed a pronounced biphasic pattern, consisting of a brief initial period of suppression followed by a marked rise to a peak value of 35.0 ± 5.5 mU/ml on day 9, i.e. a mean increase of about 250% above pretreatment levels ($P<0.01$). The magnitude of the induced LH surges, which in five out of six women occurred on day 9 and in the remaining subject (Con 01) on day 8, ranged from 23.8-58.0 mU/ml with a mean of 36.1 ± 4.8 mU/ml, a value not significantly different from that of the spontaneous midcycle LH peak (39.4 ± 2.5 mU/ml). The LH rise began during the period of ethinyl oestradiol treatment. Unlike FSH, peripheral concentrations of LH were not correlated with circulating levels of ethinyl oestradiol.

The initial decrease in concentration of gonadotrophins was associated with a rapid decline of plasma 17β-oestradiol which in five of six women remained low throughout the remainder of the test.

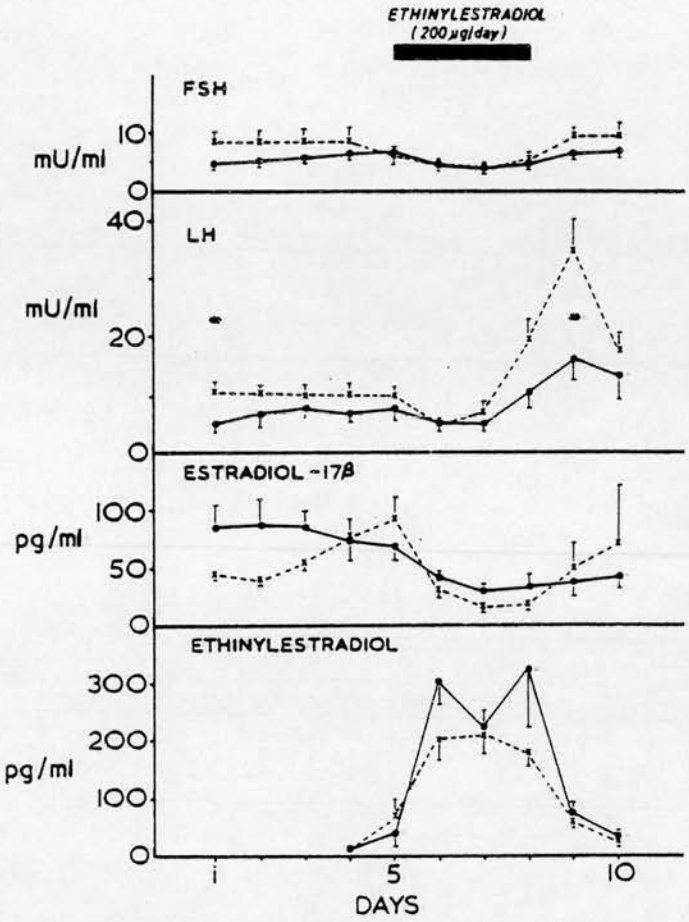


Figure 4:
Mean (±SEM) plasma concentrations of FSH, LH, 17β-oestradiol, and ethinyl oestradiol during an oestrogen provocation test in normal, early to mid follicular phase women (---) and in patients with anovulatory DUB (——). Asterisks indicate values which were significantly different between the two groups.

Women with DUB

Pretreatment values of FSH (overall mean of days 1-4, 5.51 ± 0.43 mU/ml) and LH (6.58 ± 0.80 mU/ml) in the seven patients with persistent anovulation were significantly ($P<0.05$) lower than those in control women (8.40 ± 1.02 and 10.26 ± 0.80 mU/ml), but concentrations of 17β-oestradiol were on the average about 50% higher in the former (83.2 ± 8.7 pg/ml vs. 55.0 ± 5.1 pg/ml; $n = 49$; $P< 0.01$).

Changes in the concentrations of FSH and LH during and after oestrogen administration were qualitatively similar to those seen in controls. After an initial decrease, LH levels started to rise 48-72 h after the start of treatment

and reached a peak of 16.2 ± 3.7 mU/ml on day 9. The magnitude of this oestrogen induced LH surge was significantly ($P<0.005$) smaller than that seen in controls and in only two out of the seven patients did the value fall within the range found in controls. Peak levels of LH in the four patients with a history cystic glandular hyperplasia were significantly lower than those of the remaining three patients, in whom the endometrium showed proliferative changes only (9.70 ± 2.20 vs. 25.74 ± 2.82 mU/ml; $P<0.01$). Differences in peak magnitude between patients or between patients and controls could not be attributed to differences in plasma ethinyl oestradiol concentrations. Circulating levels of the latter hormone were on the average higher in patients (mean of days 6-8, 285.0 ± 67.3 pg/ml) than in controls (197.7 ± 16.6 pg/ml), but the difference was not significant.

As in control women, ethinyl oestradiol treatment did not stimulate the release of FSH; and changes in the plasma level of FSH were inversely related to plasma concentrations of ethinyl oestradiol ($y = 142.1 - 70.1 \log x$, where y = the percentage of change in FSH from basal value and x = the plasma level of ethinyl oestradiol; $n = 35$; $r = 0.6678$; $P<0.001$). Neither slope nor intercept of this regression line was significantly different from the corresponding values found in control women, suggesting that the negative feedback sensitivity of FSH secretion to oestrogen was similar in the two groups.

GnRH test

The administration of GnRH to control women resulted in a rapid increase in the level of both gonadotrophins within 5 min of injection. The magnitude of the pituitary response was greater for LH (23.9 ± 4.7 mU/ml) than for FSH (11.0 ± 2.7 mU/ml; Fig.5). The FSH response in the patients' group (8.5 ± 0.5 mU/ml) was on the average somewhat smaller and LH response (26.2 ± 8.1 mU/ml) was somewhat greater than those of controls, but the differences were not statistically significant.

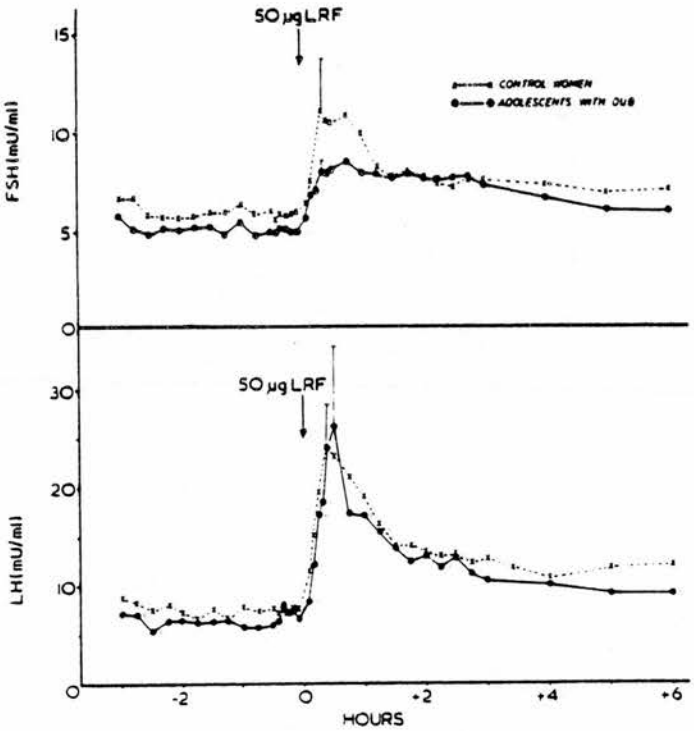


Figure 5:
Mean (±SEM) plasma levels of FSH (upper panel) and LH (lower panel) in normal women (---, n = 6) and patients with anovulatory DUB (—, n = 7) before and after the injection of 50 µg synthetic GnRH.

In every subject, the GnRH-induced rise in gonadotrophins was followed by a significant increase in the circulating levels of 17β-oestradiol, which became manifest 4-5 h after GnRH injection. The relative magnitude of this rise varied between 111 and 387% of the pre-GnRH value and was similar in both groups.

DISCUSSION:

In all patients in this study dysfunctional uterine bleeding had been present for 5 years or more, and all seven with persistent anovulation had recently undergone therapeutic curettage with the finding of cystic glandular hyperplasia in four. By contrast approximately half of an unselected group of adolescents presenting for the first time with dysfunctional uterine bleeding will establish a pattern of normal, regular cycles within 4 years (Southam and

Richart 1966). This suggests that the more severe end of the spectrum is represented by the women studied here. This is substantiated by a history of blood transfusions in 3, diagnostic laparoscopy for infertility in one and laparotomy and unilateral salpingo-oophorectomy for a large fimbrial cyst in another.

The profiles of urinary total oestrogen excretion in these patients (Fig.2 and 3) confirm earlier work, (Brown et al 1959) which showed that urinary oestrogen levels are often elevated in anovulatory DUB, particularly if the latter is associated with cystic glandular hyperplasia. As there is no difference in oestrogen metabolism between older women with DUB and regularly menstruating women (Chapter 4) it is reasonable to assume that this enhanced oestrogen excretion is a direct reflection of an increase in ovarian secretion of 17β-oestradiol. The data shown in Fig.3 indicate that this increase becomes manifest only during the latter part of the prolonged follicular phase. The cause for this prolongation of follicular oestrogen secretion beyond the normal 14-day period found in ovulatory cycles is unknown, but may be due to the absence of the spontaneous midcycle LH surge observed in these patients (Chapter 7). This view is supported by the finding that preovulatory Graafian follicles obtained from pregnant mare serum gonadotrophin (PMSG)-treated sheep continue to secrete oestrogen for at least 7 days when cultured in vitro, but that they lose this ability when an ovulatory dose of LH is given to the animals before removal of the follicles (Moor 1974).

Despite the presence of regular apparently adequate follicular development, seven out of nine patients consistently failed to ovulate, as indicated by the absence of a significant rise in urinary pregnanediol excretion. When challenged with oestrogen, four of these women failed to release LH, and the response in the remaining three subjects was small and fell below or just above the lower limit of the range found in normal women. Thus, the

present study confirms the earlier suggestion that the failure to ovulate in these patients is associated with an inability to discharge an adequate amount of LH in response to oestrogen (Chapter 7). In addition, the present investigation indicates that this failure is probably of hypothalamic origin, as pituitary secretion of LH in response to GnRH (Fig.5) was similar to that found in women with regular ovulatory cycles. It should be emphasized, however, that this latter conclusion requires additional proof. Indeed, the amount of LH released after the acute injection of 50 μg GnRH is small compared to that secreted at midcycle and, therefore it cannot be excluded that there may be a relative failure of the pituitary to synthesize and/or secrete enough LH to induce ovulation. Further studies involving continuous infusion or repeated administration of GnRH to test the secretory "reserve" of the pituitary gland (Wang et al 1976) are indicated to investigate thus possibility.

The presence of regular, co-ordinated follicular growth and oestrogen secretion and the normal rise of plasma 17β-oestradiol after GnRH indicate that the primary defect in this condition is not of ovarian origin. Follicular cysts and variants of the polycystic ovary syndrome have been described in women with DUB (Southam and Richart 1966), but these ovarian abnormalities are probably secondary to chronic anovulation. In this respect, these patients might be comparable to adult female rats made permanently anovulatory by neonatal administration of androgens or oestrogens (Barraclough 1973).

Although the results of this study leave little doubt that oestrogen-induced LH release in young women with anovulatory DUB is deficient as compared to normal, regularly menstruating women, they also tend to indicate that this positive feedback failure is not absolute. From clinical experience, it is well known that these patients may ovulate spontaneously on occasions. The two pregnancies in one of our volunteers are direct proof of this. It is possible,

therefore, that the basic defect in this condition is a relative insensitivity of the hypothalamic-pituitary unit to the positive feedback effect of oestrogen. As the magnitude and duration of the abortive LH peaks observed in our patients are very similar to those described in female rhesus monkeys after the administration of a subthreshold dose of oestrogen (Karsch et al 1973), it may be that these patients require a more potent oestrogen stimulus than normal women in order to release an adequate amount of LH at mid-cycle. It is also possible that this defect is a failure of maturation of the hypothalamo-pituitary-ovarian axis, since the positive feedback response normally appears at mid-puberty (Reiter et al 1974).

It is of interest to note that the LH peaks in all four patients with a history of cystic glandular hyperplasia were significantly smaller than those in the remaining three women without such history. This might indicate that hypothalamic sensitivity to positive feedback in the former patients was more markedly imparied and, hence, that spontaneous ovulations were less likely to occur. Evidently, these two factors, namely the presence of markedly elevated oestrogen levels for prolonged periods of time and the absence of ovulation and, hence, of progesterone, constitute the ideal endocrine environment for the development of cystic glandular hyperplasia and possibly endometrial carcinoma (Schröder 1954; Fox 1976).

A point of clinical importance is well illustrated by this study. Subject DUB 02 (Fig.2) experienced erratic bleeding which sometimes continued even with rising oestrogen levels. This emphasises that abnormal bleeding can occur when oestrogen levels are falling, stable or rising (Brown et al 1959; Chapter 7).

SECTION 4

PERCEPTION AND MEASUREMENT OF MENSTRUAL FLOW

PERCEPTION AND MEASUREMENT OF MENSTRUAL FLOW:

Several authors have suggested that individual perception of volume of menstrual blood loss is often inaccurate, and a number of methods for the objective measurement of blood loss have been described. A correct assessment of blood loss is important for a diagnosis of "true" menorrhagia/DUB, and the alkaline haematin method used by several investigators was developed for routine use with a semi-automatic extractor. This only gave a measure of the whole-blood component of the menstrual flow and an accurate sanitary towel weighing technique was also used to assess the total fluid volume on a daily basis through one menstrual period in 28 regularly menstruating women. The percentage contribution of blood (equivalent to mixed venous blood from the cubital fossa) to the total varied very greatly from subject to subject (1.6 - 81.7%) with a mean of 36.1 ± 3.6% (±SEM). There was a highly significant correlation between total fluid loss and blood loss ($r = 0.911$; $p<0.001$). The proportion of blood remained approximately the same for different total volumes and on different days of the cycle. In the one woman with objective menorrhagia the contribution of whole blood to the total was only 52%. Subjects using no contraception or who had undergone tubal sterilization had similar ratios of blood to total fluid loss whereas IUCD users had a higher ratio ($p<0.025$) and oral contraceptive users had a lower ratio ($p<0.05$). It seems probable that the major component of the fluid loss which cannot be accounted for by blood is from endometrial tissue fluid (? a transudate) rather than vaginal or cervical secretions. Variations in the endometrial tissue fluid component may contribute to some of the errors in perception of menstrual bleeding exhibited by many women.

Sixty-nine women with a convincing complaint of menorrhagia took part in a double blind treatment trial where menstrual blood loss was accurately measured and the subjects' own perception was carefully recorded. Only 38% of the women

had objective menorrhagia with a measured loss greater than 80 mls, although 59% would qualify with an upper limit of normal of 60 mls. Overall, the measured loss in the "heaviest" periods (69.6 ± 7.3 mls) was significantly greater than the "lightest" periods (42.7 ± 4.7 mls; $p<0.001$), but there were many major errors in perception by individuals. Perceived daily blood loss volume on a 4 point rating scale gave the following group means and ranges: spotting: 2.5 mls (0.1 - 15.5 mls); light: 5.7 mls (0.1 - 63.1 mls); moderate: 16.1 mls (0.5 - 108 mls); very heavy: 22.0 mls (1.4 - 215.8 mls); illustrating very wide individual ranges of assessment. As a whole the group was also able to distinguish between a day to day volume increase or decrease, but again there were many major errors. Some subjects who experienced a reduction in measured blood loss from one day to the next actually perceived this as a large increase. Menstrual pain and duration of bleeding were not found to influence perception of menstrual blood loss volume, whereas younger subjects (26 and under) were significantly more likely to regard a moderate loss as very heavy than older women (37 and over). There was no significant correlation between number of pads/tampons used and the measured menstrual loss, and some individuals showed extreme variations between blood loss and pad usage. This study suggests that the only reliable assessment of menstrual blood loss volume and changes in volume in women complaining of menorrhagia is obtained by objective measurement of blood loss using a technique such as alkaline haematin extraction.

Chapter 9

BLOOD AND TOTAL FLUID CONTENT OF MENSTRUAL DISCHARGE:

INTRODUCTION:

Most physicians and patients implicitly assume that the fluid discharged at menstruation is whole blood or altered whole blood containing a small amount of endometrial tissue, debris and other fluids, and objective measurements of menstrual volume have usually used techniques which measure the haemoglobin present in the fluid discharge. Most of these methods give a reliable assessment of haemoglobin content and therefore presumably total blood loss (Shaw 1977). However, there is evidence from earlier studies in very small numbers of women that the haemoglobin concentration in menstrual fluid is lower than in blood. Measurement of menstrual blood collected directly from the uterus or upper vagina has given haemoglobin concentrations ranging from 40 to 103 g/ℓ (Ebert and Nold 1956; De Merre et al 1967).

Many women have great difficulty in assessing the volume of their blood loss and even in detecting major day to day and cycle to cycle changes in blood loss (Chapter 10). Some of these women made the observation that on some days the fluid in their pads or tampons appeared to be very dilute blood. It seemed possible that a major source of error in perception of the volume of menstrual bleeding could be the presence of a significant, and perhaps variable, amount of endometrial tissue fluid or cervical and vaginal secretion.

The present study was designed to assess the total volume of fluid lost per day at menstruation and compare this with the volume attributable to whole blood.

This study was planned, executed and analysed by myself, with the assistance of a research sister (Miss G. McCarron) and technical staff (Mr R. Markham and Miss T. Resta).

MATERIALS AND METHODS:

Twenty-eight women volunteers with no gynaecological

symptoms were studied on a daily basis through one menstrual period and were very carefully instructed in the details of the study. None were of asian origin. None of these women had any detectable gynaecological abnormality, menstrual cycles were regular (24-35 days) and all ovulatory except for the 5 women using oral contraceptives. All women had plasma progesterone measured by standard radioimmunoassay (Shutt et al 1975) on one occasion within ten days before menstruation to confirm ovulation. No subject complained of an excessive vaginal discharge.

All women used the sanitary pads or tampons which were provided. These were accurately weighed and placed in individually labelled self-sealing polythene bags until used. After use each pad or tampon was immediately placed back into its original bag which was resealed. Analysis was carried out within 2-3 days of the end of the menstrual period. Each pad and tampon was accurately reweighed and the total fluid content calculated by subtracting the original weight.

Alkaline haematin technique for menstrual blood loss measurement

Haemoglobin content of each daily pad and tampon collection was measured by a modification of the alkaline haematin technique of Hallberg and Nilsson (1964a) using a semi-automatic extractor (Newton et al 1977).

This is colourimetric method which quantifies the haemoglobin present in sanitary towels and tampons and involves 3 main steps: 1) the complete collection of all menstrual blood lost by the woman by the use of disposable sanitary towels; 2) the extraction and conversion of haemoglobin to alkaline haematin with sodium hydroxide; 3) followed by quantitation in a spectrophotometer.

Complete collection of the menstrual loss is crucial to the accuracy of the method, and subjects required careful verbal and written instructions to avoid inadvertent loss of "clots" while in the shower or on the toilet. This

was often best achieved by a combination of tampon and pad use, and controlled "bearing down" to expel vaginal "clots" onto the pad. Women with true menorrhagia required special care. Sanitary towels were immediately placed in thick opaque polythene bags and collected in the same bag on a daily basis. At the end of the day the bag was rolled up and sealed. All bags for one menstrual period were kept in a cool place until returned to the laboratory within a few days of the end of the period. Women were permitted to continue use of their usual brand of sanitary pads or tampons since none of the brands available in Australia contain coloured material which interferes with the photometric measurement.

Extraction procedure

The original collection bag containing one daily sanitary towel collection was placed inside a strong plastic bag (14" x 20" x 200 g thickness, Cryovac, Seward Laboratories, Bury St Edmunds, Suffolk) and 2 litres of 5% sodium hydroxide solution added. The bag was then placed inside a model 3500 Stomacher Lab-Blender (Seward Laboratories) which pummeled the bag for 15 minutes to produce complete disintegration of the towels and conversion of haem to alkaline haematin. An aliquot of homogenate was filtered through Whatman No 4 filter paper to remove suspended particles and the optical density of the supernatant at 550 nm determined at approximately 30 minutes after addition of the sodium hydroxide using a Corning 253 colourimeter.

At the sametime as the sanitary towel extraction 100 ml of 5% sodium hydroxide was added to 1 ml of peripheral venous blood from the same individual for comparison. The optical density of peripheral blood was also read at 30 minutes and usually immediately before the menstrual sample. Menstrual blood loss was calculated from the following equation:

$$\text{Menstrual blood loss (mls)} = \frac{\text{(O.D. 550 M)} \times \text{Vol}}{\text{(O.D. 550 V)} \times 100}$$

Where (O.D.550 M) = optical density (550nm) of menstrual eluate

(O.D.550 V) = optical density (550nm) of venous blood

Vol = added volume of 5% sodium hydroxide

100 = volume of 5% sodium hydroxide added to venous blood

Assessment of completeness of recovery of added blood

Two brands of tampon (Meds Super; Carefree Super) and two brands of pad (Modess Super: Sure and Natural) were tested by the addition of 10 mls, 20 mls or 100 mls of whole blood (expired blood from the Red Cross Blood Bank, Sydney). Each assessment was done in quadruplicate. The mean percentage recovery overall was 92.2 ± 0.48 (SEM; n = 32). No difference was seen in percentage recovery of different volumes of added blood (range 89.0 to 94.4% recovery). Coefficient of variation = 2.6%.

Sanitary towel storage

The method of storage of the pads and tampons in individual sealed bags following use and prior to analysis was checked for evaporation by adding known volumes of water to each of 20 test pads and accurately reweighing after storage. Twenty pads were weighed individually, approximately 10 mls of water was added and they were then individually reweighed and stored in the polythene bags at room temperature. Ten were reweighed after 2 days and 7 days and replaced in the bags immediately after each weighing. All were finally weighed at 14 days after initial storage. Fluid loss attributable to evaporation or to loss on the inner surface of the bag was 2.7% in the group weighed at 2, 7 and 14 days and 2.1% in the group weighed at 14 days only. This volume of loss was highly significant (paired t test: $t = 17.49$; $p<0.001$), but would be negligible in relation to menstrual blood loss.

Each subject kept a daily menstrual diary card with details of her perception of volume of menstrual blood

loss, dysmenorrhoea and other menstrually related symptoms rated on a 3 point scale (slight, moderate, heavy).

Statistical analyses were made using the Student t test, or linear regression correlation.

RESULTS:

Measurements of total fluid loss and blood loss from each individual subject over one complete menstrual period and arranged from heaviest to lightest are illustrated in figure 1. These indicate that those women with heaviest total fluid loss also tended to have the highest blood loss, although the proportional contribution from whole blood varied considerably at all volumes.

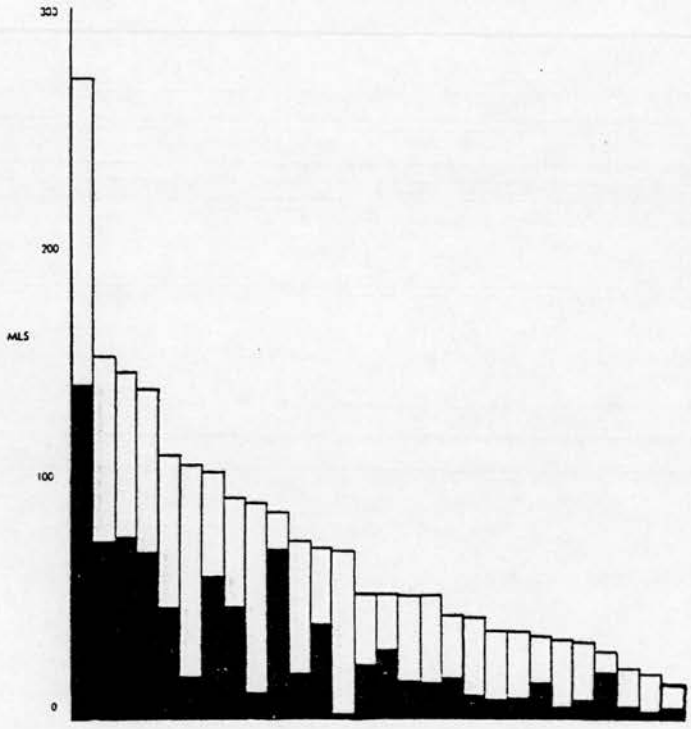


Figure 1:
Blood loss and total fluid loss at menstruation in 28 women.

= total fluid loss

= blood loss

The total fluid loss, blood loss, percentage contribution of blood to the total, and the non-blood fluid loss are listed for each subject in table 1. The mean percentage contribution from whole blood was only 36.1% ± 3.6.± S.E.M. (range 1.6-81.7%), which indicates that the majority of the fluid volume of the menstrual discharge is derived from sources other than blood.

TABLE 1 - Blood loss and total fluid loss at menstruation in 28 women using different methods of contraception.

Subject No.	Total fluid content of sanitary towels (mls)	Measured blood loss (mls)	% of blood out of total	Fluid component not due to blood (mls)	Contra-ceptive usage
1	270.9	140.7	51.9	130.2	TL
2	153.2	73.3	47.8	79.7	NC
3	146.1	75.7	51.8	70.9	IUCD
4	139.0	70.0	50.4	69.0	IUCD
5	111.1	47.6	42.8	63.5	NC
6	106.0	18.7	17.6	87.3	TL
7	104.1	60.2	57.8	43.8	IUCD
8	93.5	47.0	50.3	46.5	NC
9	91.0	11.4	12.5	79.4	NC
10	87.4	71.4	81.7	16.0	IUCD
11	74.6	19.2	25.7	55.4	NC
12	72.9	40.6	55.7	28.7	TL
13	71.7	1.2	1.6	70.0	OC
14	53.7	21.9	40.8	31.8	IUCD
15	53.5	29.8	55.7	24.6	NC
16	53.1	15.5	29.2	37.6	NC
17	53.1	14.5	27.3	38.6	NC
18	43.2	16.2	37.5	27.0	NC
19	42.4	9.8	23.1	32.6	NC
20	37.5	7.0	18.7	30.5	OC
21	37.3	8.8	23.6	28.5	NC
22	35.8	15.2	42.5	20.6	TL
23	33.7	4.5	13.5	29.2	TL
24	32.3	6.7	20.7	25.6	OC
25	28.4	19.2	67.6	9.2	NC
26	21.9	5.2	23.7	16.7	NC
27	19.7	2.1	10.6	17.6	OC
28	15.8	4.7	29.7	11.1	OC
Mean	74.4	30.6	36.1	43.3	
SEM	10.3	6.1	3.6	5.4	

TL = tubal sterilisation
IUCD = intrauterine contraceptive device
OC = oral contraceptive
NC = no contraception

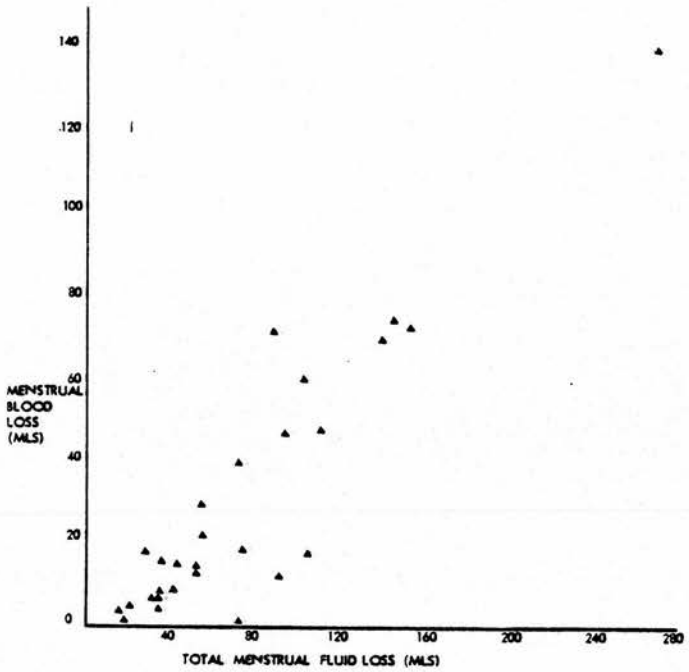
There was a highly significant overall correlation between the total fluid loss and the measured blood loss ($r = 0.911$; $p<0.001$) in spite of individual exceptions (figure 2a). There was also a significant but lesser correlation between measured blood loss and the volume of fluid which was not derived from blood (total fluid minus measured blood loss) ($r = 0.602$; $p<0.01$; figure 2b). This correlation remained significant at the 5% level when the only subject with previously unrecognised objective menorrhagia (ovulatory dysfunctional uterine bleeding) was excluded from the analysis ($r = 0.324$; $p<0.05$).

The comparison between total fluid loss and blood loss for each day of the menstrual period for the whole group is shown in figure 3. The proportion contributed by whole blood did not change significantly from day to day.

Subjects were allocated to groups according to contraceptive usage and each group analysed separately. There was no significant difference for the fluid composition of the menstrual discharge between the groups using no contraception (13 women; whole blood component = 35.5 ± 4.5%) or those who had undergone tubal interruption (5 women; 36.2 ± 8.8%) for sterilization (figure 4). However, the 5 subjects who used oral contraceptives all had a low blood loss and a low proportion of blood to total fluid which was significantly different from those not using contraception (17.3 ± 5.1%; $t = 2.281$; $p<0.05$). Those using an intrauterine device had a significantly greater proportion of blood in the total discharge (5 women; 56.5 ± 6.9%; $t = 2.490$; $p<0.025$).

Eight subjects used sanitary pads only during the study. The fluid losses per day (total fluid = 17.4 ± 4.1 mls; blood loss = 9.4 ± 2.3 mls; mean ± SEM) were slightly different from the losses in the larger group of 15 who exclusively used vaginal tampons (total fluid = 15.6 ± 1.5 mls; blood loss = 5.3 ± 0.9 mls). Blood loss but not total fluid loss, was significantly more in the pad users, due to the one subject with menorrhagia. The ratio

(a)



(b)

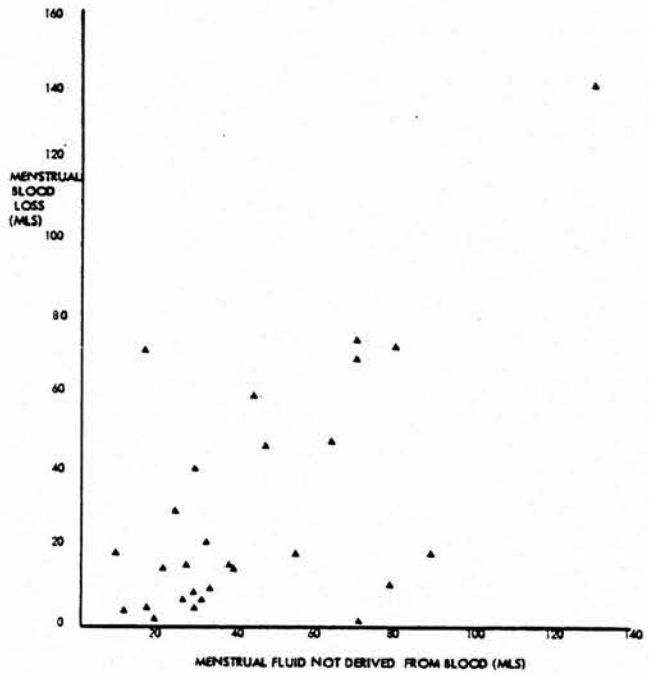


Figure 2. (a) Relationship between total fluid loss and measured blood loss in 28 women.

(b) Relationship between measured blood loss and measured fluid loss not attributable to blood in 28 women.

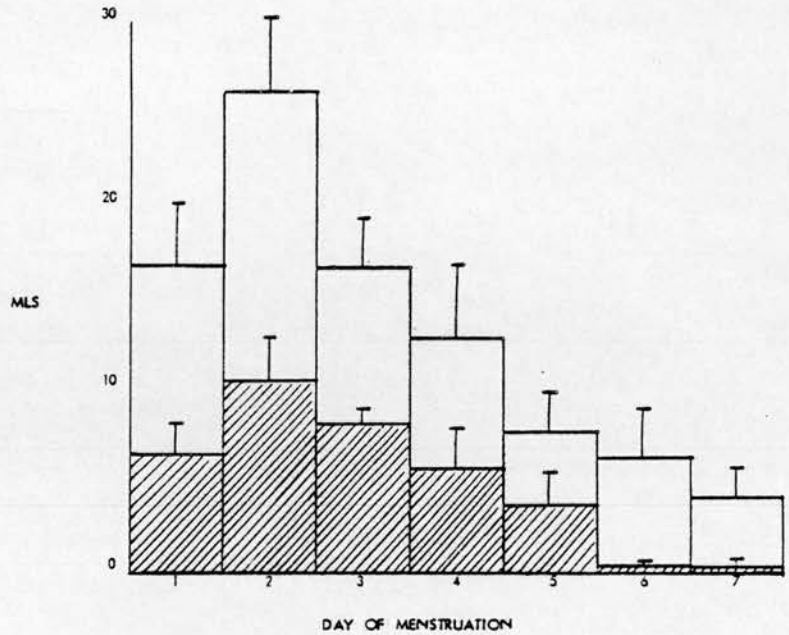


Figure 3. Comparison between total fluid loss and blood loss during each day of menses in 28 women (mean and S.E.M.).

= total fluid loss;

= blood loss.

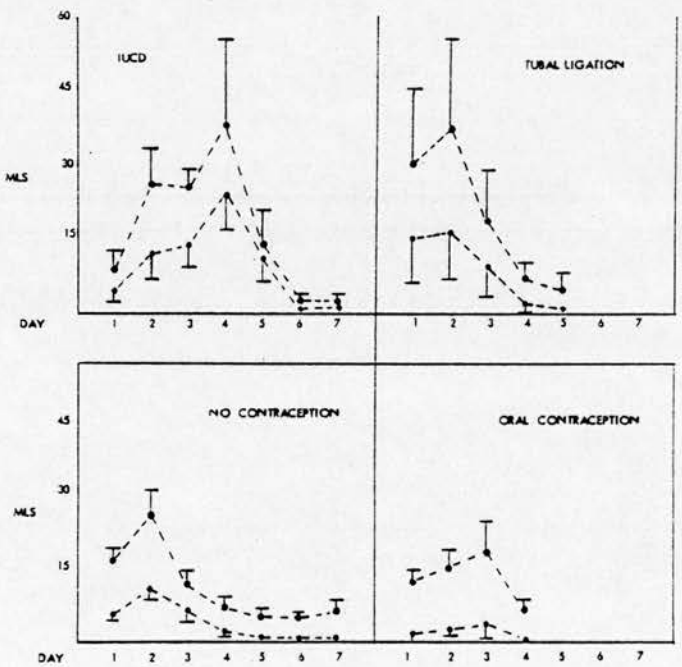


Figure 4. Daily blood loss and total fluid loss in 28 women using different methods of contraception (mean ± SEM). Upper line indicates total fluid loss. Lower line indicates blood loss.

of blood to total fluid in the pad users (50.0 ± 6.5%) was significantly higher than the tampon users (30.0 ± 4.1%; t = 2.726; p<0.02), suggesting either an increased evaporation or reabsorption of fluid in the pad users or an excessive vaginal secretion in the tampon users.

TABLE 2: Subjective rating of volume of menstrual discharge in 28 women.

Subjective rating of daily loss	Measured loss (mean ± SEM) Total fluid loss (mls)	Blood loss (mls)	Percentage due to whole blood
A) Heavy (n=6)	42.7 ± 14.6*	20.5 ± 7.7*	48.0
B) Moderate (n=21)	25.3 ± 3.1*	12.0 ± 2.1*	47.4
C) Light (n=26)	9.5 ± 1.2*	2.6 ± 0.4*	27.3

* P<0.001 = A compared with C for both total fluid and B compared with C blood loss perception

A is not significantly different from B

Subjective rating of daily menstrual blood loss volume on each point of the 3 point scale was analysed for each subject, and then as a group using the subject as the unit for analysis (table 2). Although the subjects were able to distinguish "heavy" from "moderate" from "light" bleeding days as a group, there was a substantial overlap between individuals in the absolute volumes which were rated in each category. The proportion of the total fluid loss accounted for by whole blood was virtually identical on the "heavy" and "moderate" bleeding days, whereas it was much less when bleeding was rated as light. This may indicate that vaginal and cervical secretions were making a proportionately larger contribution to the total fluid when the menstrual flow was less. To assess vaginal and cervical secretions, six subjects were asked to use tampons over a 24 hour period on 2 days when they were not menstruating. This gave a mean 24 hour tampon fluid content of 4.4 mls (± 0.4 S.E.M; range; 2.8 to 6.3 mls/24 hours).

DISCUSSION:

Blood is obviously an important component of the

menstrual discharge. However, the physiological necessity for this cyclical bleeding in women and subhuman primates is far from clear, especially when the considerable cyclical growth and regression of the endometrium which occurs without bleeding in the oestrus cycle of subprimates is considered (Finn 1977). In women an excessive monthly blood loss can be of considerable psychological concern and may even lead to a health hazard from anaemia. Yet, accurate perception of the volume of blood loss may be extremely difficult (Chapter 10). It has been known for some time that blood does not account for all the menstrual discharge (Ebert and Nold 1956; De Merre et al 1967; Hahn 1980) but little is known of the variation in blood content of the discharge and the way in which this may be manifest to the woman, that is to say in her sanitary towels.

A very detailed study of menstrual discharge collected directly from the uterus of 3 women found menstrual haemoglobin concentrations of 40, 42 and 50 g/ℓ (i.e. approximately 35% of venous concentrations) on days 1-2 of the cycle (Ebert and Nold 1956). This finding has been confirmed in the present study which relied upon the collection of fluid discharged into the vagina. In view of the agreement between these two studies it seems likely that the major contribution of fluid not derived directly from blood must come from the endometrial cavity. This is presumably derived from endometrial tissue fluid exudation and perhaps a small component from glandular secretion. It is not known whether the Fallopian tubes secrete enough fluid to make a significant contribution. Cervical and vaginal secretions must make some contribution to the fluid absorbed by sanitary tampons or pads, but the close agreement of studies of uterine and vaginal discharge suggests that the contribution of the cervical and vaginal components is usually small. The observations that the proportion of blood out of total fluid is similar on different days of menstruation and in women with different total menstrual fluid volumes would also be consistent with an endometrial origin for the majority of the fluid. Cervical and vaginal

secretions probably only make a significant contribution when total menstrual fluid loss is small. It is possible that this factor may contribute to the high total fluid to blood loss ratio in oral contraceptive users.

There is great variability in the proportion of blood in menstrual discharge and it is likely that this contributes to some errors in perception of menstrual bleeding. However, evidence for this was not found in the present study. The contribution by tissue fluid of probable endometrial origin to total fluid loss in menorrhagia is unknown and requires further investigation. The finding that the tissue fluid contribution is particularly high in oral contraceptive users is in agreement with the report of De Merre et al (1967). The demonstration of different fluid compositions of the menstrual discharge with IUCD and oral contraceptives indicates that there are factors which can indeed alter the tissue fluid component independently of the blood loss. Nevertheless, in clinical terms the volume of blood lost, or more accurately the amount of haemoglobin and iron lost from the body, is of more crucial importance to the health of the woman.

It is of interest that the proportion of "tissue" fluid in the woman with objective menorrhagia was as high as in women with less blood loss and therefore the absolute volume of fluid was much greater. If the tissue fluid component was due to exudation across a denuded surface it could be expected to be proportional to the endometrial surface area. However, the variation in surface area is not large and does not correlate with measured blood loss (Chimbira et al 1980). Therefore, it seems likely that other mechanisms may sometimes be contributing. Exudation could be greater if blood flow through the endometrium was increased or if capillary permeability was increased. The answer to this question awaits future research.

This study indicates that the alkaline haematin method of menstrual blood loss measurement generally underestimates blood loss by about 10%. However, a correction factor has

not been applied in general usage because there is a small error due to the blank value and almost certainly an added error of variable degree during the sanitary towel collection process. This will also tend to underestimate the menstrual loss. Other investigators have not applied a correction factor and it seems sensible for users of the same method to be consistent in comparison of results. The comparative study of Hallberg and Nilsson (1964a) shows a good correlation with menstrual loss measured simultaneously with the alkaline haematin and a radioactive iron labelling method. Most of the recently published work on menstrual blood loss has been based on the alkaline haematin technique, which has been found satisfactory by all groups which have used it (Hallberg et al 1966; Shaw 1973; Guillebaud et al 1966; Newton et al 1977; Shaw 1977; Haynes et al 1977). It has the added advantages of converting old and dried blood efficiently to alkaline haematin, as well as measuring most breakdown products of haemoglobin (produced by drying or micro-organisms; Hallberg and Nilsson 1964a). It does not appear to be affected by proteins or glycoproteins which may be present in menstrual "clots" (Beller 1971).

Using this method Hallberg and Nilsson (1964b) have shown a reasonable constancy of individual menstrual blood loss from month to month in normal women. Although the variation between periods may be much more in some individuals with menorrhagia the coefficient of variation for menstrual blood loss from one period to the next in a group of women with menorrhagia is the same as normal women (Haynes et al 1977). However the variation in loss between individuals is very much greater and may show substantial differences in different ethnic groups (Hallberg et al 1966; Cole et al 1971; Hefnawi et al 1979; Gao et al 1981). This has been discussed in Chapter 1.

Chapter 10

PERCEPTION OF MENSTRUAL BLOOD LOSS VOLUME IN WOMEN COMPLAINING OF MENORRHAGIA:

INTRODUCTION:

For many patients the assessment of the volume of menstrual blood loss is probably the most important single parameter of the menstrual history, since it is on this that the decision to perform the very final and invasive procedure of hysterectomy is usually based. It is widely recognised that this assessment is not very reliable, (Barer and Fowler 1936; Millis 1951; Hytten et al 1964; Jacobs and Butler 1965; Hallberg et al 1966; Chimbira et al 1980), but surprisingly little has recently been written about it.

In a large population study (Hallberg et al 1966) of 476 women in whom menstrual blood loss was objectively measured by the alkaline haematin technique (Hallberg and Nilsson 1964a) it was found that 45% of the women who rated their blood loss as "heavy" had a measured loss within the mid to low normal range. By contrast, only 4% of women who rated their loss as light had a measured loss of greater than the generally accepted upper limit of normal of 80 mls. Chimbira et al (1980) found no correlation between the patient's subjective assessment of sanitary pad usage and measured blood loss. These studies give an indication of the difficulty which some women have in assessing the absolute volume of flow but give no information on their ability to assess change in flow from cycle to cycle or day to day.

In contrast, a great deal has been written about the way in which women perceive other parameters of the menstrual cycle and the factors which may influence this. It is clear that multiple cultural, religious, familial and individual factors have an influence on this overall perception, but one important message is that retrospective recall is notoriously unreliable and should not be used in any accurate study (Gray 1980).

This Chapter presents the results of a prospective investigation of menstrual blood loss in 69 women over 4 cycles each and compares this with the patients' own subjective assessment of her menstrual loss on a day to day and cycle to cycle basis. These subjects all took part in a clinical trial of the efficacy of the prostaglandin inhibitor, mefenamic acid, for the treatment of menorrhagia (Fraser et al 1981).

Methods

Sixty-nine women who presented with a complaint of menorrhagia completed a 4 cycle double-blind placebo-controlled cross-over trial using the prostaglandin inhibitor, mefenamic acid. Each subject was interviewed in detail and was only admitted to the trial if a convincing history of menorrhagia was obtained. Virtually all subjects gave a history of passing "clots" and/or using 2 pads at a time and/or flooding underclothes or bedclothes. Almost all used "super" pads and/or tampons routinely. A full medical history and examination was recorded.

Considerable time was spent with each subject at the initial interview to explain the details of the trial and an information sheet was given. Each subject was provided with treatment bottles labelled A and B for the first two cycles and two detailed calendars for the daily prospective recording of menstrually-related symptoms, possible drug side-effects, number of capsules ingested and number of menstrual pads/tampons used. The women were treated for two cycles each with mefenamic acid and with placebo given in a randomised order. Subjects were seen again between cycles 2 and 3, and provided with bottles C and D and two further menstrual calendars. The subjects' perception of changes in menstrual symptoms, pad and tampon usage and any drug side-effects was carefully checked and recorded. Each subject was seen for a final interview after all 4 cycles were completed, and her perception of each aspect of the whole trial was carefully recorded before her individual treatment code was broken.

All subjects were carefully instructed to grade the severity of menstrually related symptoms on a four point scale (0,+,++ or +++) on a daily basis in each cycle, and to record the result on the chart each evening. Only 60 subjects provided perception data which was accurate enough for analysis. Subjects collected all their menstrual pads/ tampons on a daily basis in thick blue labelled polythene bags. They were very thoroughly instructed in the collection of all the menstrual flow including the necessity to avoid loss of "clots" down the toilet. Most subjects had no trouble with these collections.

Mefenamic acid was taken as 500 mg (two 250 mg capsules) three times daily with food from the onset until the end of menstruation. If menstruation was prolonged a maximum of 50 capsules was taken in any one cycle. In the placebo cycles two identical lactose-filled capsules were taken three times daily in the same manner.

Laboratory methods

Menstrual blood loss was measured using the alkaline haematin method of Hallberg and Nilsson (1964a) as modified by Newton et al (1977) and described in Chapter 9.

Statistical comparisons were made using the Student t test and a coefficient of variation. Measurements of menstrual blood loss have been recorded as mean ±SEM. The perception of day to day changes in measured menstrual blood loss in each category (e.g. + to ++, ++ to +++, etc) was averaged for each individual prior to comparison by Student t test. In assessing accuracy of perception from one cycle to the next an allowance was made for any error up to 15% compared with objectively measured losses. For example, if measured loss in one cycle was 80 mls, an error of ± 12 mls would be accepted as not being detectable in the subsequent cycle. Thus, any measured value between 68 and 92 mls would be regarded as indistinguishable from the prior cycle.

Results

The measured menstrual blood losses (mean of 2 consecutive placebo-treated cycles) of 69 women with a complaint of menorrhagia are illustrated in fig. 1. It should be noted that all these women gave a good clinical history of excessively heavy bleeding at the onset of the trial.

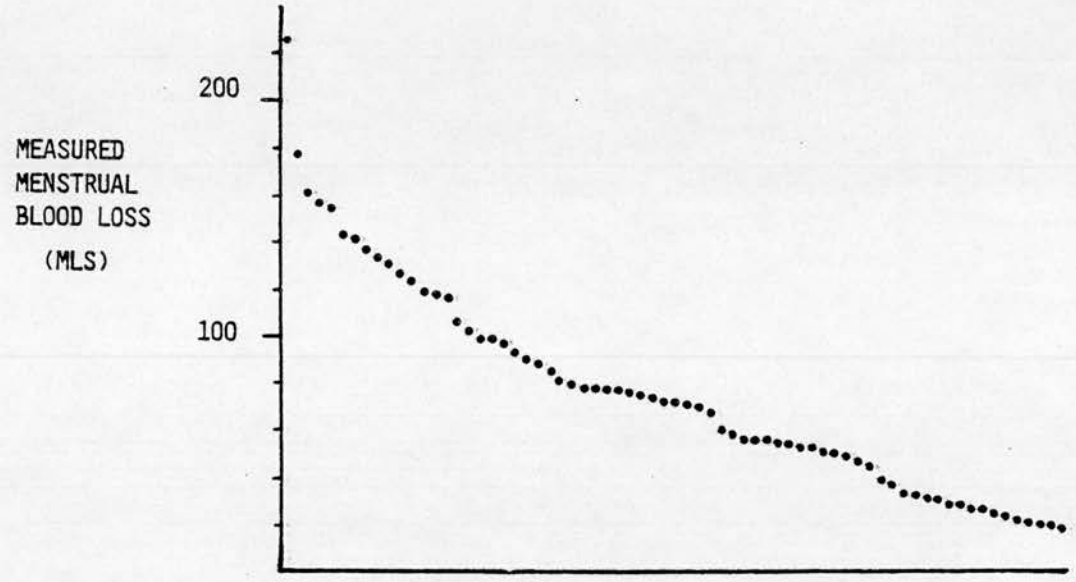


Figure 1. Measured menstrual blood loss (mean of 2 consecutive placebo treated cycles) of 69 women with a convincing complaint of menorrhagia.

However, only 26 (38%) had a mean loss greater than 80 mls, another 6 (9%) had one cycle greater than 80 mls and a further 9 (13%) had a mean loss between 60 and 80 mls. This gives a total of 41 (59%) who could be considered to have objective menorrhagia. It is noteworthy that 14 (20%) actually had a mean loss of less than 35 mls.

At midway and at the conclusion of the four study cycles each patient was asked to rank the menstrual periods from "lightest" to "heaviest" according to her perception of the volume of menstrual flow in each. Measured menstrual loss in 60 women with complete perception records in "lightest" through "heaviest" cycles was respectively 42.7 ± 4.7 mls; 49.5 ± 7.2; 61.5 ± 6.7; 69.6 ± 7.3 and the volume in the "lightest" cycles

was significantly less than in the "heaviest" cycles ($t = 3.098$; $p<0.001$). However, there were a large number of errors in the perception of lightest and heaviest cycles by individual patients, and only 45% of women correctly selected the order of all four periods from lightest to heaviest. Nineteen (32%) made an error in selecting the "lightest" period and 17 (28%) made an error in selecting the "heaviest" period. Nine (15%) selected the period with the heaviest measured loss as "lightest" or vice-versa, and in some individuals this represented a major error in volume assessment. The most unusual individual experienced small losses during placebo treatment (34.6 and 24.8 mls) and high losses during mefenamic acid treatment (70.6 and 148.0 mls), yet perceived both mefenamic acid treated cycles as being lighter than placebo cycles.

The duration of bleeding during each of the "lightest" and "heaviest" periods was compared. There was a small but not a significant difference between them (lightest = 4.7 ± 0.4 days; heaviest = 5.8 ± 0.8 days). Only 9 of the subjects had periods which regularly lasted longer than 7 days and their mean placebo cycle loss was 68.2 ± 9.4 mls. This was not significantly different from the measured loss of the remainder of the group (61.3 ± 4.9 mls).

Sixty subjects recorded the volume of blood lost each day on a four point scale of S (spotting or scanty), + (light), ++ (moderate), to +++ (very heavy). Measured menstrual blood loss and daily subjective rating are compared in Table 1. There is a highly significant difference in menstrual blood loss in the anticipated direction between the four different points on the daily rating scale in both mefenamic acid and placebo treatment categories. However, the range of measured differences in each category was extremely wide: S = 0.1 to 15.5 mls; + = 0.1 to 63.1 mls; ++ = 0.5 to 108.6 mls; +++ = 1.4 to 215.8 mls.

TABLE 1:

Comparison of measured daily menstrual blood loss and patients subjective rating of volume (mls; mean ± SEM; n = 60).

	PLACEBO TREATMENT	MEFENAMIC ACID TREATMENT	TOTAL
"spotting" (S)	2.1 ± 0.4 ***	2.8 ± 0.5 **	2.5 ± 0.3 ***
light (+)	6.0 ± 0.5 ***	5.4 ± 0.7 ***	5.7 ± 0.4 ***
moderate (++)	15.9 ± 1.3 **	16.3 ± 1.6	16.1 ± 1.1 *
very heavy (+++)	24.2 ± 2.4	19.6 ± 3.3	22.0 ± 2.2

t tests: * $p<0.02$; ** $p<0.005$; *** $p<0.001$

The ability of each individual to assess change in volume of blood loss from day to day was investigated by comparing the day to day difference in measured blood loss with the subjective ratings on successive days. These can be summarised for the group (n = 60) as follows: + to ++ = 13.1 ± 1.2 mls; ++ to + = 10.6 ± 0.9 mls; ++ to +++ = 15.6 ± 2.3 mls; +++ to ++ = 10.0 ± 2.6 mls; + to +++ = 24.9 ± 5.1 mls; +++ to + = 21.7 ± 4.6 mls. The measured difference for a perceived increase in bleeding from + to ++ compared with a major perceived increase from + to +++, was barely significant ($t = 2.252$; $p<0.05$). A similar result was found comparing perceived decreases from ++ to + with +++ to + ($t = 2.368$; $p<0.02$). Although these results indicate that the women as a group were able to make some distinction between the volume of menstrual blood loss on successive days there was a very large variation in day to day perception by most individuals. The day to day perception differences have been compared with the measured menstrual blood loss differences in Fig. 2. Figure 2a illustrates the day to day perceived differences after they have been averaged for each woman and compared with measured differences.

(a)

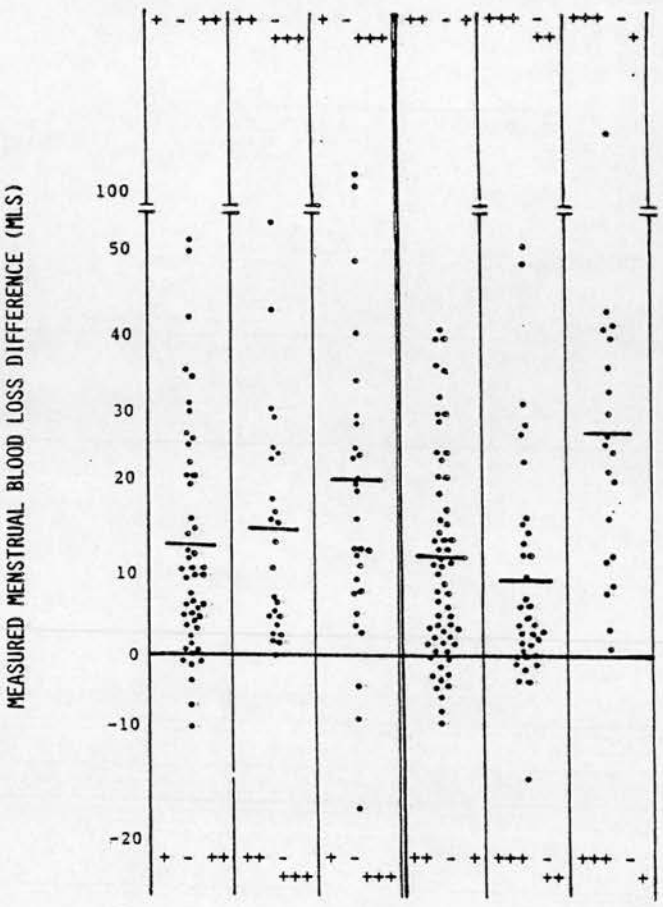
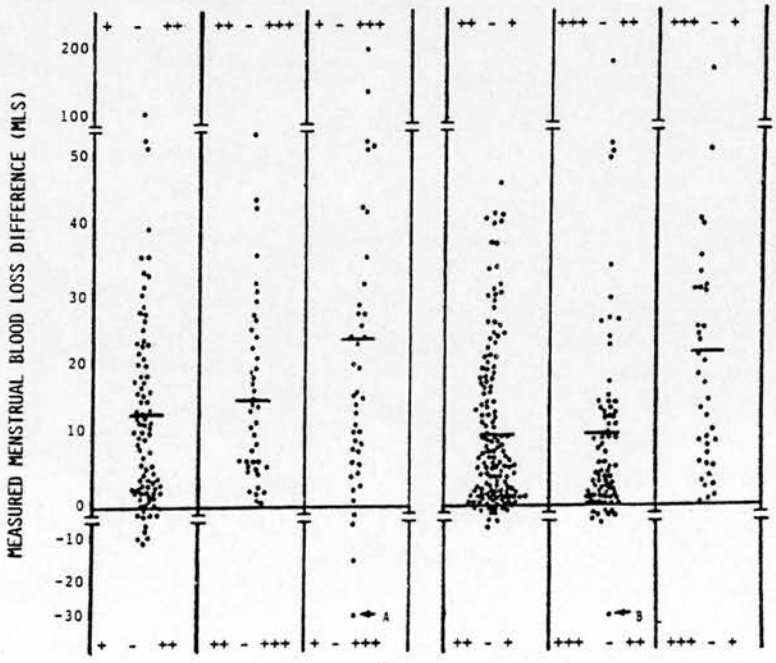


Figure 2:
Perceived day to day differences in menstrual bleeding compared with objective measurements of the actual differences in 60 women over 2 placebo-treated menstrual cycles.

(a) Average data for each woman so that each individual does not appear in any column more than once.

(b) All data from all cycles to indicate the range of variation in perception.

(b)



In figure 2b every day to day change in the whole trial has been recorded rather than using the patient as the unit for analysis. This clearly illustrates the difficulty which most women had in assessing individual day to day changes in blood loss. It is only necessary to look at the third column (+ to +++) in figure 2b to see that four subjects who actually experienced a reduction in blood loss have perceived this as a large rise (from light to very heavy)!

In discussing these changes with the women in retrospect it was not possible to identify any factors which were obviously responsible for influencing errors in perception. However, it was suspected that accompanying dysmenorrhoea might have some influence. This proved to be quite incorrect when perception was compared with measured menstrual loss on those days when pain was mild or absent and on those days when pain was recorded as moderately or extremely severe. When pain was mild or absent perception of volume of loss (+, ++ or +++) compared with measured loss was as follows: + = 7.5 ± 0.7 mls; ++ = 19.4 ± 1.6 mls; +++ = 26.6 ± 4.1 mls; and on days with severe pain the comparison was very similar: + = 6.3 ± 1.2 mls; ++ = 14.2 ± 1.6 mls; +++ = 23.6 ± 3.0 mls.

It seemed that age of the patient might also be a factor which influenced perception, and a clear trend was indeed identified. There were very few teenagers in the study so that a "younger" and an "older" group (aged 26 and under, n = 9; and 37 and over, n = 15) were selected for comparison. In the under 27 group perception compared with daily measured loss as follows: + = 5.1 ± 1.7 mls; ++ = 13.8 ± 2.4 mls; +++ = 11.6 ± 2.2 mls. In the over 36 group the corresponding figures were: + = 10.1 ± 1.6 mls; 20.1 ± 1.8 mls; 32.0 ± 3.3 mls. There was a significant difference between the age groups in their perception of moderate ($t = 2.118$; $p<0.05$) and very heavy loss ($t = 4.421$; $p<0.001$). The younger age group had the same measured loss for days which they had recorded as moderate (++) or very heavy (+++).

There was no significant correlation between the numbers of menstrual pads or tampons used per cycle and the measured menstrual blood loss in the same cycles for all 69 women followed over 4 cycles each (figure 3; $r = 0.138$; $p>0.1$).

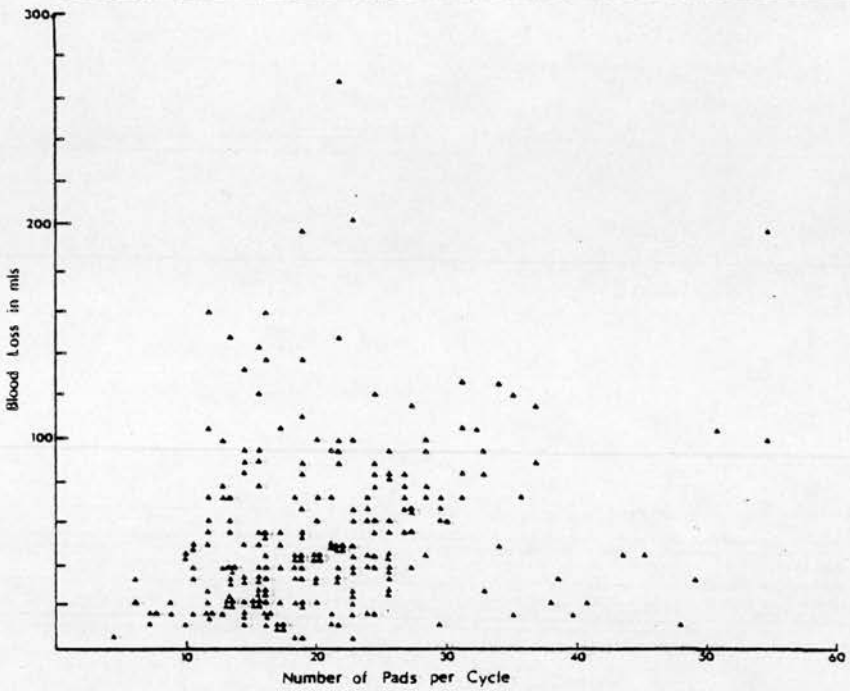


Figure 3:
Scattergram comparing average number of menstrual pads or tampons used per cycle from 2 placebo-treated cycles with the average measured menstrual blood loss per cycle from the same 2 placebo cycles.

In Table 2 it can be seen that the coefficient of variation of number of pads/tampons used per menstrual period is much less than the coefficient of variation of measured menstrual blood loss in the same periods, suggesting that the adjustment which most women make to their pad usage from cycle to cycle is less than any change in actual menstrual loss.

Five examples of the wide variation which can occur is given by the following examples:

1. Subject A used 58 pads/tampons in each of 2 successive cycles. Measured loss in one was 102.5 mls and in the next was 202.2 mls.

2. Subject B used 21 pads in cycle one where the measured loss was 101.1 mls. In cycle 2 she used 23 pads and measured loss was 275.8 mls. Six cycles later she also used 21 pads and the measured loss was 336.3 mls.

3. Subject C used 11 pads/tampons in cycle one where measured loss was 103.7 mls. In cycle 2 she used 8 pads/tampons and the measured loss was only 15.1 mls.

4. Subject D used 19 pads in cycle one where measured loss was 15.6 mls. In cycle two where the measured loss was also 15.6 mls she used 29 pads.

5. Subject E used 48 tampons in one cycle where the loss was 9.6 mls, and 2 cycles later used 42 tampons when the loss was 16.1 mls.

TABLE 2:

Comparison of pad/tampon usage with measured menstrual blood loss during menstruation (2 cycles with each treatment in each of 60 women).

	Coefficient of variation of number of pads and/or tampons per period	Coefficient of variation of measured menstrual blood loss	Mean (±SEM) pad/tampon usage per period	Mean (±SEM) measured menstrual blood loss per period (mls)
Placebo cycles	15.9%	36.7%	23.5 ± 1.2	64.0 ± 4.3
Mefenamic acid treatment cycles	25.1%	46.9%	20.3 ± 1.1	48.5 ± 4.6

DISCUSSION:

This treatment trial was carefully planned to allow an assessment of subjective perception by the patient of her menstrual blood loss for comparison with the objective measurement of changes in blood loss. The women were very carefully instructed and given an information sheet at the outset of the trial and were questioned again in detail midway and at the end of the trial.

The first striking finding was that the majority of women who were enrolled into the trial and who gave a clear history of excessively heavy bleeding did not really have objective "menorrhagia", if the criterion of over 80 mls per menstrual period is taken (Hallberg et al 1966). Hallberg and colleagues have calculated the 95th percentile of the normal range at 76.4 mls but

have indicated that some women who lose between 60 and 80 mls per cycle may also be at some risk of developing increasing iron deficiency. If this criterion is taken then 59% of the women in the present study could be said to qualify as suffering from true menorrhagia. Sixty mls may be a more realistic upper limit of normal. However, this still leaves a substantial proportion (41%) who cannot by any objective measurement be said to exhibit menorrhagia, but who still perceive their periods as being excessively heavy. Many of these women use large numbers of pads and/or tampons per period although some of these pads are barely discoloured after use. In one extreme case 48 tampons were used when the measured loss was only 9.6 mls. This finding accords with the Swedish population study of Hallberg et al (1966) who recorded that 45% of the women who reported "heavy" periods had measured blood loss of less than 40 mls, and 13% less than 20 mls. However, it is not so clearly in agreement with the study of Haynes et al (1977) who investigated women presenting to a gynaecological clinic in the United Kingdom with heavy periods. These authors found that 53% of the women had a measured loss which was repeatedly greater than 80 mls and of the 24% with measured loss consistently less than 80 mls almost all admitted to a recent subjective decrease in loss and required no treatment.

The present study indicates that when a woman states that she has had very scanty bleeding or spotting on a particular day she will probably have lost about 2.5 mls. However, the range of daily blood loss volumes recorded for "scanty" or spotting was from 0.1 up to 15.5 mls! Findings in the other 3 categories indicate a similar wide variation: with "light" bleeding equivalent to 5.7 mls (0.1 to 63.1 mls); moderate = 16.1 mls (0.5 to 108.6 mls); very heavy = 22.0 mls (1.4 to 215.8 mls).

It has been suggested that some women confuse duration of menstruation with "heaviness" of the menstrual flow (World Health Organization 1979) and it has been demonstrated previously that women with longer menstrual periods do tend

to have a heavier loss (Barer and Fowler 1936). No evidence for a relationship between perception of volume and duration has been found in the present study. It is generally accepted that "normal" menstrual periods may last between 2 and 8 days (Guillebaud and Bonnar 1978), and in this study only 13% of subjects had a menstrual flow of greater than 7 days. Also in this study, it has not been possible to implicate simultaneous dysmenorrhoea as a factor in the incorrect perception of heavy menstrual bleeding. It does seem likely that age may be a factor in the perception of heavy bleeding, in that younger women may tend to have a lower tolerance of heavy bleeding, or older women may tend to accept heavier bleeding than they would have been prepared to tolerate when younger. The younger women in this study did have lighter measured menstrual loss than the older women and also perceived their daily menstrual loss as being excessively heavy when the older women perceived a similar loss as light or moderate. Larger population studies will be needed to confirm these findings.

It was demonstrated many years ago (Barer and Fowler 1936) that the number of "napkins" used per menstrual period was not very reliable as a measure of the volume of menstrual flow in an individual. However, that study did suggest that in general the number of napkins used paralllelled the menstrual blood loss. The criterion of pad counting has been successfully used to demonstrate the response of patients with menorrhagia to treatment (Jacubowicz and Wood 1979), but under most circumstances this method cannot be considered satisfactory. In the present study no significant relationship could be demonstrated between pad and tampon counts and the measured blood loss, and there were some extreme examples of lack of correlation in individuals. Some of the women in this study felt that at some stages of their period they were losing fluid which was very dilute compared with pure blood. It appeared that the perception of volume might be based partly on a loss of endometrial, cervical or vaginal fluid which was not measured by a method

based on detection of haemoglobin. In normal women the percentage contribution of blood to the total menstrual loss varies greatly from subject to subject with a mean of only 35% (Chapter 9). No information is available in women complaining of menorrhagia. It has been stated that completely wet pads will absorb approximately 90 mls of blood while a tampon will absorb approximately 40 mls (Beller and Schweppe 1979), but it is quite obvious that no woman will allow a single pad or tampon to reach such a degree of saturation. Therefore, it is likely that frequency of changing of pads and tampons will be influenced by many factors apart from volume of flow, and these may include personal factors (ideas of hygiene and cleanliness), age, racial factors, education, climate, physical activity, menstrually-related urinary frequency or diarrhoea, availability of toilet facilities and, recently, recommendations about tampon use in relation to toxic shock syndrome.

This trial has demonstrated that many women have great difficulty in determining whether their menstrual loss is heavy or light and in detecting any change in blood loss from one cycle to the next and from one day to the next with any accuracy. There is much need for a detailed study of those factors which influence each woman's perception of her blood loss, with a view to defining, if possible, means of obtaining a more accurate historical assessment of menstrual blood loss.

Many doctors are not aware of the extent of errors in menstrual perception and it seems likely that large numbers of hysterectomies are performed for "menorrhagia" which cannot be objectively confirmed. One physician has even gone so far as to say "I am unaware of any other important organ that is electively removed without first assessing its degree of malfunction" (Greenberg 1981). In Australia it is calculated that about 40% of women will undergo hysterectomy (Selwood and Wood 1978) and in many the indication is likely to be "menorrhagia".

It should be recognised that, at present, the only reasonably accurate means of assessing volume of menstrual blood loss is to measure it by an objective technique such as that described by Hallberg and Nilsson (1964a) and modified by Newton et al (1977). This should become a standard part of the investigative armamentarium of the gynaecologist.

SECTION 5

ENDOMETRIAL BLOOD FLOW

ENDOMETRIAL BLOOD FLOW:

Two methods utilizing inert gas clearance have been developed for the measurement of endometrial blood flow (EBF) in non-pregnant women. A Krypton-85 rebreathing technique allowed measurement of superficial EBF by use of an intra uterine semiconductor β-detector probe. Intra-uterine instillation of Xenon-133 in saline allowed EBF measurement by gamma camera recording of the rate of clearance. Both these methods permitted calculation of absolute rates of EBF and comparison of cyclical changes.

Several types of equipment have been developed and evaluated in women and a computer analysis programme has been developed. The two techniques have been directly compared in 3 women with close correlation, and the Xe-133 technique has been carried out on 2 or 3 successive occasions on the same day in 5 women with similar close correlation. Vaginal blood flow measured by Xe-133 clearance (3 women) was always much lower than EBF measured simultaneously by Xe-133 clearance in the same women.

The Kr-85 (10 comparisons) and Xe-133 (11 comparisons) methods have both been assessed by direct comparison with a radioactively-labelled microsphere (15 μ diameter) technique in sheep. In seven instances, the Kr-85 clearance curve had only one component while in the remaining 3 instances the curves could be resolved into 2 components. The flow rates derived from the single component curve and the mean flow rates calculated from the curves with 2 components were of the same order as, and highly correlated with, estimates of capillary blood flow obtained with microspheres for either the caruncles,* the intercaruncular endometrium or the total endometrium ($r = 0.832$, $r = 0.822$ and $r = 0.841$, respectively; $p<0,005$). There was no correlation between the flow rates obtained with the Kr-85 technique and estimates of myometrial capillary blood flow obtained with microspheres.

Seven of the Xe-133 clearance curves in sheep exhibited

* Caruncles are specialised areas of endometrium found in sheep and related species on which placental adherence occurs during pregnancy.

2 components ("fast"and"slow") with flow rates approximating the rate of capillary blood flow in the caruncular and inter-portions of the endometrium, respectively. In each of the remaining 4 curves which provided a single estimate of flow, the derived blood flow rate was similar to the rate of capillary blood flow in both the caruncular and inter-caruncular portions of endometrium.

Overall comparison with caruncular ($r = 0.974$; $p<0.001$) and intercaruncular ($r = 0.959$; $p<0.001$) flow was excellent, and the respective mean flow rates did not differ significantly. No relationship existed between myometrial capillary blood flow and the flow rates derived from Xe-133 clearance curves.

The bicornuate uterus of the ewe allowed an assessment of local effect of an intrauterine probe on endometrial capillary blood flow. A significant elevation of local endometrial flow was found in the region in contact with the probe while myometrial flow was usually unchanged.

A detailed study of EBF was carried out in 17 women with normal menstrual cycles (28 estimations) and 20 women with dysfunctional uterine bleeding (32 estimations; 6 anovulatory and 14 ovulatory women). A mean blood flow of 27.7 ml/100 g/min (± 2.6, SEM) was recorded in the women with normal cycles, but marked changes were seen at different phases of the menstrual cycle: early follicular phase 19.3 ml/100 g/min ($n = 6$); mid follicular 39.6 ± 5.5 ($n = 8$); periovulatory 12.5 ($n = 4$); early luteal 23.5, ($n = 3$), mid luteal 32.8 ($n = 4$); late luteal 30.1 ($n = 3$). A significant correlation with plasma oestradiol levels was found in the normal follicular phase ($r = 0.760$; $p<0.05$). Luteal phase EBF did not correlate with plasma levels of oestradiol or progesterone.

Similar cyclical changes were seen in women with ovulatory DUB, but mid follicular phase EBF was significantly lower than in controls (25.2 ± 3.5 ml/100 g/min; $n = 9$; $p<0.05$). As with normal controls a moderately high rate of EBF was recorded in the late luteal phase (38.8 ml/100 g/min). Anovulatory women exhibited variable and frequently elevated rates of EBF.

Chapter 11

METHODOLOGY OF ENDOMETRIAL BLOOD FLOW MEASUREMENT IN WOMEN:

INTRODUCTION:

It seems probable that there is a relationship between the volume of blood lost at menstruation and the rate of blood flow through the endometrium over the same period of time. However, there is virtually no information on changes in endometrial blood flow through the menstrual cycle in women with normal or abnormal cycles.

Several techniques have been used in an attempt to study different aspects of human uterine blood flow. Electromagnetic flowmeters can give a measure of flow through a single uterine artery for a short period of time during operation and immediately following a period of acute handling of the vessel (Klingenberg 1973). A variety of clearance techniques have been investigated including heat (Loeser 1948; Prill and Götz 1961; Åkerlund, Bengtsson and Carter 1975) hydrogen (Klingenberg 1969; 1974) and Xenon-133 (Munck et al 1964; Jansson 1969; Secher et al 1973; Forssman 1973). Heat clearance allows repeated or continuous measurements of relative changes in flow in the conscious patient but does not give an absolute measure of flow. However, it is sensitive to very rapid changes in flow. The inert gas clearance techniques will theoretically give a measure of absolute flow in a portion of tissue, and can be used repeatedly but not continuously in the conscious woman. Inert gas clearance can only be used to assess slow changes in flow rate (e.g. hour to hour rather than minute to minute). There are no published studies where two methods have been simultaneously compared, and with some techniques it is not always clear exactly what is being measured.

In this chapter two different inert gas clearance techniques are described. These methods should both be capable of providing an absolute measure of blood flow in the human endometrium with safety and with only minor

inconvenience to the subject. Validation of these techniques in ewes is described in Chapter 12. Results of application of the Xenon-133 clearance in women is described in Chapter 13.

The work described in Chapter 11, 12 and 13 was designed, executed and analysed solely by the author apart from valuable technical assistance from Dr P. E. Mattner and Mr B. W. Brown with the sheep experiments, Dr D. Macey with refinement of the clearance techniques and Mr B. Hutton with development of the computer programmes.

MATERIALS AND METHODS:

Isotope characteristics and radiation hazards.

1. Krypton-85 (Kr^{85})

Greater than 99% of radioactive emission is in the β-particle range; maximum energy 670 KeV. Maximum range of penetration is about 230 mg/cm^2 which is equivalent to about 2.2 mm in tissue. It is calculated that greater than 90% of the observed counts by a surface β-detector arise from the superficial 0.7 mm of tissue (Thorburn, Casey and Molyneux 1966). Less than 1% of radioactivity is ɣ-emission with peak energy of 514 KeV. Radioactive half-life is 10.6 years.

2. Xenon-133 (Xe^{133})

Main radioactive emissions is in the ɣ-range with an energy peak at 81 KeV. A weak β-particle with energy of 340 KeV (maximum tissue penetration of 1.1 mm) and ɣ-rays of 34 KeV are also released. Radioactive half-life is 5.3 days. Gamma rays will penetrate through soft tissue without significant attenuation .

3. Krypton and Xenon are both biologically and chemically inert, non-toxic gases which diffuse very rapidly through tissues. Ninety five percent of each is excreted from the bloodstream in one passage through the lungs (Bentivoglio et al 1963), and therefore recirculation is minimal.

Although fat has a 9-fold greater affinity for these gases than other body tissues, it is still rapidly cleared from fat into the bloodstream as blood levels are reduced through excretion by the lungs. The equilibrium concentration of Xenon in air in contact with water is 10 times higher than the concentration in water (Ladefoged and Andersen 1967). The principles of inert gas washout techniques have been studied in several tissues (Kety 1951; Lassen 1971; Hoedt-Rasmussen and Veall 1971).

Radiation hazards are very low. For example a 1 m Ci intravenous injection of Xe^{133} delivers only 14 mrads to the lung and 0.1 to 0.4 mrads to the gonads (International Commission on Radiological Protection 1971). Intrauterine Xe^{133} (100-200 μCi) will give a gonadal radiation dose of less than 0.5 mrads (Forssman 1974). Rebreathing techniques for Krypton-85 and Xenon-133 will give slightly higher gonadal exposures from 0.7 to 1.2 mrads (calculated from data of Jones 1950). Lung exposure in rebreathing techniques with 0.35 m Ci/ℓ is 10-20 mrads.

Isotopes were obtained commercially from the Radiochemical Centre, Amersham, Bucks, from New England Nuclear, Boston, Mass., and as a special order from the Australian Atomic Energy Research Establishment, Lucas Heights, New South Wales.

Description of techniques.

1. Krypton-85 clearance from the endometrium measured with an intrauterine detector probe sensitive to β-particles.

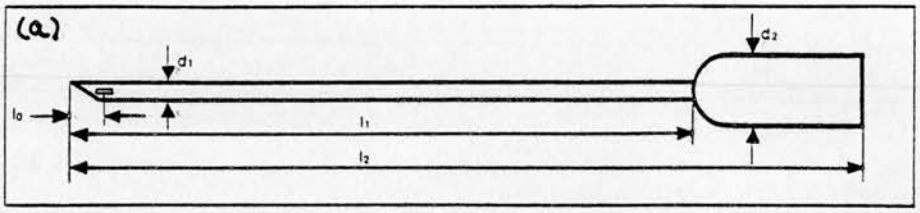
In principle the endometrium is partially saturated to a steady state with Krypton-85 while a sensitive β-detector probe lies in the lumen of the uterus. Krypton administration is then abruptly ceased by switching the rebreathing apparatus to open circuit. The gas in solution in endometrial tissue is then removed at a rate which is determined by the rate of blood flow through the endometrial capillaries, by the rate of transfer of Krypton across cell membranes and through tissue (which is rapid) and by the blood/tissue

partition coefficient. The rate of this tissue "washout" from a cylindical core of endometrium 1-2 mm thick is detected by the luminal β-probe, and the blood flow can then be calculated (see below).

An intrauterine probe of diameter less than 4 mm is inserted througth the cervix until the fundus is gently touched and then withdrawn 2-3 mm. Antiseptic precautions are observed as for insertion of an intrauterine device, the cervix being cleaned with 1% Cetrimide solution and the probe previously being sterilised by immersion in a 3% Cetrimide solution. Care is always taken to minimise handling of the uterus and a tenaculum is not used if it can be avoided. In this way it is hoped to minimise iatrogenic influences on local blood flow. The patient is allowed 15 minutes resting time following insertion before any recordings are made, and flow measurements are always carried out in a quiet environment.

Background counts are measured twice before the subject partially saturates her tissues by rebreathing Kr^{85} (2 m Ci in air) from a 5 litre closed system spirometer for 90 seconds. Preliminary studies indicated that 60 seconds was sufficient to achieve maximum counts. The three way valve is then switched to open circuit through an outdoor vent and excretion of absorbed Kr^{85} permitted through lungs. The rate of decrease in radioactivity in the endometrium is detected by the luminal β-probe and registered by standard recording equipment at 10 or 20 second intervals over a 45 minute period. The analysis is discussed below.

Three semiconductor β-detector probes were tested inside the uterus. The most sensitive was a Studsvik needle detector (Type 5435-B-100; A.B. Atomenergi, Nyköping, Sweden) of outside diameter 2.2 mm with a lithium drifted silicon detector of sensitive volume 12.5 mm^3 (figure 1). This was connected through a preamplifer and amplifer to a standard Ortec Counting System (discriminator, single channel analyser, ratemeter and printer; Ortec Inc.,



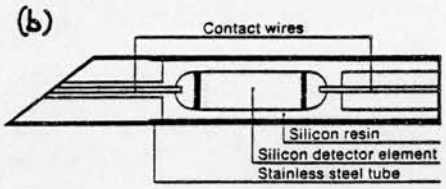


Figure 1:
Studsvik needle detector (type 5435-B100)
(a) complete probe in outline (l_o = 8mm; l_1 = 100 mm; l_2 = 114 mm; d_1 = 2.2 mm; d_2 = 4.8 mm)
(b) Enlarged probe tip containing detector.

Oakridge, Tenn, USA). This gave acceptable β-sensitivity for the purposes of this study, but manufacture of the probe was discontinued in 1975 and further supplies could not be obtained. A 4 mm probe was developed specially by the United Kingdom Atomic Energy Research Establishment, Harwell, Bucks, but even with careful attention to the amplification system could not be made sensitive enough to detect the small amounts of Kr^{85} β-particle emission from the endometrium. A third type of semiconductor probe of teflon coated catheter type and 3 mm external diameter (Catelix probe, Tokyo Shibaura Electic Co; Ueda et al 1969) was found satisfactory for the high levels of Kr-85 administered in sheep (Chapter 12) but was not adequately sensitive for human studies.

2. Xenon-133 clearance from saline solution instilled into the uterine cavity and recorded by a gamma camera.

In principle, Xe^{133} is instilled in a small volume of normal saline solution into the uterine fundus. This is followed by steady uptake into endometrial tissue and clearance into the venous circulation followed by pulmonary excretion. The depth of diffusion before removal by the circulation is unknown and was a concern before animal testing was carried out (Chapter 12). Radioactivity in the uterine cavity is detected by an external gamma camera

(Searle Pho-Gamma 4; with 28 cm field and a high sensitivity collimator) and blood flow calculated (see below).

A fine catheter with moderate pliability (Portex Instruments, Hythe, Kent; Size 4FG, external diameter: 1.34 mm) is inserted through the cervix in a manner which has recently become popular with embryo transfer technology. Immediately before insertion the catheter is filled with Xe^{133}-saline solution from a preloaded syringe whose radioactivity has been measured, and extreme care is taken to ensure that there is no air bubble at the tip. Care is taken to minimise manipulation of the uterus and a tenaculum is not used. The cervix is gently cleansed with 1% cetrimide solution before insertion. The catheter is advanced slowly until the fundus is lightly touched and then withdrawn about 2 mm. Accurate positioning under the gamma camera is then confirmed (figure 2). Xe^{133} (100-400 μCi) is instilled within 5 seconds in a volume of approximately 50 μl of normal saline solution. The catheter is then withdrawn slowly and recording immediately resumed. Background counts are recorded prior to insertion of the catheter and subtracted from counts before analysis. The rate of clearance of radioactivity is recorded over a 45 minute period by a computer interfaced with the gamma camera. Preliminary studies were recorded as described in Chapter 12 and analysed by hand.

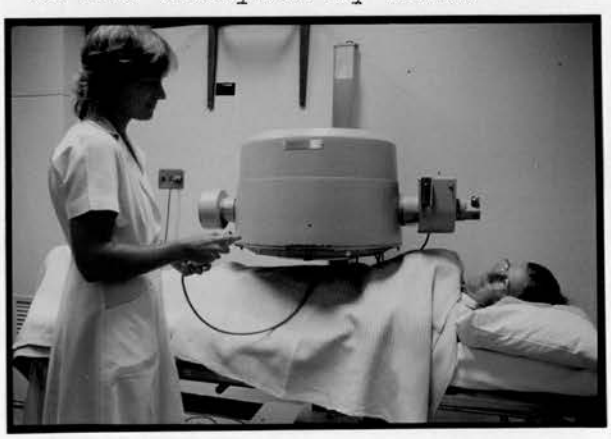


Figure 2:
Gamma camera centred over lower abdominal region of subject about to undergo endometrial Xe-133 clearance studies.

THEORETICAL CONSIDERATIONS:

The theoretical aspects of inert gas clearance from tissue have been well described and evaluated by Kety (1951) and have been applied by a number of investigators to the study of human myometrial (Munck et al 1964; Jansson 1969; Klingenberg 1969; Secher et al 1973; Forssman 1973) and endometrial blood flow (Secher et al 1973).

In applying these methods it is assumed that a highly diffusible inert gas such as Xenon-133 or Krypton-85 will achieve diffusion equilibrium between blood and tissue in a single passage through a capillary bed, provided the intercapillary distance is relatively small (Kety 1951). Under these conditions, the rate of clearance of Kr^{85} or Xe-133 will provide a measure of nutrient or capillary blood flow. Flow through arterio-venous anastomoses will not be measured.

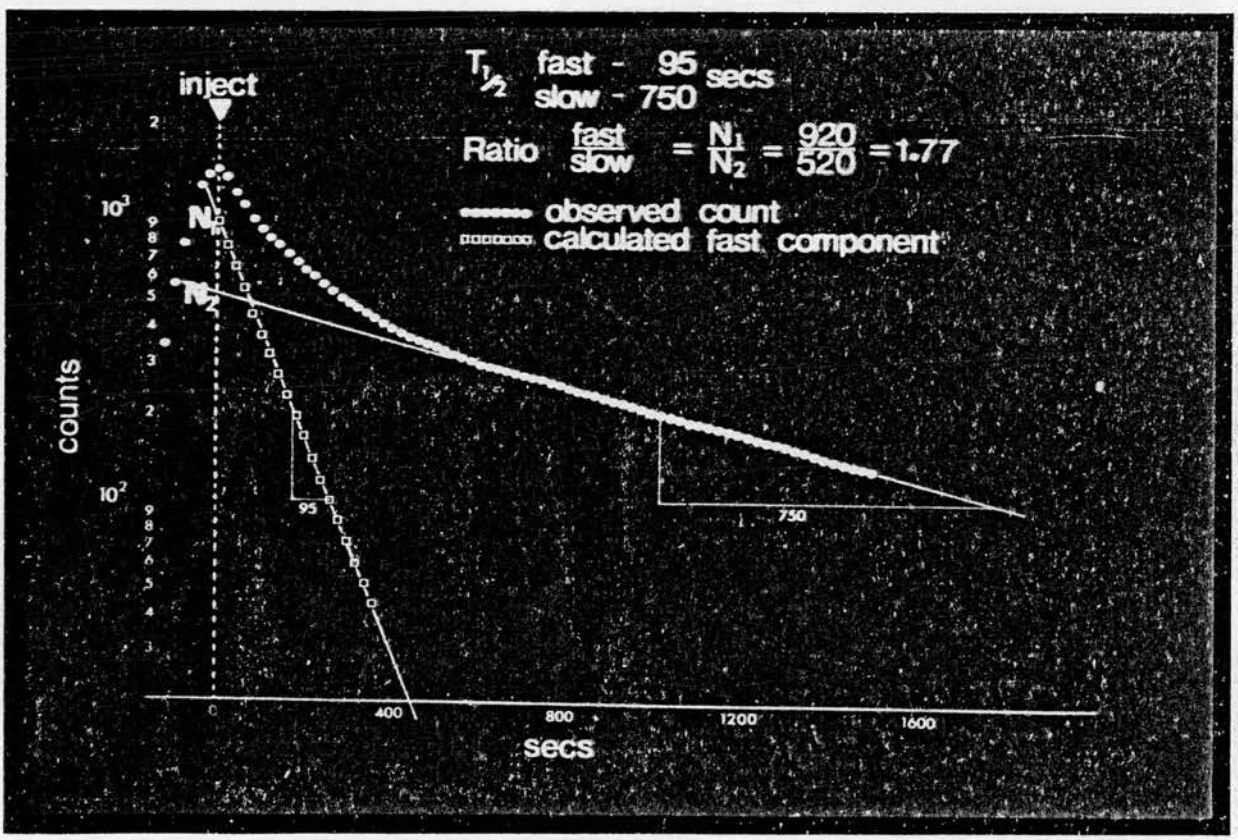


Figure 3. Simplified Xe-133 clearance curve recorded by gamma camera following intrauterine instillation of 200 µCi in 0.05 mls of saline; also illustrating the method of calculation of fast component and the relative sizes of the two compartments (N_1 & N_2).

The washout curves are analysed graphically by hand or by computer into 2 or 3 single exponential components on log-linear scales (figure 3). The analysis of the curves obtained assumes a bimodal distribution of blood flow into 2 or 3 distinct homogeneously perfused compartments which are arranged in parallel. The flow rates (F) in each compartment are calculated from the following equation assuming a tissue/blood partition coefficient for Krypton and Xenon of 1.0 (Thorburn, Casey and Molyneux 1966; Chapter 12), and a specific gravity for endometrium of 1.0:

$$F = 100\ k\ ml/100\ g/min$$

where $$k = \frac{0.693}{t_{\frac{1}{2}}}$$

and $t_{\frac{1}{2}}$ = the half time of the exponential in minutes

The relative sizes of each of the compartments as a percentage of total tissue mass (m'% and m"%) was calculated by back extrapolation of the exponents to time zero to obtain the initial distribution of counts to each compartment (Ao' and Ao"):

$$Ao' = \frac{F' \times M'\%}{F'' \times M''\%}$$

And the mean endometrial blood flow ($\bar{F}$) from the relation:

$$\bar{F} = \frac{(F' \times m'\%) + (F'' \times m''\%)}{100}$$

Experimental data from Chapter 12 indicate that the mean flow obtained using both compartments gives a more accurate assessment of total endometrial flow for intra luminal Xe-133, while the slow compartment (compartment I) can be discounted for intraluminal probe use with Kr-85.

Analysis of curves by hand involves initial identification of the slowest component (after background subtraction) which is then subtracted from the original raw data curve to leave the faster component. In occasional experiments an ultra fast 3rd component was identified when component 2 was

subtracted. The existence of different components or compartments points to the existence of portions of endometrial tissue with different blood flow rates. The time in minutes taken for counts in each component to fall by 50% is recorded as washout half time ($t_{\frac{1}{2}}$).

Analysis is now carried out by computer programme, although initial studies described in this chapter were plotted and calculated by hand. Data were analysed with a computer programme (modified by Dr. Brian Hutton) by fitting a multi-exponential curve by the least-squares method utilizing an iterative function minimisation programme (Powell 1964). The formula for the washout curve was:

$$A(t) = \sum_{j} [Bj \exp(\lambda jt]$$

Where A is the activity at time t and Bj and λj (j = 1,2 or 3) are the unknown constants to be estimated for a 2 or 3 component exponential.

Early development of the computer programme allowed variable sized moveable windows to be drawn around the area of maximum radioactivity on the visual display screen. This allowed an assessment of the extent of any leakage into the vagina (which occurred only once, during the study series in a patient who was menstruating at the time) or into the Fallopian tubes (not detected). The computer used was manufactured by Digital Equipment Corporation (Model PDP 11/34 Gamma II). Steps in the computer analysis are illustrated in Figure 4.

Ethical approval for studies described in Chapters 11 and 13 was obtained from the Medical Research Council (United Kingdom), University of Oxford, Oxford Area Health Board, National Health and Medical Research Council (Australia), Australian Radiation Laboratories, Royal Prince Alfred Hospital Ethics Review Committee and the University of Sydney Medical Ethical Review Committee. It was recommended that no more than 3 separate measurements be carried out in each individual subject.

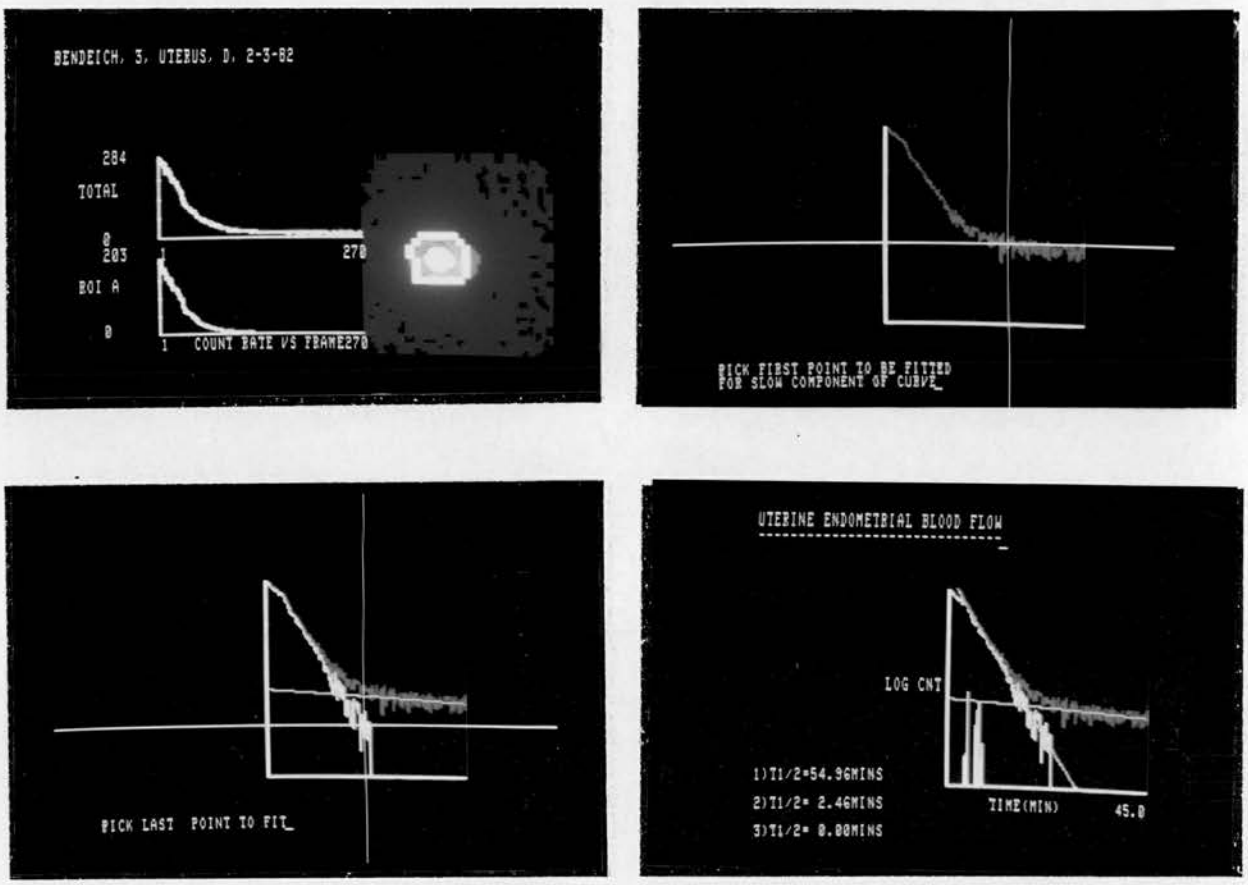


Figure 4. Calculation of Xe-133 clearance data using computer.

(a) Xe-133 clearance over 45 minutes (10 second segments) from the specifically delineated uterine area (ROI A) and total screen area.

(b) Log-linear plot of count rate against time over 45 minutes. Computer instruction is asking for manual selection of the section of the curve most representative of the slow component.

(c) Computer has automatically subtracted the slow component and is asking for selection of the section of subtracted curve most likely to represent a straight line fast component.

(d) Computer has automatically calculated clearance data for each component and checked the best fit to the original data by the method of least squares; full details of the data appear on the typed print-out.

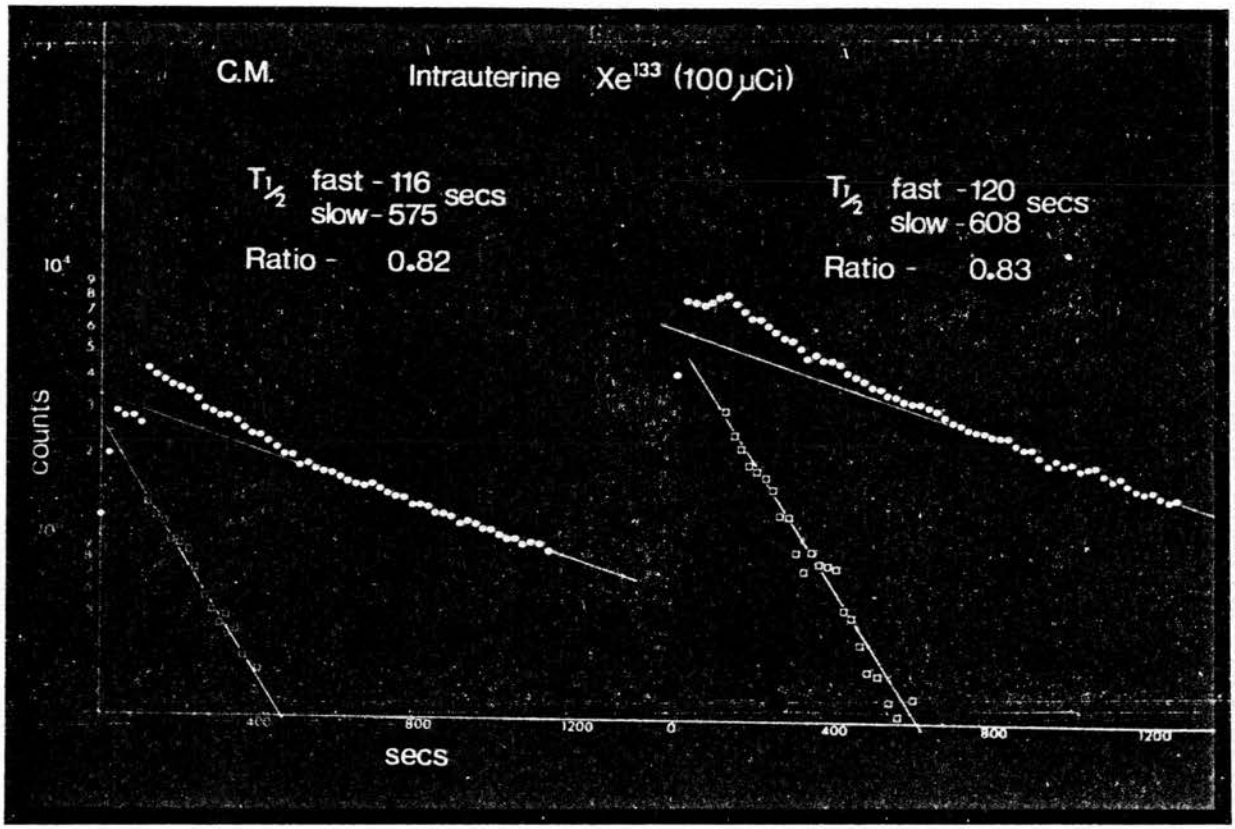


Figure 5. Two successive Xe-133 clearance curves from the same subject carried out 20 minutes apart. Only the first 20 minutes of each curve is shown.

Informed consent to participation was obtained by the author from all individuals who took part in the studies, and a detailed information sheet was provided.

RESULTS:

Krypton-85 rebreathing.

Six women were studied with this method utilising the Studsvik needle probe (Table 1). The slow component (component 1) was always greater than 50 mins. Flows varied between 14.1 and 59.2 ml/100g/min. Insufficient numbers were studied to assess menstrual cycle changes with any accuracy, but three of these women also had intraluminal Xe-133 studies on the same day (Table 2). There was a close correlation between the two methods, although blood flow measurements using Kr-85 were higher than the Xe-133 calculations in all cases.

Intraluminal Xenon-133 instillation.

A series of investigations were carried out to assess the validity of the technique.

(i) Repeatability was assessed by giving 2 successive instillations to 5 women on the same day (Table 3; Figure 5). An excellent correlation was obtained; the mean (±SEM) of the first measurement being 28.9 (± 6.0 ml/100g/min) compared with 27.3 ± 4.9 for the second measurement.

(ii) Volume of instillation fluid was increased in 3 women until spill was recorded in the Vagina by the gamma camera. Volumes of 0.1; 0.5 and 1.0 mls were used. Spill into the vagina was recorded in all cases with 1.0 ml, in one case with 0.5 ml and no cases with 0.1 ml. The only exception to this rule was seen later when one woman on day 2 of menstruation expelled a small volume of isotope into the vagina about 3 minutes after instillation of Xe-133 in less than 0.1 mls of saline.

Spread within the endometrial cavity was assessed by instilling 0.1 mls of crystal violet solution into the uterus of 3 women after hysterectomy and measuring the area of spread

TABLE 1:

Endometrial blood flow measured by Kr-85 rebreathing Technique in 6 women.

Subject	Age	Cycle Length	Cycle day during study	Plasma Steroid Levels E_2 pg/ml	Plasma Steroid Levels P_4 ng/ml	Endometrial Histology	Kr-85 component 2 $t_{½}$ (secs)	Kr-85 component 2 Flow (ml/100g/min)	Idealised cycle day
K-1	41	25-30	7	555	<1	P	1.17	59.2	10
K-2	42	20-29	13	—	—	ES	2.80	24.8	17
K-3	46	25-28	22	18	17.5	MS	3.07	22.6	24
K-4	37	29-33	30	230	5.2	LS	2.53	27.4	26
K-5	28	26-33	12	620	<1	LP	1.23	56.3	12
K-6	40	29-34	17	140	2.1	ES	4.93	14.1	15

P = Proliferative; LP = Late proliferative; ES = Early secretory
MS = Mid secretory; LS = Late secretory

TABLE 2:

Comparison of blood flow measurements in the same women using Kr-85 rebreathing and Xe-133 Instillation in succession on the same day.

Subject Number (Kr-85 Study)	Subject Number (Xe-133 Study)	Kr-85 Blood Flow (ml/100g/min)	Xe-133 Blood Flow (ml/100g/min) Component 1	Component 2	Mean
K-1	C1	59.2	1.9	55.7	53.5
K-2	OD2	24.8	3.3	22.9	21.3
K-5	C3	56.3	3.9	49.1	48.0

TABLE 3:

Repeated measurements of endometrial blood flow on the same day with intraluminal Xe-133 in the same women.

Subject Number	1st measurement Component 1 $t_{1/2}$	Component 1 F'	Component 2 $t_{1/2}$	Component 2 F"	Mean Flow $\overline{F}$	2nd measurement Component 1 $t_{1/2}$	Component 1 F'	Component 2 $t_{1/2}$	Component 2 F"	Mean Flow $\overline{F}$
X-1	9.6	7.2	1.9	35.9	20.1	10.1	6.8	2.0	34.7	22.8
X-2	18.6	3.7	1.7	40.8	38.2	22.8	3.0	1.9	36.3	33.8
X-3 (C3)	17.9	3.9	1.4	49.1	48.0	26.2	2.6	1.6	44.1	43.2
X-4 (C6)	15.7	4.4	3.6	19.2	17.1	16.3	4.3	3.5	20.1	18.3
X-5 (OD4)	23.7	2.9	3.2	22.0	21.1	28.5	2.4	3.5	19.9	18.6
Mean					28.9					27.3
SEM					6.0					4.9

after 5 minutes. Spread was a little irregular but in 2 instances reached the region of one tubal ostium and in each case was 2-3 cms diameter.

(iii) Air bubbles were a major concern because of the possibility of Xe-133 sequestration in a bubble. In one case a normal washout was obtained and then followed by a washout when 0.1 mls of air was also deliberately instilled. The washout component half times changed from 78 and 320 secs to 102 and 560 secs suggesting that very small volumes of air are unlikely to make major differences.

(iv) Vaginal blood flow was compared with endometrial blood flow at the same time in 3 women (Table 4) utilising instillation of 0.1 mls of Xe-133 in saline into the posterior fornix. Those instillations were clearly shown as separate areas of γ-activity on the computer screen (figure 6) and could be analysed independently. The vaginal slow component was small and greatly prolonged in 2 cases and absent in one.

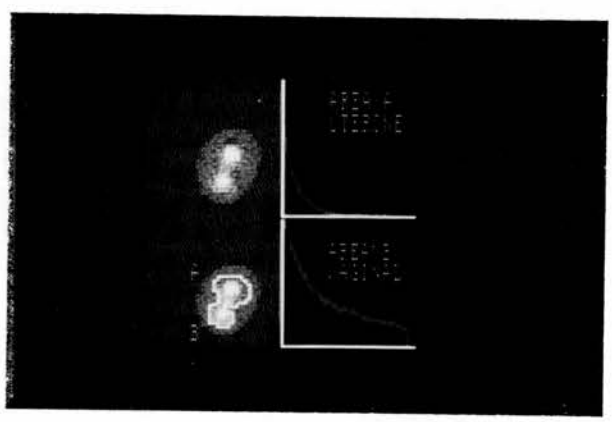


Figure 6. Gamma camera recording of Xe-133 instillation simultaneously into uterine cavity and vagina. Clearance curves for each area (A = uterine; B = vaginal) are shown separately. Clearance from the vagina is substantially slower than from the uterine cavity.

TABLE 4:

Simultaneous intrauterine and intravaginal instillation of Xe-133 in 3 women with measurement of local blood flow.

Subject Number	Intrauterine Component 1 $t_{½}$	Intrauterine Component 1 F'	Intrauterine Component 2 $t_{½}$	Intrauterine Component 2 F"	Intrauterine Mean Flow $\overline{F}$	Intravaginal Component 1 $t_{½}$	Intravaginal Component 1 F'	Intravaginal Component 2 $t_{½}$	Intravaginal Component 2 F"	Intravaginal Mean Flow $\overline{F}$
1	44.4	1.6	2.3	30.5	30.1	>100	< 1	4.7	14.7	14.7
2	14.7	4.7	2.8	24.8	23.5	80.4	1.7	6.1	11.4	8.5
3	15.5	4.5	4.6	15.1	14.6	75.5	1.4	5.9	11.7	9.4

Mean vaginal flow was substantially less than endometrial flow in all cases but they were not closely correlated. Statistical comparisons were not attempted in view of the very small numbers.

(v) Computer analysis of components using the method of least squares was compared with curve fitting by eye in 10 cases (Table 5) with generally close correlation. In all cases except one computer calculated mean flow was less than that derived from curve fitting by eye. Computer-derived mean flow was 24.5 ± 2.1 (SEM, ml/100 g/min) and eye-derived mean flow was 26.9 ± 2.3. This is unlikely to lead to major variation by comparison with biological variation in flow.

DISCUSSION:

Two techniques for the measurement of human endometrial blood flow have been described in this chapter. Both techniques give a valid measure of endometrial blood flow (see Chapter 12), but each may be measuring blood flow in different depths of tissue. Kr-85 may only give a measure of very superficial endometrial flow while Xe-133 may diffuse deeper into the tissue before it is removed. Kr-85 gives a single component curve relating to endometrial flow while each of the 2 or 3 components of the Xe-133 curves appear to relate to some aspect of endometrial flow (Chapter 12).

At first it appeared that Kr-85 might give a measure of flow which was more relevant to abnormalities of menstrual bleeding by assessing superficial flow only. In addition, the method should remain valid during menstruation, whereas Xe-133 in solution was sometimes expelled into the vagina after intrauterine instillation during menses if bleeding was heavy. However, studies in sheep clearly indicated that the presence of the intrauterine β-probe induced a significant local increase in endometrial blood flow (Chapter 12). In view of this and of the difficulty in obtaining adequately sensitive β-probes the Kr-85 studies were discontinued. Nevertheless, the small number of studies carried out showed a close correlation with blood flow calculated from Xe-133

TABLE 5:

Comparison of best fit of two components as determined by eye and by computer analysis using method of least squares.

Subject Numbers	Component 1				Component 2				Mean flow	
	By eye		Computer		By eye		Computer			
	$t_{\frac{1}{2}}$	Flow	$t_{\frac{1}{2}}$	Flow	$t_{\frac{1}{2}}$	Flow	$t_{\frac{1}{2}}$	Flow	By eye	Computer
C14	38.44	1.80	87.21	0.79	2.17	31.94	2.48	27.94	28.4	26.7
C12	50.44	1.37	26.06	2.64	2.03	34.14	1.93	35.91	33.8	34.7
C15	49.57	1.40	>100	< 1	2.23	31.08	2.76	25.11	28.9	25.1
OD7	44.52	1.56	>100	< 1	1.69	41.01	1.94	35.72	37.9	35.7
OD12	20.93	3.31	>100	< 1	3.12	22.21	3.65	18.99	21.1	19.0
C10	18.14	3.83	16.39	4.23	2.37	29.24	2.70	25.67	28.1	24.6
C14	32.83	2.11	34.92	1.98	3.94	17.59	4.77	14.53	14.2	12.9
C15	13.09	5.29	31.70	2.19	2.52	27.5	4.07	17.03	22.4	16.3
C15	54.96	1.26	>100	< 1	2.46	28.17	3.13	22.14	25.8	22.1
C13	14.92	4.64	14.88	4.66	2.44	28.40	2.48	27.94	27.9	27.5
Mean									26.9	24.5
SEM									2.1	2.3

clearance, and the cyclical pattern was not substantially different from endometrial blood flow calculated for rhesus monkey ocular auto transplants (Markee 1950) and for the human using heat clearance (Prill and Götz 1961; Figure 7).

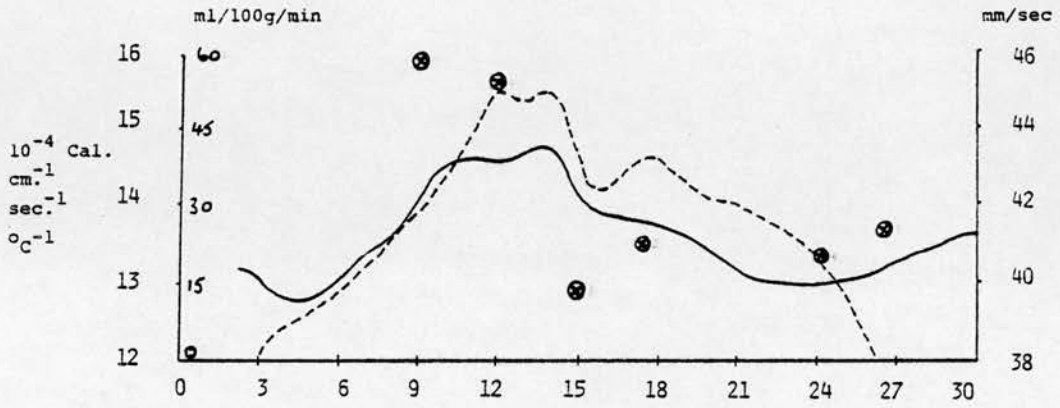


Figure 7. Comparison of endometrial blood at different stages of the menstrual cycle measured by Kr-85 clearance in 6 women (ml/100g/min), and compared with the data of Markee (1950) in rhesus monkeys (mm/sec;dotted line) and prill and Götz (1961) using a heat clearance technique in women (10^{-4} Cal. cm^{-1}. sec^{-1}. $^{o}C^{-1}$; continuous line).

The theory of inert gas clearance as a measure of capillary blood flow in several tissues including the uterus has been thoroughly discussed by several authors, and has been addressed earlier in this chapter. Validation of the techniques described here has been carried out with radio-active-labelled microspheres in sheep (Chapter 12). However, it is necessary to pay moderate attention to detail in order to obtain consistent results. This includes resting the subject for 15-30 minutes beforehand in a supine position in a quiet room with two investigators only. Very gentle manipulation of the uterus was attempted and great care was exercised to ensure that air bubbles were not instilled with the Xenon.

Some consideration was given to the method of analysis

and it was decided that compartment analysis (Thorburn et al 1966; Forssman 1975) would give a more accurate result than the initial slope method (Secher et al 1973). Computer analysis using the least squares method should give more accurate curve fitting than an attempt by eye, but direct comparison of these two approaches did not show marked differences in the assessment of mean flow with Xe-133.

The origin of the 2 or 3 components of the Xe-133 clearance curves is obscure, but presumably relates to regions within the endometrium which have genuinely different flows. Theoretically, one might expect different rates of flow in the superficial endometrium, in the stroma and perhaps around the glands. It is also possible that endometrium over the fundus might have different flow from regions nearer the cervix. This requires study using precise microsphere techniques in animals.

Initially, great care was taken to assess the safety of these isotope techniques and radiation dosage calculations were reviewed. The inert gases, Krypton and Xenon, have rapid diffusion properties and rapid lung excretion that minimise radiation risk and several prestigious Scientific and Ethical Committees reviewed the protocols before human studies began.

These techniques clearly have considerable value for assessing slow changes in flow with repeated assessments at intervals of hours, days or weeks. However, they are laborious and time consuming and are not capable of assessing rapid changes in flow. In fact, rapid changes in flow of moderate magnitude during Xe or Kr clearance could make calculation of the clearance components difficult. This was not found to be a problem in practice.

In 3 women vaginal blood flow was measured simultaneously with endometrial flow and was found to be substantially lower (36-64% of endometrial flow). Calculations of vaginal flow were in the same range as those recorded by Wagner and Otteson (1980), using the same technique.

Chapter 12

VALIDATION OF INERT GAS CLEARANCE TECHNIQUES IN EWES:

INTRODUCTION:

Theoretical considerations suggested that both Krypton-85 (Kr^{85}) and Xenon-133 (Xe^{133}) techniques should give reasonable measures of endometrial blood flow without any interference from flow in the myometrium. However, these techniques had never been compared directly or validated against any other method. This could only be done in an animal species with a uterus large enough for insertion of a β-probe, with an endometrium greater than 1 mm in thickness and in whom the radioactively labelled microsphere technique could also be used. Ideally, a subhuman primate such as the rhesus monkey (macaca mulatta) or baboon (papio sp.) with endometrial spiral arterioles and regular menstruation would have been preferred, but these were not available. The sheep was chosen because it fitted the essential criteria and a great deal is known about its reproductive (oestrous) cycle.

With appropriate care and attention to experimental details the radioactively-labelled microsphere technique will provide an accurate measure of absolute capillary blood flow in a portion of excised tissue (Hales 1973) and for this reason was chosen as the yardstick for comparison with the inert gas clearance techniques.

This study was planned, executed and analysed by myself with the invaluable technical assistance of Dr. P. Mattner and Mr B. Brown.

MATERIALS AND METHODS:

Measurement of capillary blood flow with the microsphere technique.

The procedures used to measure capillary blood flow and cardiac output with microspheres were those of Hales (1973). Radioactive microspheres were injected into the left ventricle. These are distributed to body tissues in

proportion to the blood flow to each tissue. Thus, if the dose of microspheres administered is D counts per minute (cpm) and d cpm/g is located in a tissue:

$$\text{Fraction of cardiac output to tissue} = \frac{d}{D}$$

If the cardiac output (CO, mℓ/min) is known, a quantitative measure of blood flow ($\dot{Q}$, mℓ/min) is obtained:

$$\dot{Q} = CO \times \frac{d}{D}$$

Cardiac output may be measured from this equation by introducing an artificial reference organ into the system, in the form of a constant rate withdrawal pump; if blood is sampled at f mℓ/min and d' cpm is contained in the sample then:

$$CO = f \times \frac{D}{d^1}$$

Spheres were injected into the left side of the heart and sampled through the right femoral artery. In the present study f was calculated from the weight and specific gravity of the blood and the duration time of sampling. By combining the above equations measurement of tissue capillary blood flow rates ($\dot{Q}$ mℓ/100 g/min) may be obtained without accurately determining the dose of isotope, thus obviating a possible source of error:

$$\dot{Q} = f \times \frac{d}{d^1} \times 100$$

Microspheres (15 ± 5 μm; mean ± SD diameter) were suspended in isotonic saline and a drop of Tween 20 added. Samples were ultrasonicated before injection to prevent aggregation. A sample from each new batch was examined through the light microscopic to check diameter and uniformity of shape.

For the Xenon-133 experiments the intraventricular dose contained 9-14 x 10^6 microspheres labelled with either ^{51}chromium, ^{46}scandium or ^{113}tin (New England Nuclear Company, Boston, MA, U.S.A.) and was delivered in 0.9% (w/v) saline at 39°C. For the Krypton-85 experiments 15 μm microspheres from the 3M Company, St Paul, Minnesota

(^{141}Cerium, ^{46}Scandium and ^{85}Strontium) were used. Doses were prepared using an RIDL pulse height analyzer and scintillation probe (Model 910, Nuclear Chicago, Des Plaines, Ill.). Certified nuclide standards accurate to ± 2% (Australian Atomic Energy Commission) were used in the calibration of instruments. The 'reference organ' blood sample from the right femoral artery was withdrawn at 25 mℓ/min^{-1}. At autopsy, a length of the left uterine horn extending 1.5 cm either side of the site at which ^{133}Xe had been injected or the β-probe tip had been positioned was removed. The section was divided into its myometrial and its caruncular and inter-caruncular endometrial components. These were weighed and the radioactive content of each was determined in an autogamma spectrometer (Packard Instruments, Model 5320, La Grange, Ill. U.S.A.; ^{51}Cr, 250-350 keV; ^{46}Sc 840-1220 keV; ^{113}Sn, 355-445 keV). Subsamples were taken from each kidney, from fallopian tubes and ovaries, and sometimes from other tissues to confirm adequate mixing and bilateral distribution of microspheres. Portions of tissue counted always yielded counts of >1000 per minute. The gamma spectrometer count was markedly influenced by geometry of the sample, but this effect was avoided by filling sample tubes to less than 2 cm high.

Krypton-85 experiments.

Animals

Six mature, parous, Merino ewes were used. Three ewes were near oestrus and three were at the mid-luteal stage of the oestrous cycle. The day of oestrus (Day 0) was determined using a vasectomized ram.

Surgical procedures

On the day prior to the experiment, each ewe was anaesthetized with halothane while polyethylene catheters (1.0 mm ID, 1.5 mm OD) filled with heparin saline were inserted into (a) the left ventricle via a small puncture in the left carotid artery, (b) the left and right femoral arteries via the saphenous arteries and (c) the left recurrent tarsal vein. The following day, each ewe was

anaesthetized with sodium pentobarbitone for the duration of the experiment (1-2h). After Laparotomy, the tip of the detector probe was inserted into the uterine lumen through a needle puncture made in the middle third of one uterine horn (fig. 1). It was advanced until the end window lay in the middle or posterior region of the anterior third of the same horn and was held in position with a fine suture through the serosa The genital tract was handled as little as possible during these procedures. The catheter in the left femoral artery was advanced so that the tip was approximately 4 cm cranial to the bifurcation of the abdominal aorta. After closure of the laparotomy incision, the animal was kept in a supine position for approximately ½ h before administration of ^{85}Kr. Rebreathing of expired ^{85}Kr was prevented by the use of polyethylene

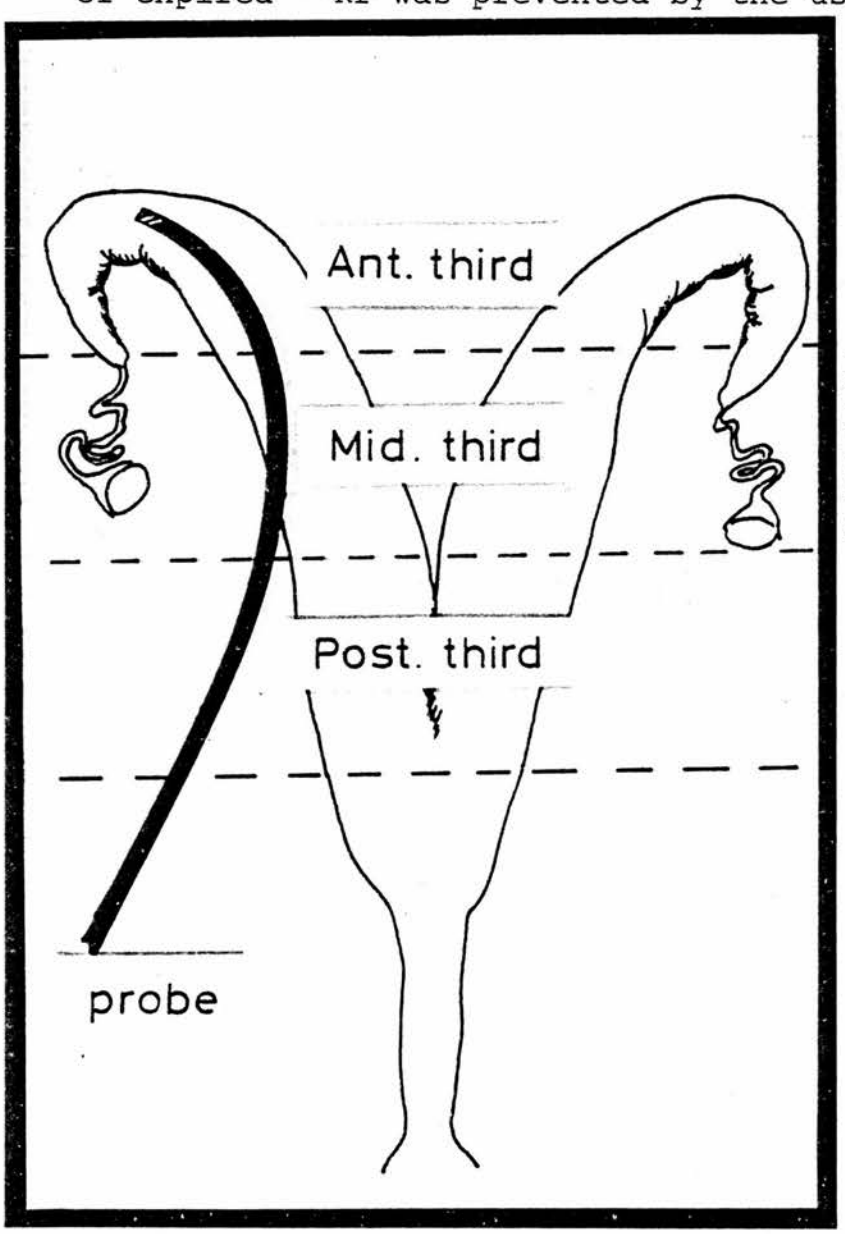


Figure 1:
Location of the intra-uterine β-detector probe in the lumen of the anterior hour of the sheep uterus.

Radiation detector probe.

A 3.0 mm diameter, catheter-type, semi-conductor, silicon p.i.n. type, β-radiation detector (Catelix probe, Tokyo Shibaura Electric Co.) with a teflon-coated end window mounted in the tip [Ueda, Sasaki, Iio, Kaihara, Machida, Ito, Takayanagi, Kobayashi and Sugita, 1969] was used to monitor β-emissions from ^{85}Kr. The probe output was analysed by a preamplifier, an amplifier, a single channel analyser and a counter (Ortec Incorporated, Oakridge, Tennessee). The printout appeared on a line printer controlled by a counter timer.

Experimental procedures.

A dose of 7-14 mCi of ^{85}Kr (Australian Atomic Energy Commission, Lucas Heights, N.S.W.) in 5-10 ml of saline was injected through the aortic catheter and the outputs of the probe over 10 sec periods were recorded for 40-60 min. Approximately 5 min after the administration of ^{85}Kr, a dose of 15 μm diameter microspheres (9-14 million spheres) labelled with either ^{141}Cerium, ^{46}Scandium or ^{85}Strontium (3M Company, St. Paul, Minnesota) were injected into the left ventricle whilst arterial blood was being withdrawn from the right femoral artery (at approximately 25 $m\ell/min^{-1}$ over 1 min 10 sec) for the determination of cardiac output. In four ewes, a second dose of ^{85}Kr and of microspheres with an alternate label to that used previously was administered after the counts had fallen to background levels.

After completion of each experiment, the animal was killed with an overdose of anaesthetic and the position of the probe tip within the uterine horn was determined and marked with a small suture through the serosa.The probe was withdrawn and a 2-3 cm length of the uterine horn which had previously contained the tip of the probe was excised and separated as described above.

Preliminary testing showed that, with the probe lying in the uterine lumen, doses of microspheres labelled with ^{141}Ce, ^{46}Sc or ^{85}Sr had no effect on the level of background

counts recorded by the probe.

Test for retention of ^{85}Kr by the detector probe.

In preliminary studies on the clearance of ^{85}Kr from ovine uteri each clearance curve had a component that gave evidence of there being a compartment of extremely slow flow. This flow was much slower than the capillary blood flow rate in any of the uterine tissues. The possibility that this component was an artefact produced by temporary retention of ^{85}Kr by the probe [Sasaki, Wagner, Iio, Murao, Takayanagi and Kobayashi, 1971; Takayanagi and Iio, 1974] was investigated as follows. ^{85}Kr was injected via an indwelling catheter into the left uterine artery whilst the probe was positioned either (a) in the left uterine horn, (b) in the right uterine horn, or (c) initially in the left uterine horn but transferred into the right uterine horn a few minutes after the injection of ^{85}Kr.

Analysis of data.

The methods described by Thorburn, Casey and Molyneux [1966] were used to analyse the ^{85}Kr clearance curves. Counts of β-activity (corrected for background activity) were plotted against time on log-linear paper, the curves were analysed by graphical peeling into their various components and the blood flow rate (F) for each was calculated using the equation:

$$F = 100k \text{ ml } 100 \text{ g}^{-1}\text{min}^{-1}$$

where $k = 0.693/t_{\frac{1}{2}}$

$t_{\frac{1}{2}}$ = half time of the exponential in minutes assuming a tissue/blood coefficient for ^{85}Kr of 1.0 and the specific gravity of uterine tissue to be approximately 1.0.

When multiple compartments of flow were present, the relative compartment sizes were calculated after back extrapolation of the exponents to time zero and the mean flow rate (F_m) was determined using the methods of Thorburn et al. [1966].

Estimates of capillary blood flow obtained with the two methods were compared using paired t test.

Xenon-133 experiments.

Animals.

Six mature, parous Merino ewes were used. Three were examined at day 8-11 and three at day 15-16 of the oestrous cycle (day of oestrus equals day 0) to broaden the range over which comparisons of estimates of blood flow could be made.

Surgical procedures.

On the day prior to the experiment, each ewe was anaesthetized with halothane while polyethylene catheters (1.0 mm i.d., 1.5 mm o.d.) filled with heparinized saline were inserted into (a) the left ventricle via the left carotid artery, (b) the right femoral artery via the saphenous artery, and (c) the left recurrent tarsal vein.

On the day of the experiment, each ewe was anaesthetized with pentobarbitone sodium (administered via the catheter in the recurrent tarsal vein) for the duration of the experiment (1-1½ h). With the animal in a supine position, the uterus was exposed via a mid-ventral abdominal incision and a puncture wound was made with a 25 S.W.G. hypodermic needle through the wall of the left uterine horn in its middle third. The uterus was returned to the abdominal cavity and 2 min later, a dose of microspheres was administered into the left ventricle (see below). Five minutes later, a dose of radioactive ^{133}Xe (Radiochemical Centre, Amersham) was injected into the lumen of the left uterine horn through a 25 S.W.G. hypodermic needle inserted at the site of the previous puncture wound. In five ewes the procedures were repeated 45 min later using microspheres labelled with an isotope different from that used initially. On the conclusion of the experiment, the ewe was killed with an overdose of pentobarbitone sodium.

Measurement of blood flow with the Xenon-133 clearance technique.

A dose of 3.0-5.5 MBq of ^{133}Xe in 0.2 ml of saline (0.9% w/v) at 39° was injected into the lumen of the left uterine horn as previously described. The uterus was immediately returned to the abdominal cavity and the abdomen was temporarily closed with towel clips. A scintillator probe containing a 5 cm sodium iodide crystal was quickly positioned over the abdomen immediately above the uterus and the γ emissions were subsequently monitored for 30 min. The delay in positioning of the scintillator probe was 10-20 s. The probe was connected to an amplifer, a single channel analyser and a counter system (Ortec Incorporated, Oakridge, TN, U.S.A.) and the accumulated counts for each 12 s interval appeared on a line printer controlled by a counter-timer.

The data were analysed with a computer program as described in Chapter 11.

RESULTS:

Krypton-85 experiments.

(a) Retention of ^{85}Kr by the detector probe.

Following the injection of ^{85}Kr into the left uterine artery, a clearance curve of the type shown in Fig. 2 was obtained when the probe was in the left uterine horn. However, no counts were detected when the probe was in the right uterine horn throughout the procedure. When the probe was transferred from the left to the right horn partway through a clearance study, it continued to register radioactivity, the levels declining at a rate which approximated that of the usual slow component. Over 10 clearance studies, the contribution of this artefactual component was equivalent to a flow rate of 1.7 ± 0.14 ml 100 $g^{-1}min^{-1}$.

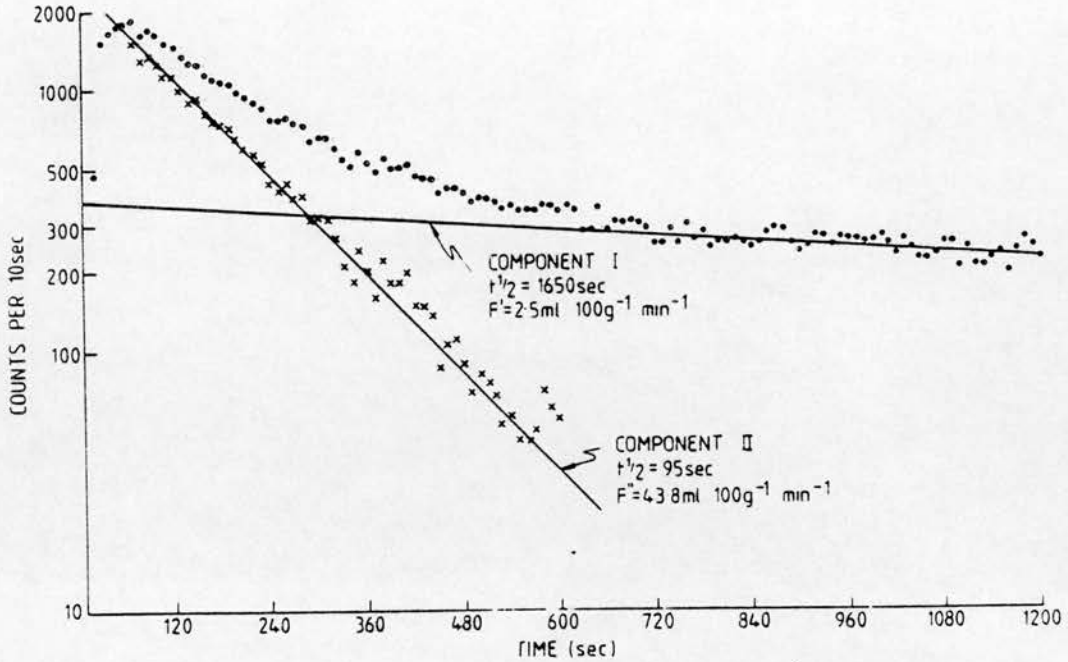


Figure 2. A two component exponential curve for the clearance of ^{85}Kr from an ovine uterus. Only the initial 20 min of the 60 min clearance time is shown. The two components are represented by the straight lines. (O) Totals counts of β-particles per 10 sec interval. (x) Total counts minus corresponding values derived from component I to define component II {Thornburn et al., 1966} see Analysis of data.

(b) Estimates of blood flow.

The clearance curves plotted from the original counts (background-corrected) always had at least two components (see Fig.2) but on three occasions, three components were present. As the slowest component of each curve was an artefact associated with temporary adsorption of ^{85}Kr to the probe (see section (a) above) the contribution of this component was subtracted from the original counts. The resultant curve was described by a single exponential function in seven instances and by two exponential components in three others. The blood flow rates (F) calculated from the former curves, together with the mean flow (F_m) calculated from the latter curves are compared in Table 1 with estimates of capillary blood flow ($\dot{Q}$) obtained for the uterine tissue with the microsphere technique.

Considering only the seven clearance curves with a single component, the estimates of blood flow obtained with the ^{85}Kr method were highly correlated with $\dot{Q}$ for either

TABLE 1:

Comparison of uterine blood flow values (ml 100 $g^{-1}min^{-1}$) obtained with the Krypton-85 clearance and microsphere technique in 6 anaesthetized ewes.

Blood Flow assessed by ^{85}Kr		Capillary blood flow assessed by microspheres			
Sheep No.	Flow rate	Caruncles	Inter-caruncular endometrium	endometrium	Myometrium
700 (a)	84.4**	79.8	69.0	71.9	38.5
(b)	43.8	59.6	53.5	55.1	24.9
743	43.3	37.7	22.4	26.8	38.5
7876* (a)	64.0	65.4	73.1	69.3	18.1
(b)	69.0	75.9	72.5	74.2	83.7
9023*	90.4	71.3	66.2	69.0	41.4
9031* (a)	65.6**	82.5	61.0	70.0	51.6
(b)	48.4**	43.9	28.9	35.1	75.6
9036 (a)	39.6	38.3	24.9	28.7	35.7
(b)	36.2	20.7	23.0	22.3	73.2

(a) and (b) refers to first and second observation respectively where two ^{85}Kr clearance curves were obtained in the same ewe. *Refers to ewes near oestrus; all other ewes were at the mid-luteal stage. **Mean flow rate (two exponential components present in curve).

the caruncles, the inter-caruncular endometrium or the total endometrium (caruncles plus inter-caruncular endometrium) ($r = 0.819$, $r = 0.799$ and $r = 0.920$, respectively, $p<0.05$) but not with $\dot{Q}$ for the myometrium. The mean $\dot{Q}$ values for each of these tissues did not differ significantly from each other or from the mean of the flow rates obtained with the ^{85}Kr method. In the three instances in which the clearance curves had two exponential components, the blood flow in the compartment with the faster flow accounted for 20-40% of the mean flow. However, in each instance, neither of the two flow rates were similar to $\dot{Q}$ for either the caruncles, the inter-caruncular endometrium or the total endometrium but the mean flow rate approximated the $\dot{Q}$ values for these tissues. Considering all data, there was little change in the correlation coefficient for estimates of blood flow (F together with F_m) obtained with the ^{85}Kr method and $\dot{Q}$ for the caruncles, inter-caruncular endometrium and total endometrium ($r = 0.832$, $r = 0.822$ and $r = 0.041$, respectively, $p<0.005$, see Fig.3).

The mean (±SEM) capillary blood flows (measured with microspheres for the left and right kidneys were 284.8 ± 22.0 ml 100 $g^{-1}min^{-1}$ and 298.5 ± 35.9 ml 100 $g^{-1}min^{-1}$ respectively and were of the same order as previously published values obtained in anaesthetized sheep [Bruce and Moor, 1976]. This suggests that the microspheres were distributed to the tissues in the same proportion as was the cardiac output and thus provided reasonable estimates of capillary blood flow ($\dot{Q}$) in the uterine tissues.

(c) Local influence of probe on endometrial blood flow.

Presence of the β-detector probe in one uterine horn produced a significant local effect on endometrial but not myometrial blood flow (Table 2). Endometrial and caruncular blood flows in the segment of uterine horn containing the β-probe were significantly higher than in the equivalent segment of the opposite horn (endometrial flow = 53.2 versus 33.7 mls/100g/min; paired $t = 3.907$, $p<0.01$; caruncular flow: 54.4 versus 38.7 mls/100g/min; paired $t = 3.764$, $p<0.02$).

TABLE 2:

INFLUENCE OF β-DETECTOR PROBE ON LOCAL BLOOD FLOW IN UTERINE HORNS IN 7 SHEEP.

ANIMAL NO.	Blood flow in segment of horn containing β-Probe (ml/100g/min)				Blood flow in equivalent segment of opposite horn (no probe; ml/100g/min)			
	TOTAL	CARUNCLES	ENDOMETRIUM	MYOMETRIUM	TOTAL	CARUNCLES	ENDOMETRIUM	MYOMETRIUM
9031	26.3	37.7	26.8	38.6	27.1	19.9	18.5	43.3
743	78.8	82.5	70.0	51.6	57.5	53.5	54.6	60.8
9017	71.6	-	82.5	57.6	36.9	-	35.9	35.3
9023	74.2	71.3	68.0	41.4	54.8	46.8	43.0	45.5
4876	83.1	75.9	74.2	83.7	85.7	68.7	55.0	79.5
9036	45.6	38.3	28.7	35.7	43.5	24.8	16.9	54.9
9014	71.6	20.7	22.3	73.2	77.3	18.6	11.9	83.6
Mean	64.5	54.4	53.2	54.5	54.7	38.7	33.7	57.6
±SEM	7.8	10.3	9.8	6.9	8.0	8.4	8.4	6.9

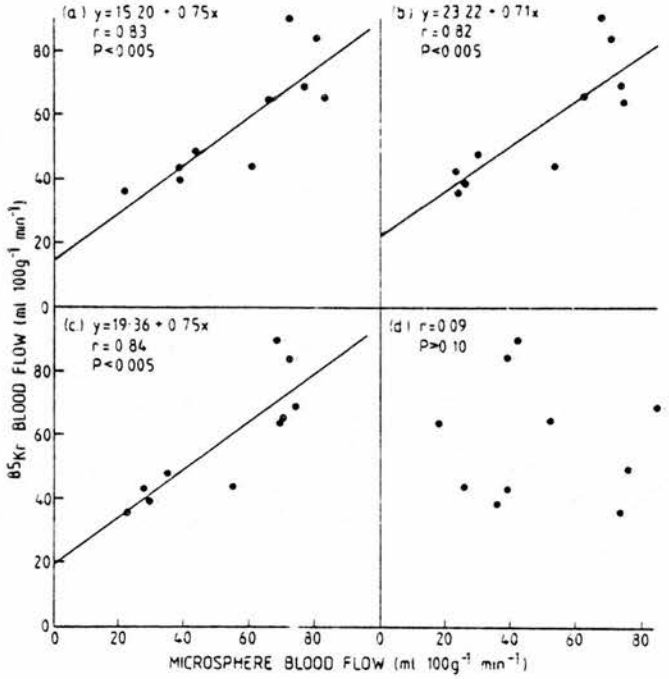


Figure 3. Relationship between estimates of blood flow obtained by the ^{85}Kr and microsphere methods for uterine tissue in six anaesthetized ewes. (a) caruncles, (b) inter-caruncular endometrium, (c) total endometrium (caruncles plus inter-caruncular endometrium), (d) myometrium.

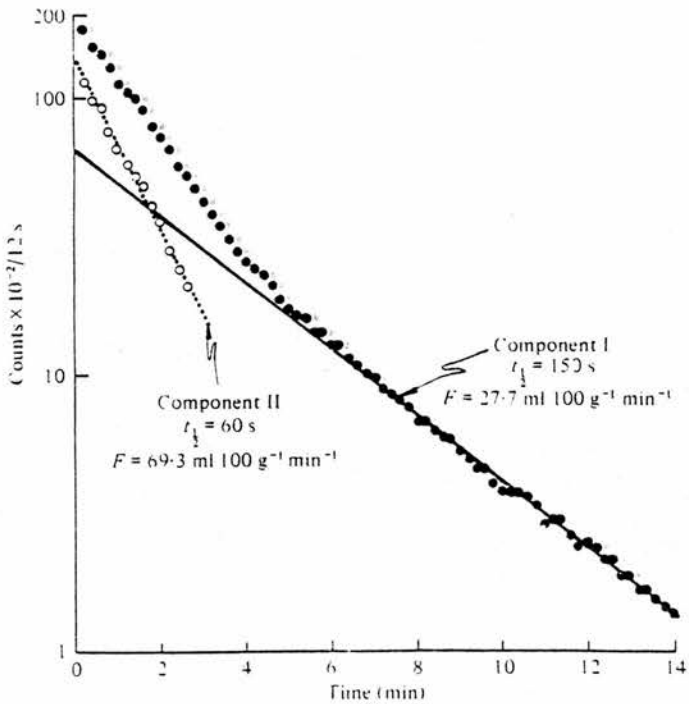


Figure 4. A two-component exponential curve for the clearance of ^{133}Xe from the ovine uterus. The two components are represented by the straight lines. O, total counts of γ particles per 12 second intervals. O, total counts minus corresponding values derived from component I to define component II.

There was no significant difference in myometrial flow. Total flow in the segment was greater but did not reach statistical significance.

Xenon-133 experiments.

The clearance curves obtained after intra-uterine injection of ^{133}Xe comprised eight with two components (see Fig.4) and three with a single component. A comparison of the blood flow rates (F) calculated from the curves and the estimates of uterine capillary blood flow ($\dot{Q}$) obtained with the microsphere technique is shown in Table 3.

With curves containing two components (curve 5172a excepted), the fast flow (F_1) was generally of a similar value to that of $\dot{Q}$ for the caruncles, the flow in the slow compartment (F_2) approximated $\dot{Q}$ for the inter-caruncular endometrium and neither the F_1 nor the F_2 value resembled $\dot{Q}$ for the myometrium. In each of the three curves described by a single exponential (7009b; 7009a,b), derived flow rate F and the $\dot{Q}$ values for both the caruncles and the inter-caruncular endometrium were of a similar order.

In the case of curve 5172a, the slow component appeared to be an artifact because the derived compartment size (7%) was minute compared to that of the slow compartment in all other experiments (63.5%, SEM 6.4) and its flow rate bore no relation to $\dot{Q}$ for any of the uterine tissues. Consequently, it appears that the curve was more correctly represented by a single exponential component with a flow rate approximating $\dot{Q}$ in the caruncles and in the inter-caruncular endometrium as in the above three instances (7008b; 7009a,b).

Taken together, the F_1 values from the two-compartment curves and the F values from single-compartment curves were highly correlated with the corresponding $\dot{Q}$ values for caruncles ($r = 0.974$, $p<0.001$) and the respective means (± SEM) did not differ significantly (55.8 ± 10.3 cf. 59.9 ± 11.4 ml 100 $g^{-1}min^{-1}$). Similarly, when considered together, the F_2 values from two-component curves and the F value for

TABLE 3:

Comparison of uterine blood flow values (ml 100 $g^{-1}min^{-1}$) obtained with Xenon-133 clearance and microsphere techniques in six anaesthetized ewes.

	Blood flow assessed with Xenon-133		Capillary blood flow assessed with microspheres		
Sheep No.	Fast compartment or only compartment	Slow compartment	Caruncles	Inter caruncular endometrium	Myometrium
*706 (a)	69.3	27.7	68.5	34.2	95.5
(b)	44.7	19.3	42.5	17.7	36.7
5170 (a)	64.2	18.2	85.6	45.8	98.9
(b)	43.3	14.0	37.5	15.8	30.8
5172 (a)	138.6	**18.9	149.5	122.3	55.6
(b)	77.0	25.3	87.0	30.3	39.2
*9011 (a)	69.3	31.5	71.1	47.7	39.9
*7008 (a)	43.3	16.5	34.0	25.7	43.3
(b)	19.8	——	24.5	20.3	65.5
7009 (a)	23.9	——	26.2	27.4	122.4
(b)	20.4	——	32.0	22.7	99.6

(a) and (b) refer to first and second observation respectively where two ^{133}Xe clearance curves were obtained in the same ewe. *Refers to ewes near oestrus; all other ewes were at the mid-luteal stage of the oestrous cycle. **As this value appears to be an artifact (see Results section), it was excluded from the analysis and curve 5172 (a) was regarded as being a single-component curve with an F value of 138.6.

single-component curves were highly correlated with the corresponding $\dot{Q}$ values for inter-caruncular endometrium ($r = 0.959$, $P<0.001$) and the respective means (±SEM) were not significantly different (32.3 ± 10.7 cf. 37.3 ± 9.1 ml $100\ g^{-1}min^{-1}$). There was an extremely poor relationship between $\dot{Q}$ for the myometrium and either the F_1 or the F_2 values derived from the ^{133}Xe clearance curves ($r = 0.274$ and $r = 0.156$, respectively, $p>0.3$).

DISCUSSION:

Krypton-85 experiments.

Since the average range of penetration of β-particles in tissue is less than 1 mm [Casey and Thorburn, 1965; Thorburn et al., 1966; Einer-Jensen, 1977] and the thickness of the endometrium in sections of uteri taken from the experimental ewes varied from 1.7 to 2.2 mm, it would be expected that the majority of the β-emissions detected by a probe lying within the lumen of the uterus would arise from the endometrium only. The extremely good correlation between the blood flow rates obtained with the ^{85}Kr method and $\dot{Q}$ values for the endometrial tissues as well as the general similarity of the estimates of flow obtained with the two methods are in accord with this expectation.

In an earlier and very preliminary study [Brown, Fraser and Mattner, 1977] in which the efficiency of β-detection by the probe used was only 20-25% of that for the probe in the present study, the F values though highly correlated with $\dot{Q}$ for the endometrium ($r = 0.985$, $p<0.005$), were many times smaller than $\dot{Q}$. Thus, it appears that the sensitivity of the probe used is an important factor in determining whether the F value obtained is merely an index or a reasonable estimate of the endometrial capillary flow. Nevertheless, differences between individual $\dot{Q}$ and F values could be expected to occur. Estimates obtained with microspheres relate to flow during lodgement of the microspheres (approximately 30 sec; Hales and Cliff [1977] while those provided by the ^{85}Kr method represent mean values over the initial

10-15 min of the clearance time.

On the three occasions when the ^{85}Kr clearance curve was composed of two single exponential components, neither compartment of flow could be related to any region or tissue within the endometrium. Nor could the occurrence of two compartments be attributed to effects associated with stage of oestrous cycle. Additionally, with sheep No. 700, two components were evident in only the first of the two clearance curves obtained (see Table 1). When two components were present, the mean flow rate approximated $\dot{Q}$ for the endometrial tissue. It is possible that factors such as handling of the uterus or contact of the probe with the endometrium may have at times caused local changes in blood flow within the endometrium without the total blood flow to the areas involved being altered. An increase in flow rate in some capillary beds and an associated decrease in flow rate through adjacent beds could result in heterogeneity of blood flow being recorded. $\dot{Q}$ being a mean value would not reflect such changes.

This study clearly shows that there is a local effect of the β-probe on endometrial blood flow in the region in contact with the probe. This effect was seen as an increase in endometrial blood flow with no effect on myometrial flow. This study was unable to assess whether the effect persisted with time, but does raise major concerns about the advisability of using a probe technique to measure absolute blood flow changes in endometrium under different circumstances. It was encouraging to find that a similar local effect was not seen in the experiments where Xe-133 was injected into one horn. The mechanism of this local disturbance of flow could involve changes in prostaglandin secretion (or other locally released vasoactive substances) of a degree which is insufficient to influence the myometrium.

Xenon-133 experiments.

The data from the present study in ewes indicates that when ^{133}Xe diffuses from the uterine lumen into the

endometrium, it is cleared by the vasculature before it reaches the underlying myometrium. Thus, whether the clearance curves contained one or two components, the derived blood flow rates were related to $\dot{Q}$ in the caruncular and intercaruncular portions of the endometrium and not to $\dot{Q}$ in the myometrium. The introduction of even a minute amount of air into the uterus during deposition of the ^{133}Xe solution would allow some of the ^{133}Xe to occur in the gaseous phase with a consequent delay in the rate of clearance of that fraction of the marker from the uterus. Such an occurrence may have contributed to the apparent presence of a second very slow compartment of flow in observation 5172a in the present study.

On well-established theoretical grounds, a correction factor corresponding to the tissue-blood coefficient of the gas (0.7 for xenon in most tissues; Andersen & Ladefoged, 1967) is usually included in the calculation of tissue blood flow rate (F) from xenon clearance curves. However, in the present study, in which reference values for the capillary blood flow ($\dot{Q}$) in the uterine tissues were provided with the microsphere method, there was general agreement between the F values calculated when a value of 1.0 was assigned as the tissue-blood partition coefficient and the Q values for the relevant tissues. It appears, therefore, that when ^{133}Xe is introduced into the uterine lumen, the effect of the endometrium-blood diffusion equilibrium on the rate of clearance of the gas from the endometrium is counterbalanced by some other factor, possibly the endometrium-luminal fluid diffusion equilibrium, with the result that realistic estimates of endometrial blood flow rates may be obtained without reference to the tissue-blood coefficient. Complete agreement between paired estimates of endometrial blood flow rates determined with the gas clearance and the microsphere methods would not be expected because the period over which the flow is assessed is considerably longer with the former than with the latter method.

Chapter 13

ENDOMETRIAL BLOOD FLOW IN WOMEN USING XENON-133 CLEARANCE:

INTRODUCTION:

In this chapter a series of measurements of endometrial blood flow in women with normal menstrual cycles and with dysfunctional uterine bleeding is described. After extensive preliminary investigations described in Chapter 11 and 12 the technique of Xenon-133 clearance from the uterine cavity was chosen to give a confident and repeatable measure of blood flow in superficial endometrium at different stages of the menstrual cycle.

These studies were carried out both in Oxford (Department of Radiation Physics, Churchill Hospital) and in Sydney (Department of Nuclear Medicine, Royal Prince Alfred Hospital).

CLINICAL MATERIAL AND METHODS:

Endometrial blood flow measurement was carried out using the intrauterine Xe-133 clearance technique as described in detail in Chapter 11.

Subjects studied included 17 women with normal menstrual cycles (Table 1) and 20 women with dysfunctional uterine bleeding (6 anovulatory; Table 2). These women were studied at different stages of the menstrual cycle and 15 were studied on more than one occasion (7 normal cycles; 8 DUB).

Almost all subjects also had plasma oestradiol and progesterone measured at the time of blood flow studies, and most had several samples taken at other stages in the same cycle. All women kept an accurate note of data of each preceding and succeeding menstrual period and a few maintained basal body temperature and vaginal mucus charts. A combination of these pieces of information permitted dating of each blood flow measurement to an idealised 28 day menstrual cycle, except in those women with a history of anovulatory DUB.

TABLE 1:

Clinical data on 17 women with normal menstrual cycles who underwent endometrial blood flow studies.

Subject Number	Age	Cycle Length	Days Since L.M.P.	Plasma Steroid Levels Oestradiol (pg/ml)	Progesterone (ng/ml)	Endometrial Histology	Idealised Cycle Day
C1	41	25-28	7	555	< 1	P	10
C2	39	30-35	7	115	< 1	P	5
C3	28	26-33	12	620	< 1	LP	12
C4	37	28-31	4	-	-	P	4
C5	25	28-35	23	160	14.7	MS	22
C6	36	30-35	20	185	9.8	ES	18
C7	32	24-28	8	285	< 1	P	10
C8	37	28-35	10	255	< 1	P	9
C9	39	29-32	26	410	15.2	-	24
			24	365	12.6	MS	21
C10	30	27-32	25	290	8.5	-	23
			5	115	< 1	-	5
			11	345	< 1	-	11
C11	32	28-34	14	490	< 1	-	12
C12	28	27-30	26	290	6.1	-	26
			18	360	10.9	-	19
C13	31	24-29	5	385	< 1	-	8
			1	135	1.1	-	1
			3	150	< 1	-	3
C14	35	27-29	14	335	< 1	-	14
			9	220	< 1	-	8
			28	105	1.3	-	27
C15	30	30-34	15	200	1.8	-	15
			29	265	5.3	-	27
			7	155	< 1	-	6
C16	21	27-32	17	330	2.1	-	15
C17	28	26-30	25	265	12.0	-	25
			16	290	4.6	-	16

P = Proliferative; LP = late proliferative; ES = early secretory; MS = midsecretory

TABLE 2:

Clinical data on 20 women with dysfunctional uterine bleeding who underwent endometrial blood flow studies

Subject Number	Age	Cycle Length	Days Since LMP	Plasma Steroid Levels Oestradiol (pg/ml)	Progesterone (ng/ml)	Endometrial Histology	Type of D.U.B.	Idealised Cycle Day
AD1	46	14-50	22	600	< 1	LP	ANOV	(22)
AD2	43	20-60	16	380	< 1	LP	ANOV	(16)
AD3	45	28-50	20	555	< 1	CGH	ANOV	(20)
AD4	41	25-60	2	-	-	-	ANOV	(2)
			9	540	< 1	-		(9)
AD5	29	25-35	23	490	< 1	-	ANOV	(23)
			9	720	< 1	-		(9)
AD6	31	18-40	26	205	< 1	-	ANOV	(26)
OD1	39	28-35	31	335	4.6	LS	OV	27
OD2	42	21-30	13	-	-	ES	OV	17
OD3	36	24-30	1	-	-	M	OV	1
OD4	35	27-40	28	340	24.7	MS	OV	21
OD5	31	28-35	12	195	< 1	P	OV	8
OD6	36	29-35	9	135	< 1	-	OV	5
			12	200	< 1	-		9
			28	225	10.8	-		23
OD7	34	25-28	8	670	< 1	-	OV	10
			21	180	7.6	-		25
			24	145	4.9	-		26
OD8	31	26-35	12	420	< 1	-	OV	12
			14	135	6.3	-		16
			35	105	2.1	-		27
OD9	40	27-30	11	455	< 1	-	OV	11
			9	390	< 1	-		9
OD10	38	29-35	6	90	< 1	-	OV	4
OD11	29	28-31	26	145	8.3	-	OV	25
OD12	34	27-32	15	430	< 1	-	OV	11
			14	275	10.4	-		15
			24	280	14.5	-		24
OD13	41	26-31	10	645	< 1	-	OV	11
			19	295	11.8	-		19
OD14	37	21-25	5	585	< 1	-	OV	10

ANOV = Anovulatory; OV = Ovulatory; P = Proliferative; LP = Late Proliferative; ES = Early Secretory; MS = Mid Secretory; LS = Late Secretory; M = Menstrual; CGH = Cystic Glandular Hyperplasia.

TABLE 3:

Endometrial blood flow in 17 women with normal menstrual cycles

Subject Number	Idealised cycle day	Component 1 $t_{\frac{1}{2}}$ (mins)	Component 1 flow (ml/100g/m)	Component 1 size %	Component 2 $t_{\frac{1}{2}}$ (mins)	Component 2 flow (ml/100g/m)	Component 2 size %	Component 3 $t_{\frac{1}{2}}$ (mins)	Component 3 flow (ml/100g/m)	Component 3 size %	Mean flow (ml/100g/min)
C1	10	36.6	1.9	4	1.2	55.7	96	-			53.5
C2	5	>100	< 1	< 1	4.5	15.5	100	-			15.5
C3	12	17.9	3.9	2.5	1.4	49.1	97.5	-			48.0
C4	4	84.4	< 1	1.2	6.1	11.4	98.8	-			11.3
C5	22	28.3	2.4	5	1.8	37.5	95	-			35.8
C6	18	15.7	4.4	14	3.6	19.2	86	-			17.1
C7	10	21.2	3.3	3	3.3	21.0	40	1.4	49.5	57	36.8
C8	9	40.4	1.7	6	2.2	31.4	94	-			29.6
C9	24	>100	< 1	< 1	1.7	40.2	100	-			40.2
	21	29.9	2.3	12	1.8	38.6	88	-			34.3
C10	23	>100	< 1	< 1	2.3	29.9	100	-			29.9
	5	>100	< 1	< 1	2.6	27.0	100	-			27.0
	11	16.4	4.2	5	2.7	25.7	95	-			24.6
C11	12	>100	< 1	< 1	6.7	10.3	39	0.66	105.0	61	68.1
C12	26	26.1	2.6	< 1	1.9	34.9	99+	-			34.7
	19	12.6	5.5	2.6	3.5	19.6	97.4	-			19.2
C13	8	11.6	6.0	17	2.0	34.0	83	-			29.2
	1	14.9	4.7	1.9	2.5	27.9	98.1	-			27.5
	3	27.6	2.5	14	4.9	14.1	86	-			12.5
C14	14	34.9	2.0	13	4.8	14.5	87	-			12.9
	8	87.2	0.8	1.5	2.5	27.1	98.5	-			26.7
	27	28.9	2.4	4	2.2	31.7	96	-			30.5
C15	15	31.7	2.2	5	4.1	17.0	95	-			16.3
	27	>100	< 1	< 1	2.8	25.1	100	-			25.1
	6	>100	< 1	< 1	3.1	22.1	100	-			22.1
C16	15	39.4	1.8	48	4.4	15.8	52	-			9.1
C17	25	18.2	3.8	6	2.6	26.7	94	-			25.3
	16	22.4	3.1	6	5.7	12.1	94	-			11.6
Mean											27.7
SEM											2.6

TABLE 4:

Endometrial blood flow in 20 women with dysfunctional uterine bleeding (DUB).

Subject Number	Idealised cycle day	Component 1 t½	Component 1 Flow	Component 1 Size %	Component 2 t½	Component 2 Flow	Component 2 Size %	Component 3 t½	Component 3 Flow	Component 3 Size %	Mean Flow (ml/100g/min)
AD1	22	16.3	4.3	35	1.1	63.0	65	-			42.5
AD2	16	25.6	2.7	8	3.8	18.2	51	1.7	40.8	41	26.2
AD3	20	19.1	3.7	22	1.5	46.2	88	-	-		41.5
AD4	2	13.7	5.1	29	4.8	14.3	71	-			11.6
	9	14.4	4.8	14	2.5	28.2	86	-			24.9
AD5	23	20.4	3.4	2.8	1.7	42.0	97.2	-			40.9
	9	19.5	3.6	1.7	1.2	57.8	98.3	-			56.9
AD6	26	31.5	2.2	9	1.6	42.2	91.	-			38.6
OD1	27	26.7	2.6	2	1.3	52.8	98	-			51.8
OD2	17	21.0	3.3	8	3.0	22.9	92	-			21.3
OD3	1	13.6	5.1	10	2.5	27.2	90	-			25.0
OD4	21	23.7	2.9	4.8	3.2	22.0	95.2	-			21.1
OD5	8	18.8	3.7	12	4.8	14.5	58	2.5	27.3	30	17.0
OD6	5	82.0	0.85	2.6	3.4	20.1	97.4	-			19.6
	9	20.0	3.5	11	2.9	23.6	50	2.1	33.6	39	25.3
	23	49.4	1.4	< 1	1.7	40.8	99+	-			40.6
OD7	10	14.0	5.0	6	2.2	31.5	94	-			29.9
	25	> 100	< 1	< 1	1.9	35.7	100	-			35.7
	26	29.4	2.4	< 1	2.0	35.5	99+	-			35.3
OD8	12	15.5	4.5	5	4.6	15.1	95	-			14.6
	16	15.2	4.6	43	5.5	12.6	57	-			9.2
	27	> 100	< 1	< 1	2.4	29.4	100	-			29.4
OD9	11	18.9	3.7	7	3.1	22.7	93	-			21.4
	9	18.6	3.7	< 1	4.3	16.3	99+	-			16.2
OD10	4	61.5	1.1	7	7.3	9.5	93	-			8.9
OD11	25	22.5	3.1	3	2.1	33.5	97	-			32.3
OD12	11	14.7	4.7	6	2.8	24.8	94	-			23.5
	15	> 100	< 1	< 1	3.7	19.0	100	-			19.0
	24	100	1	1	3.0	23.4	100	-			23.4
OD13	11	44.4	1.6	1.4	2.3	30.5	98.6	-			30.1
	19	12.0	5.8	10	6.3	11.0	42	2.3	29.7	48	19.5
OD14	10	> 100	< 1	< 1	1.4	48.5	100	-			48.5
MEAN											28.2
SEM											2.2

RESULTS:

Xenon-133 washout half-times and supine endometrial blood flow calculations for each subject are summarised in Tables 3 and 4. The overall mean (± SEM) endometrial blood flow in women with normal cycles was 27.7 ± 2.6 ml/100 g/min when all measurements were considered together. Control subjects and women with ovulatory and anovulatory dysfunctional uterine bleeding have been considered separately according to stage of cycle in Table 5.

TABLE 5:

Endometrial blood flow measurements at different stages of the menstrual cycle in women with normal cycles or dysfunctional uterine bleeding.

	Early-Follicular Day 1-7	Mid-Follicular Day 8-12	Peri-Ovulatory Day 13-16	Early Luteal Day 17-21	Mid-Luteal Day 22-25	Pre-Menstrual Day 26-28	Persistent Follicular (Anovulatory)
Normal cycles	19.3	39.6	12.5	23.5	32.8	30.1	—
n	6	8	4	3	4	3	
Ovulatory DUB	17.8	25.2	14.1	20.6	33.0	38.8	—
n	3	9	2	3	4	3	
Anovulatory DUB	11.6	40.9	—	—	—	—	37.9
n	1	2					5

Although numbers in each group are small there does not appear to be much difference between women with normal menstrual cycles and those with ovulatory dysfunctional uterine bleeding (Figure 1 & 2).However, blood flow in normal women in the mid-follicular phase (days 8-12) is significantly higher than the women with ovulatory dysfunctional uterine bleeding (39.6 and 25.2 ml/100 g/min; $t = 2.274$, $p<0.05$). One woman with ovulatory DUB had a high rate of flow (51.8 ml/100 g/min) on the day before menstruation but others with DUB were in the

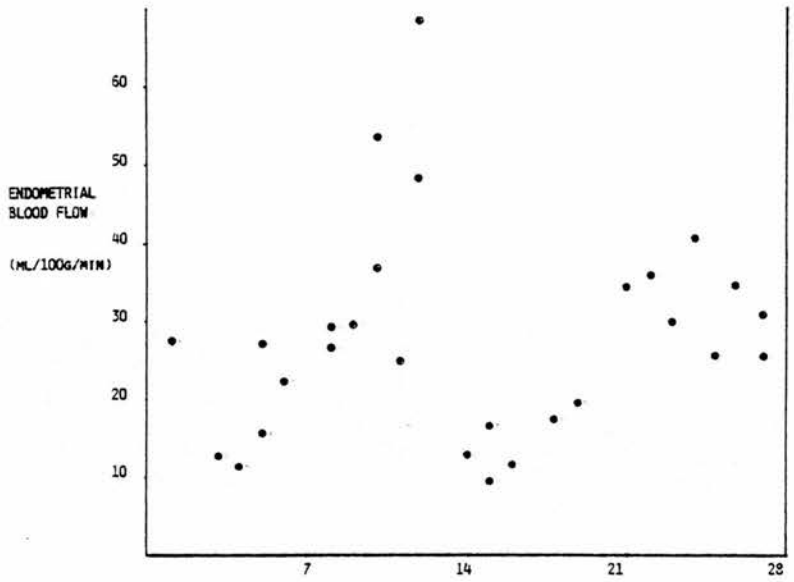


Figure 1. Endometrial blood flow (ml/100g/min) measured using Xe-133 clearance at different stages of the menstrual cycle in women with normal cycles. Data have been adjusted to fit into an idealised 28 day menstrual cycle.

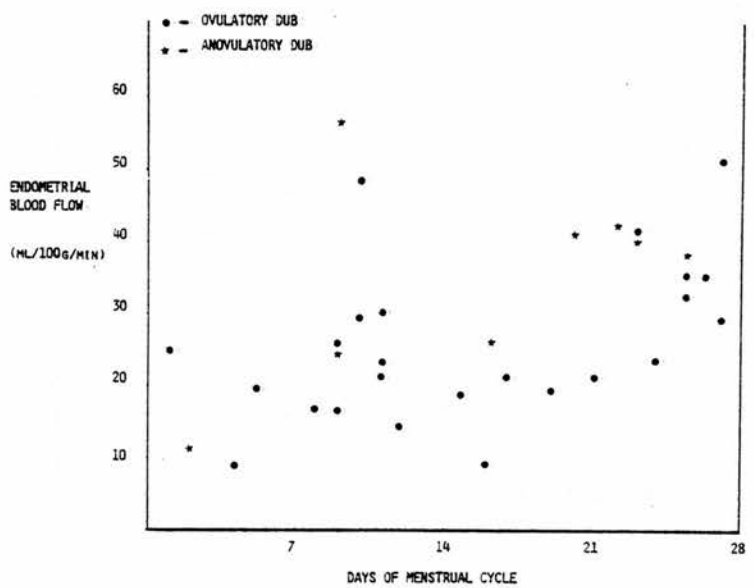


Figure 2. Endometrial blood flow (ml/100g/min) measured using Xe-133 clearance in women with ovulatory and anovulatory dysfunctional uterine bleeding. Data for women with ovulatory DUB have been adjusted to fit into an idealised 28 day menstrual cycle.

same range as controls in the premenstrual period.

The numbers of women with anovulatory DUB are too small to make any comment except that very variable blood flows were recorded and these overlapped with normal levels.

Women with normal cycles and ovulatory DUB have an obvious cyclical variation in endometrial blood flow with high levels in the mid-late follicular phase which fall substantially around ovulation. A secondary rise occurs through the early and mid-luteal phases and is maintained until the onset of menstruation. In 2 women (C13 and OD3) measurements were carried out just after the onset of premenstrual spotting and a few hours before the onset of full flow. In both these cases endometrial flow was still maintained at a fairly high level (27.5 and 25.0 ml/100 g/min). Later in menstruation and during the early follicular phase flow was slower.

Only 6 examples of 3 component curves were recorded, occurring in both DUB groups as well as in normal cycles. There did not appear to be any particular pattern to their occurrence, and mean flows were quite variable (17.0-68.1 ml/100 g/min).

A clear correlation between plasma oestradiol levels and endometrial blood flow was recorded for all studies undertaken when progesterone levels were less than 1 ng/ml ($r = 0.622$; $n = 31$; Figure 3) and for women with normal cycles when progesterone was less than 1 ng/ml ($r = 0.760$; $n = 13$), but not in situations where progesterone was elevated (total: $r = 0.016$, $n = 25$). No correlation between plasma progesterone levels and endometrial blood flow was found for luteal phase studies (total: $r = 0.073$; $n = 25$). This poor correlation was particularly seen when endometrial blood flow did not fall substantially during the premenstrual period at a time when oestradiol and progesterone were falling rapidly.

DISCUSSION:

Most previous work on genital tract blood flow in

TABLE 6:

Blood flow measurement in different uterine compartments as reported by different investigators.

INVESTIGATOR	TECHNIQUE	BLOOD FLOW (ml/100g/min) ENDOMETRIUM	MYOMETRIUM	INTERVILLOUS (PLACENTA)
SECHER ET AL (1973)	Intraluminal Xe-133 (Supine)	17.8 (13.5 - 29.0)	-	-
SECHER ET AL (1973)	Intramyometrial Xe-133 (Supine)	-	18.8 (5.2 - 37.9)	-
SECHER ET AL (1973)	Intramyometrial Xe-133 (Standing)	-	13.5 (3.9 - 33.5)	-
KLINGENBERG (1974)	Intramyometrial hydrogen (Supine)	-	13.5 (36.5 - 172.9)	-
FORSSMAN (1975)	Intramyometrial Intraarterial Xe-133 (Supine)	-	15.3 (3.8 - 35.2)	-
REKONEN ET AL (1976)	Intravenous Xe-133 (Supine)	-	77 ± 2.5 (SEM) pregnancy	135 ± 49 (77 - 322)
FRASER	Intraluminal Xe-133 (Supine)	27.7 (9.1 - 68.1) normal women	-	-

women has been directed towards study of myometrium, myomata, endometrial cancer, amenorrhoea, dysmenorrhoea and changes in posture, and no previous systematic studies have been carried out in normal and abnormal cycles, or in relation to menstruation. This investigation has provided the first assessment of changes in absolute blood flow in human endometrium through the menstrual cycle, as well as in women with dysfunctional uterine bleeding.

Cyclical changes in endometrial blood flow have been clearly demonstrated, with high levels in the mid to late follicular phase, a large fall at around ovulation and a secondary luteal phase rise which was maintained up till menstruation. The preovulatory measurements showed a weak correlation between circulating levels of oestradiol and endometrial blood flow. This correlation was lost during the luteal phase. Other factors separate from plasma oestradiol and progesterone must influence endometrial blood flow at least during some stages of the cycle. There was a major fall in blood flow at around ovulation which seemed to slightly precede the midcycle rise in progesterone. Unfortunately, the progesterone assays used in the study were not reported sufficiently accurately in the lower ranges to exclude a very small rise in progesterone in two subjects (C14 and OD8) who had low blood flow levels at day 12 and 14.

In the latter part of the luteal phase when luteolysis was occurring there was no evidence of any related fall in endometrial blood flow. In fact, blood flow was still relatively high (27.5 and 25.0 ml/100 g/min) in 2 subjects who were experiencing premenstrual spotting and who began bleeding heavily within 3-4 hours.

Two previous studies have provided evidence of the occurrence of cyclical changes in endometrial blood flow during the menstrual cycle. Markee (1950) studied endometrial auto transplants in the anterior chamber of the eye of rhesus monkeys, and measured blood flow by a stroboscopic

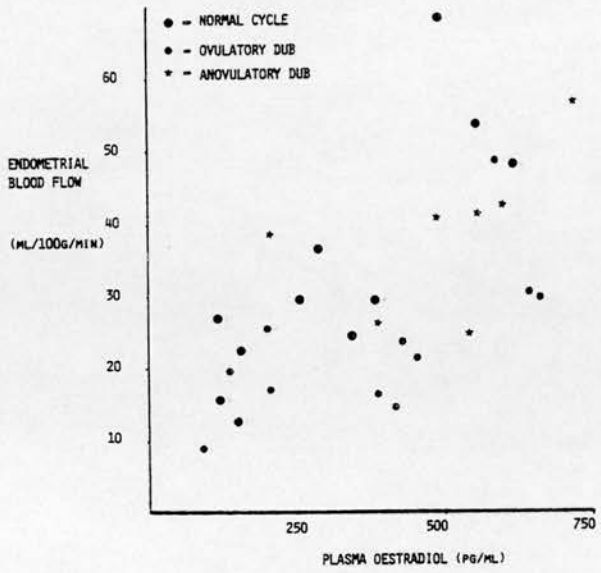


Figure 3:
Comparison of endometrial blood flow with plasma levels of oestradiol in women with normal cycles and ovulatory or anovulatory dysfunctional uterine bleeding. Only follicular phase measurements (plasma progesterone less than 1 ng/ml) have been included.

technique which assessed the speed of movement of red cells through stromal capillaries in mm/sec. Prill and Götz (1961) used a heat clearance probe technique to study cyclical changes in human endometrial blood flow in 10^{-4}cal.cm^{-1}.sec^{-1} °c^{-1}, although this type of technique cannot be exactly recalibrated from one day to the next. It is also pertinent to note that an intrauterine probe may cause artificial changes in local blood flow (Chapter 12). A comparison of the cyclical changes demonstrated by these two groups of investigators with the present Xe-133 study is shown in Figure 4. A comparison of these studies with the Kr-85 clearance technique has been shown in Chapter 11. All studies show a preovulatory peak with a post ovulatory fall and a secondary rise in the luteal phase, although clear differences are also apparent. The inert gas clearance techniques have demonstrated a much more marked periovulatory fall in endometrial flow with a very slow rise in the luteal phase. With the heat clearance technique a slow premenstrual rise in flow is seen which continues through until day 2 - 3 of menstruation. In the rhesus monkey a marked premenstrual fall in flow was seen. Much of our current understanding of the morphology of menstruating endometrium is based on

Markee's studies (1940), especially with respect to the occurrence of dramatic vasoconstriction in the endometrium just before and at the onset of bleeding. The present study and that of Prill and Götz (1961) suggests that this may not occur, at least to the same extent in humans. In which case the coagulation mechanisms studied by Christiaens et al (1982) may play a more dominant role in the maintenance of haemostasis in the human uterus. Nevertheless, in pathological conditions such as spasmodic dysmenorrhoea excessive uterine contractions may produce substantial falls in endometrial blood flow during menstruation (Akerlund et al 1976). There is also some histological evidence that arteriolar vasoconstriction may sometimes occur in human luteal phase endometrium (Salvatore 1968).

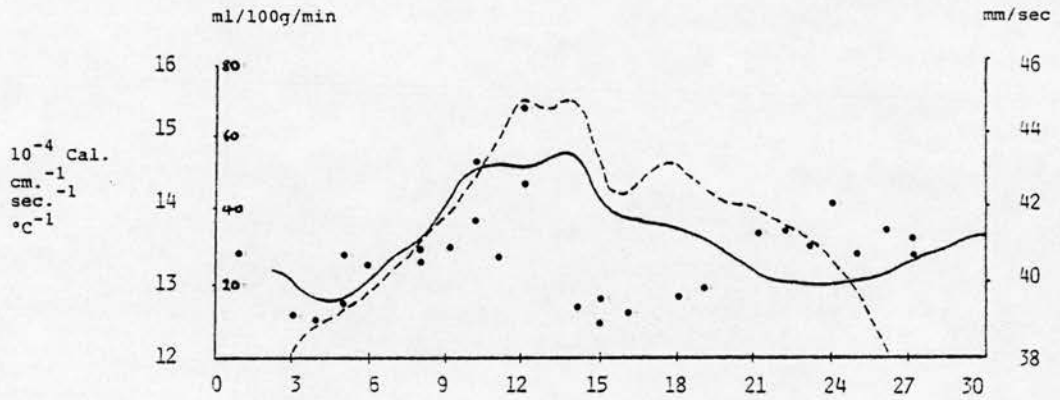


Figure 4. Comparison of endometrial blood flow at different stages of the menstrual cycle measured by Xe-133 clearance in normal women (ml/100g/min) and compared with the data of Markee (1950) in rhesus monkeys (mm/sec; dotted line) and Prill and Gotz (1961) using a heat clearance technique in women (10^{-4} Cal. cm^{-1} . sec^{-1}. $^{o}C^{-1}$; continuous line).

Only one previous investigation has reported absolute flow measurements in human endometrium (Secher et al 1973). This study utilised a different technique of clearance analysis which may not always be appropriate for uterine blood flow studies (Forssman 1975). However, this does report the first measurements of absolute flow in human endometrium (Table 6), and although the mean level is lower than the present study the range overlaps greatly. It is of interest that all the Xe-133 studies of blood flow in human myometrium are in the same range of 3.8 - 37.9 ml/100 g/min (Table 6). This is very similar to the measurements of endometrial flow, although it has been demonstrated that endometrial and myometrial flow may show large independent variations (Chapter 12).

It is of interest to speculate on the mechanism of elevation of endometrial blood flow in the late luteal phase in women. In the light of recent evidence of the importance of vasoactive prostaglandins in endometrial function during menstruation it seems likely that they may be involved in these blood flow changes. However, an influence of other vasoactive substances such as catecholeamines (Cohen et al 1964) or angiotensin (Johnson 1980) should not be discounted.

In normal endometrium most investigators have demonstrated a rise in both $PGF_{2\alpha}$ and PGE_2 in the late luteal and menstrual phases (Downie et al 1974; Singh et al 1975; Lundström and Green 1978) but little is known about cyclical changes in thromboxane A_2 (TXA_2) and prostacyclin (PGI_2). $PGF_{2\alpha}$ and TXA_2 are generally vasoconstrictive while PGE_2 and PGI_2 are vasodilatory, with TXA_2 and PGI_2 also having potent and opposite effects on platelet aggregation. It seems that a balance between these 4 compounds may be important.

Recent evidence has been presented for a decrease in endometrial secretion of $PGF_{2\alpha}$ in anovulatory DUB and an increase in secretion of PGE_2 and PGI_2 in ovulatory DUB (Willman et al 1976; Smith et al 1981a; Smith et al 1981b; Smith et al 1982). Although this study has not demonstrated

any difference in endometrial blood flow between women with normal cycles and menorrhagia in the late luteal phase it does not exclude differences in flow at the onset of heavy bleeding. A significant difference in blood flow in the mid-late follicular phase with lower rates in women with ovulatory DUB (Table 5) could be a pointer to differences in prostaglandin secretion even at that early stage of the cycle.

From the results presented in this study it can be seen that endometrial blood flow is slowly increasing at the time of implantation, and it seems likely that this change is important to aid the success of this crucial event in the early development of the embryo. This increase in flow may also stimulate secretion of chorionic gonadotrophin from the early trophoblast.

Further application of endometrial blood flow studies in normal women, and women with various gynaecological disorders and hormonal therapies promises to help in the understanding of menstruation, its disorders and their management, and perhaps also in some cases of infertility.

SECTION 6

GENERAL DISCUSSION

Chapter 14

GENERAL DISCUSSION:

This thesis describes a series of investigations along a developing theme through various aspects of the condition called dysfunctional uterine bleeding. It covers these disturbances of bleeding in all reproductive age groups and includes studies which address the problems of definition, incidence, perception and function of pituitary, ovary and uterus in women with this condition. All the investigations have raised new questions which merit more detailed study in the future.

Dysfunctional uterine bleeding is a confusing and controversial clinical diagnosis requiring exclusion of local pelvic disease, pregnancy and certain generalised medical diseases. Recent evidence suggests local and hormonal mechanisms may sometimes be responsible for the clinical symptoms. In this thesis an attempt has been made to study different types of DUB in several ways. In several studies it has also been necessary to investigate changes in women with normal cycles before changes seen in women with DUB could be interpreted.

In Section 2 oestrogen dynamics have been studied in women undergoing hysterectomy in the mid to late reproductive years for DUB or for conditions which did not interfere with the menstrual cycle. At the time of the investigations there was some basic knowledge about changes in small numbers of normal women (Baird et al 1969; Longcope et al 1968; Hembree et al 1969). Our studies confirmed the earlier work and were able to demonstrate that oestradiol is virtually an exclusive secretory product of the developing follicle and the corpus luteum. This permitted measurement of ovarian secretion rates of oestradiol and oestrone throughout the cycle. Both are secreted in a biphasic pattern which is directly reflected in the circulating plasma levels, although much oestrone also comes from other sources.

Little was known about the secretion of oestrogens

in women with DUB although urinary excretion of oestrogens in women with anovulatory DUB had been extensively studied by Brown and Colleagues (Brown, Kellar and Matthew 1959; Brown and Matthew 1962). Studies described in this thesis demonstrated that there was no qualitative difference in the secretion and metabolism of oestradiol and oestrone in women with DUB but that the pattern of secretion was different in women with anovulatory DUB. A surprising number of these older women with DUB were found to have double ovulations or simultaneous development of more than one follicle. Great variability in the occurrence of ovulation and the degree of oestrogen effect on the endometrium from cycle to cycle was seen in most of these older women with DUB.

Although numerous studies have been reported of ovarian blood flow in various animal species the only information on ovarian blood flow in women is described in Chapter 4. This indicates capillary flows of 4.6 - 36.4 ml/min (equivalent to a mean of 2.3 ml/g/min), which are of a simular order to those recorded in animals. Since the highest flows were recorded in the luteal phase it is probable that human corpora lutea have a similar high rate of capillary blood flow to that seen in animals. Ovarian blood flow was in the same range in 4 women with anovulatory dysfunctional bleeding.

One of the first studies of oestradiol and oestrone concentrations in follicular fluid is described in Chapter 5. This was planned in such a way that simultaneous studies of oestrogen levels could be carried out in blood from both ovarian veins and a peripheral vein. The highest oestradiol and oestrone levels were found in large follicles (>1cm) in the preovulatory phase. Oestradiol levels were found to be 6,000 times higher in fluid from large "preovulatory" follicles than in peripheral blood, 100 times higher than in ipsilateral ovarian venous blood and 1,200 times higher than in contra-lateral ovarian venous blood (not containing a developing follicle), indicating almost exclusive oestradiol secretion from the preovulatory follicle. In women with dysfunctional uterine bleeding multiple large ovarian follicles were all

found to be actively secreting oestradiol, suggesting a disturbance of intraovarian follicle control mechanisms in these women.

Levels of oestrone in follicular fluid were very much lower than oestradiol and did not show such large increases with follicle growth, confirming that oestradiol is a much more important product of follicle secretion. Much of the circulating oestrone arises from peripheral conversion (Chapter 2; Baird et al 1969).

In recent years many new studies of follicle endocrinology have been published (McNatty et al 1976; McNatty et al 1979; Hodgen 1982) but there is very little new information on follicle dysfunction in dysfunctional uterine bleeding. Extension of these studies into the investigation of disturbances of intraovarian follicle control mechanisms and other aspects of follicle endocrine pathophysiology would be very valuable.

Adolescent DUB is commonly anovulatory, and in those women with recurrent symptoms the prognosis is poor with a high incidence of long term reproductive disturbance (Southam and Richart 1966; Chapter 6). This is especially so for those women in whom endometrial cystic glandular hyperplasia (CGH) is found. However, this particular condition at the severe end of the spectrum of adolescent anovulatory DUB is rare with an annual incidence in the South East of Scotland of only 1 per 14,700 adolescent girls.

In a group of 4 women with adolescent anovulatory DUB and CGH daily plasma sampling over 3 month periods revealed normal patterns of follicular oestrogen production but a failure to trigger the midcycle LH surge (Chapter 7). This even occurred when excessive follicle growth and oestradiol production was stimulated with clomiphene. More detailed studies with 9 young women with anovulatory DUB and 6 normal controls using an oestrogen provocation test and a GnRH stimulation test confirmed that the abnormality was due to a failure of the oestrogen positive feedback mechanism

presumably at a hypothalamic level (Chapter 8). In these women the negative feedback mechanism and pituitary GnRH action are both intact. It is possible that the defect is a maturational phenomenon since positive feedback normally develops in midpuberty, later than negative feedback (Reiter et al 1974), and a few of these adolescents do gain normal function later. Further investigation of these women is indicated to see whether ovulation can be reliably induced by timed midcycle injection of hCG.

Absence of the midcycle LH peak permitted a study of oestradiol secretion by the maturing follicle in a situation where ovulation did not occur (Chapter 8). In these women urinary oestrogen excretion continued to rise for a further 5-6 days and reached levels more than double those seen in controls (62.8 ± 12.6 μg/24 hour compared with 30.7 ± 3.1), before falling in an exponential fashion over the next 10-14 days. Bleeding usually began 10-12 days after the oestrogen peak. A study of the mechanisms of follicle atresia under these circumstances would be most interesting.

Further studies were planned to investigate GnRH and oestrogen feedback actions in perimenopausal women with DUB and in women with polycystic ovarian (PCO) disease (Van Look et al 1977; Baird et al 1974). None of the women with PCO and only 3 out of 5 with perimenopausal anovulatory DUB had a positive feedback defect, indicating clearly that different mechanisms for anovulatory DUB exist. One of the women reported in Chapter 7 experienced an episode of bleeding which indicates the diversity of factors bearing on such disturbances of bleeding. In a cycle showing defective luteal function she started bleeding heavily in the mid to late follicular phase at a time when ovarian oestrogen secretion was rapidly increasing. The mechanism of endometrial bleeding in this situation remains unexplained, as it does under many other circumstances.

Recently, a high incidence of coagulation disorders in adolescents with menorrhagia has been reported (Claessens and Cowell 1981). Adolescents with these diagnoses were

specifically looked for and excluded from our studies.

Early on in these investigations it became obvious that many women were experiencing difficulty in assessing the volume of their menstrual loss and one report provided some objective evidence for this (Hallberg et al 1966). As a consequence later studies included objective measurements of menstrual blood loss using the alkaline haematin technique of Hallberg and Nilsson (1964a) as modified by Newton et al (1977). This permitted development of the concept of "subjective" and "objective" menorrhagia (Fraser et al 1981). In this study of 69 women with a convincing clinical complaint of menorrhagia only 38% satisfied the criterion (blood loss greater than 80 mls) for objective menorrhagia (Fraser et al 1981), and as a consequence it was decided to study perception of menstrual blood loss and changes in loss further in these women (Chapter 10). As a group the women were able to distinguish the difference between "heaviest" and "lightest" periods (69.6 ± 7.3 mls; mean ± SEM; compared with 42.7 ± 4.7 mls), but there were many major errors in perception by individuals. This was particularly clearly seen in the perception of daily blood loss when on days where bleeding was perceived as excessively heavy measured loss varied between 1.4 and 215.8 mls (mean 22.0 mls). No correlation was found between numbers of sanitary towels used and measured blood loss. Two other studies carried out over the same period of time encountered similar errors in the perception of loss (Chimbira et al 1980; Gao Ji et al 1981).

This study indicates that the only reasonably accurate means of assessing volume of menstrual blood loss for research or clinical studies is to measure it by an objective technique such as that described by Hallberg and Nilsson (1964a). It is recommended that this technique should be available to all gynaecologists.

Some of these women noticed that their menstrual discharge was relatively dilute, and a study of 28 normally

menstruating women confirmed that only 36% of the total menstrual fluid loss could be accounted for by blood (Chapter 9). However, there was a wide individual range from 1.6 to 81.7%. This is in agreement with the very limited data from earlier studies (Ebert and Nold 1956; De Merre et al 1967). There was a highly significant correlation between total fluid loss and blood loss even in the one woman with objective menorrhagia. The main volume of menstrual fluid is probably a transudate of endometrial tissue origin. Since this increases in parallel with blood loss it is unlikely to be related to endometrial surface area (Chimbira et al 1980), and may be associated with variations in capillary permeability or rate of endometrial blood flow in women with DUB. Some variations in the proportional content of blood were seen with different methods of contraception but these require confirmation. Much further research is required to study mechanisms and factors influencing the fluid composition of the menstrual discharge. Prostaglandins, lysosomes and coagulation factors clearly merit further investigation. In fact, data on the composition of menstrual blood under most circumstances are very limited (Hahn 1980). Detailed investigation of factors influencing perception of blood loss should also be a high priority.

It seemed possible that abnormalities of endometrial blood flow might exist in women with DUB, and a major study was initiated to test this hypothesis (Chapter 11, 12 and 13). This also necessitated an extensive study of normal women. The only existing techniques for blood flow measurement which seemed applicable to the study of changes in absolute blood flow in human endometrium were a Krypton-85 rebreathing and clearance technique with an intrauterine β-probe and a Xenon-133 intrauterine clearance technique monitored by an external gamma camera (Chapter 11). These two methods were carefully evaluated (Chapter 11) and were then validated by direct comparison with a radioactively labelled microsphere technique in sheep (Chapter 12). None

of the methods previously used for the measurement of human uterine blood flow had been validated in any such way.

Only one study of absolute levels of human endometrial blood flow has been reported (Secher et al 1973) and these levels fall within the lower part of the range reported in the present study (Chapter 13). The study of Secher et al (1973) indicated that endometrial and myometrial blood flow were in the same close range. However, our studies in sheep have demonstrated that endometrial and myometrial blood flow can vary quite independently, especially in the presence of an intrauterine probe or with uterine artery cannulation (Chapter 12; Brown, Fraser and Mattner 1984). Partly for this reason the intrauterine instillation of Xe-133 was chosen for the main human study rather than the intraluminal β-probe with Kr-85.

This study demonstrated a clear cyclical variation in endometrial blood flow in normal women with an early to midfollicular phase rise, preovulatory peak, (mean 39.6 ml/100 g/min) marked periovulatory fall and slow midluteal phase rise which was maintained up to the onset of menstruation and then fell during menstruation (Chapter 13). These values may be compared with the very high rates of flow found in the human ovary (mean 230 ml/100 g/min; Chapter 4). The preovulatory blood flow measurements correlated fairly closely with plasma levels of oestradiol ($r = 0.760$), but this correlation was lost after ovulation because of the low early-mid luteal and elevated late luteal phase flow rates. A tendency to a late elevation of luteal phase flow was also shown by Prill and Gotz (1961) and may indicate differences between women and rhesus monkeys (Markee 1940; 1950) on which species much of our current understanding of menstrual vascular changes is based. It is possible that arteriolar vasoconstriction is not such an important mechanism for limiting the volume of menstrual loss in the human as in the monkey. However, many vasoactive substances are present in endometrium and local changes in blood flow

or sudden changes after the onset of menstruation may still occur in women. Endometrial vessels have unusual morphological appearances which must relate to function and probably blood flow. The spiral arterioles have been well described (Bartelmez 1933; Fanger and Barker 1961; Farrer-Brown et al 1970 a; Ancla and de Brux 1964) but unusual ultrastructural features such as bizarre endothelial protrusions in small vessels (Fraser 1980, and unpublished observations) have still to be clearly defined. The data are limited for normal endometrium and are almost non-existent for DUB.

Women with ovulatory DUB also exhibited cyclical changes in endometrial blood flow. These only differed from normal women in being significantly lower in the mid-late follicular phase. No differences were demonstrated in the late luteal or premenstrual phases but some individuals may show abnormalities. Women with anovulatory DUB exhibited very variable but generally high levels of flow.

The series of investigations described in this thesis has raised numerous questions which require further study in almost every area addressed by the thesis. These relate particularly to the perception and measurement of bleeding, endometrial function and composition of the menstrual discharge, factors influencing endometrial blood flow and many aspects of therapy.

SECTION 7

REFERENCES

REFERENCES

Abdul-Karim R W and Bruce N W. Blood flow to the ovary and corpus luteum at different stages of gestation in the rabbit. Fertil Steril (1973) 24, 44-47.

Abel M. Production of prostaglandins by the human uterus: are they involved in menstruation? Research and Clinical Forums (1979) 1, 33-37.

Ahren K and Hamberger L. Follicle stimulating hormone and ovarian metabolism. Progress in Endocrinology (Gual C, editor) Excerpta Medica Amsterdam (1969) 75-82.

Ahren K, Janson P O and Selstam G. Search for arterio-venous shunts in the rabbit ovary in situ using perfusion of microspheres. J Reprod Fert (1974) 41, 133-142.

Akerlund M, Bengtsson L P and Carter A M. A technique for monitoring endometrial or decidual blood flow with an intrauterine thermistor probe. Acta Obstet Gynecol Scand (1975) 54, 469-477.

Aksel S and Jones G E. Etiology and treatment of dysfunctional uterine bleeding. Obstet Gynecol (1974) 44, 1-13.

Alford F P, Baker H W G, Burger H G, de Kretser D M, Hudson B, Johns M W, Masterton J P, Patel Y C and Rennie G C. Temporal patterns of integrated plasma hormone levels during sleep and wakefulness. J Clin Metab (1973) 37, 848-860.

Ancla M and de Brux. Etude au microscope electronique des arterioles spiralies de l'endometre humain. Annal Anat Pathol (Paris) (1964) 9, 209-222.

Andersen A M and Ladefoged J. Partition coefficient of ^{133}Xenon between various tissues and blood in vitro. Scand J Clin Lab Invest (1967) 19, 72-78.

Anderson A B M, Haynes P J, Guillebaud J, Turnbull A C. Reduction of menstrual blood loss by prostaglandin synthetase inhibitors.Lancet (1976) 1, 774-776.

Aparicio S R, Bradbury K, Bird C C, Foley M E, Jenkins D M, Clayton J K, Scott J S, Ragah S M and McNicol G P. Effect of intrauterine contraceptive device on uterine haemostasis: a morphological study. Brit J Obstet Gynaecol (1979) 86, 314-324.

Arey L B. The degree of normal menstrual irregularity: an analysis of 20,000 Calendar Records from 1,500 individuals. Amer J Obstet Gynecol (1939) 37, 12-26.

Baird D T. A method for the measurement of oestrone and oestradiol 17β in peripheral human blood and other biological fluids using ^{35}pipsyl chloride. J Clin Endocrinol Metab (1968) 26, 244-259.

Baird D T. Secretion of androgens and oestrogens from the ovary and adrenal gland. Reproductive Endocrinology (Irvine W J, editor) Churchill Livingstone Edinburgh (1970) 95-99.

Baird D T. Steroids in blood reflecting ovarian function. Control of Gonadal Steroid Secretion (Baird D T and Strong J, editors) Edinburgh University Press Edinburgh (1971).

Baird D T. The secretion of oestrogens from the ovary in normal and abnormal menstrual cycles. Proc IV Int Congr Endocrinol Washington June 1972. Excerpta Medica Amsterdam (1973) 59-64.

Baird D T, Burger P E, Heavon-Jones G D and Scaramuzzi R J. Androstenedione: a secretory product of the Graafian follicle. J Endocrinol (1973) 59, xxxvi.

Baird D T, Collett R A, Fraser I S, Kelly R W, Land R B and Wheeler A G. Progesterone Secretion from the ovary in the ewe following infusion of uterine venous plasma. J Reprod Fertil (1973) 35, 13-22.

Baird D T, Corker C S, Fraser I S, Hunter W M, Michie E A and Van Look P F A. Pituitary-ovarian relationships in polycystic ovarian disease. J Endocrinol (1975) 64, 53P-54P.

Baird D T and Fraser I S. Disorders of the hypothalamic-pituitary-ovarian axis. Clinics in Endocrinology and metabolism (1973) 2, 469-487.

Baird D T and Fraser I S. Blood production and ovarian secretion of estradiol-17β and estrone throughout the menstrual cycle in women. J Clin Endocrinol Metab (1974) 38, 1009-1017.

Baird D T and Fraser I S. Oestradiol and oestrone concentrations in ovarian venous blood and follicular fluid in women. Clin Endocrinol (1975) 4, 259-266.

Baird D T, Giles M and Cockburn F. The PO_2,PCO_2, pH and oxygen content of ovarian venous blood of sheep. J Endocrinol (1973) 57, 405-411.

Baird D T, Goding J R, Lehikawa Y and McCracken J A. The secretion of steroids from the autotransplanted ovary in the ewe spontaneously and in response to systemic gonadotrophin. J Endocrinol (1968) 42, 283-295.

Baird D T and Guevara A. Concentration of unconjugated estrone and estradiol in peripheral plasma in non-pregnant women throughout the menstrual cycle. J Clin Endocrinol Metab (1969) 29, 149-156.

Baird D T, Horton R, Longcope C and Tait J F. Steroid dynamics under steady state conditions. Rec Prog Horm Res (1969) 25, 611-656.

Baird D T, Uno A and Melby J C. Adrenal secretion of androgens and oestrogens. J Endocrinol (1969) 45, 135-136.

Bardin C W and Lipsett M B. Testosterone and androstenedione blood production rate in normal women and women with idiopathic hirsutism or polycystic ovaries. J Clin Invest (1967) 46, 891-895.

Barer A P and Fowler W M. The blood loss during normal menstruation. Amer J Obstet Gynecol (1936) 31, 979-988.

Bartelmez G W. Histological studies on the menstruating mucus membrane of the human uterine. Contrib Embryol Carneg Inst (1933) 24, 143-187.

Barlow J J and Logan C H. Estrogen Secretion, Biosynthesis and metabolism: their relationship to the menstrual cycle. Steroids (1966) 7, 309-315.

Barraclough C A. Sex steroid regulation of reproductive neuroendocrine process, In: (Greep R O and Astwood E B, editors) Handbook of Physiology section 7 Endocrinology vol 2 part 1 Washington DC American Physiological Society (1973) 29-40.

Beazley J M. Dysfunctional uterine haemorrhage. Brit J Hosp Med (1972) 7, 573-578.

Beilby J O W, Farrer-Brown G, Tarbit M H. The microvasculature of common uterine abnormalities excluding fibroids. J Obstet Gynaecol Brit Cwlth (1971) 78, 361-368.

Beller F K. Observations on the clotting of menstrual blood and clot formation. Amer J Obstet Gynecol (1971) 111, 535-546.

Beller F K and Schweppe K W. Review of the Biology of Menstrual Blood. In: The Biology of the Fluids of the female Genital Tract (Beller F K and Schumacher G F B, editors). Elsevier North Holland New York and Amsterdam (1979) 231-245.

Bentivoglio L G, Beerel F, Bryan A C, Stewart P B, Rose B and Bates D V. Regional pulmonary function studied with Xenon-133 in patients with bronchial asthma. J Clin Invest (1963) 42, 1193-1200.

Bohnen P. Wie weit wird das endometrium bei der menstruation abgestossen? Archiv fur Gynakologie (1927) 129, 459-471.

Bonte J. Beneficial effects of contraceptive doses and large doses of medroxyprogesterone in the prevention and treatment of uterine hyperplasia. Gynak. Rdsch (1978) 18, 172-184.

Boon R C, Schalch D S, Lee L A and Reichlin S. Plasma gonadotrophin secretory patterns in patients with functional menstrual disorders and Stein-Leventhal Syndrome: response to clomiphene therapy. Amer J Obstet Gynecol (1972) 112, 736-748.

Brown B W, Emery M J and Mattner P E. Ovarian arterial velocity measured with Doppler ultrasonic transducers in conscious ewes. J Reprod Fert (1980) 58, 295-300.

Brown B W, Fraser I S and Mattner P E. Comparison of microsphere and Krypton washout techniques for measuring uterine blood flow in sheep. Theriogenology (1977) 8, 146.

Brown B W, Fraser I S and Mattner P E. Changes in reproductive tract blood flow during the oestrous cycle of the ewe and effects of arterial cannulation. Aust J Biol Sci (1984) in press.

Brown B W, Hales J S R and Mattner P E. Capillary blood flow in sheep ovaries, measured by iodoantipyrine and microsphere techniques. Experientia (1974) 30, 914-915.

Brown J B. Assessment of ovarian function by measurement of urinary oestrogens and pregnanediol. Control of gonadal steroid secretion (Baird D T and Strong J editors) Edinburgh University Press (1971) 127-140.

Brown J B, Kellar R, Matthew G D. Preliminary observations on urinary oestrogen excretion in certain gynaecological disorders. J Obstet Gynaecol Brit Emp (1959) 66, 177-211.

Brown J B, MacLeod S C, Macnaughton S, Smith M A and Smyth B. A rapid method for estimating oestrogens in urine using a semi-automatic extractor. J Endocrinol (1968) 42, 5-15.

Brown J B, Matthew G D. The application of urinary estrogen measurements to problems in gynecology. Rec Prog Horm Res (1962) 18, 337-373.

Bruce N W and Moor R M. Bibliography and review on ovarian blood flow and function. Bibliog Reprod (1975) 25, 581-586, 695-697.

Bruce N W and Moor R M. Capillary blood flow to ovarian follicles, stroma and corpora lutea of anaesthetized sheep. J Reprod Fertil (1976) 46, 299-304.

Buttram V S, Reiter R C. Uterine leiomyomata: etiology, symptomatology and management. Fertil Steril (1981) 36, 433-445.

Casey B H and Thorburn G D. The distribution of blood flow in the skin using radioactive Krypton^{85} clearance technique. In: Biology of the Skin and Hair Growth (Lyne A G and Short B F editors) Sydney Angus and Robertson Ltd (1965) 603-615.

Chamberlain J and Contractor S F. A gas-liquid chromatographic method for the rapid estimation of pregnanediol and allopregnanediol in non-pregnancy urine. Am J Obstet Gynecol (1968) 101, 649-657.

Chamlian D L, Taylor H B. Endometrial hyperplasia in young women. Obstet Gynecol (1970) 36, 659-665.

Channing C P and Couvert S P. Contribution of granulosa cells and follicular fluid to ovarian estrogen secretion in the rhesus monkey in vivo. Endocrinol (1976) 98, 590-597.

Chimbira T H, Anderson A B M, Cope E and Turnbull A C. Preliminary results on clinical and endocrine studies in the treatment of menorrhagia with danazol. Postgrad Med J (1979) 55 (Suppl 5) 90-94.

Chimbira T, Anderson A B M, Turnbull A C. Relation between measured menstrual blood loss and patients subjective assessment of loss, duration of bleeding, number of sanitary towels used, uterine weight and endometrial surface area. Brit J Obstet Gynaecol(1980) 87, 603-609.

Christiaens G C M L, Sixma J J and Haspels A A. Hemostasis in menstrual endometrium: a review. Obstet Gynecol Surv (1982) 37, 281-303.

Claessens E A, Cowell C A. Acute adolescent menorrhagia. Amer J Obstet Gynecol (1981) 139, 277-280.

Clara M. Der arterio-venosen anastomosen, 2nd edition. Springer-Verlog, Vienna (1956).

Cohen B J B, Gibor Y. Anaemia and menstrual blood loss. Obstet Gynecol Surv (1980) 35, 597-618.

Cohen S, Bitensky L, Chayen J, Cunningham G T and Tussell J K. Histochemical studies of the endometrium. Lancet (1964) 2, 56-58.

Cole S K, Billewicz W Z, Thomson A M. Sources of variation in menstrual blood loss. J Obstet Gynaecol Brit Cwlth (1971) 78, 933-939.

Collier H O J, Sweatman W J F. Antagonism by fenamates of prostaglandin F_{2_a} and of slow reacting substance on human bronchial muscle. Nature (1968) 219, 864-866.

Cope E. Dysfunctional uterine bleeding. Brit Med J (1971) 2, 631-633.

Cox L W. The Australian human pituitary induction of ovulation programme. Aust NZ J Obstet Gynaecol (1976) 16, 106-110.

Crawford P. Attitudes to menstruation in seventeenth-century England. Past and Present (1981) 91, 47-73.

Dallenbach-Hellweg G, Dawson A B and Hisaw F L. The effect of relaxin on the endometrium of monkeys. Amer J Anat (1966) 199, 61-78.

Damarawy H, Toppozada M. Control of bleeding due to IUDs by a prostaglandin biosynthesis inhibitor. I R C S Medical Science, Reprod Obstet Gynaecol (1976) 4, 5-7.

Davies A J, Anderson A B M. Turnbull A C. Reduction by Naproxen of excessive menstrual bleeding in women using intrauterine devises. Obstet Gynecol (1981) 57, 74-78.

De Merre L J, Moss J D and Pattison D S. The hematological study of menstrual discharge. Obstet Gynecol (1967) 30, 830-839.

Dewhurst C J, Cowell C A and Barrie L C. The regularity of early menstrual cycles. J Obstet Gynaecol Brit Cwlth (1971) 78, 1093-1099.

Dignam W J, Parlow A F and Daane T A. Serum FSH and LH measurements in the evaluation of menstrual disorders. Amer J Obstet Gynecol (1969) 105, 679-693.

Donney J, Nanters M, Thomas K. Luteal function after tubal sterilisation. Obstet Gynecol (1981) 57, 65-69.

Doring G K. The incidence of anovular cycles in women. J Reprod Fertil (1969) Suppl 6, 77-81.

Downie J, Poyser N L, Wunderlich M. Levels of prostaglandins in human endometrium during the normal menstrual cycle. J Physiol (1974) 236, 465-469.

Droegemueller W, Greer B and Makowski E. Cryosurgery in patients with dysfunctional uterine bleeding. Obstet Gynecol (1971) 38, 256-258.

Ebert R and Nold B. Gerinnungsphysiologische studien am menstrualblut. Schweiz Med Wochenschr (1956) 36, 999-1009.

Edgerton W D. Late complications of laparoscopic sterilisation. J Reprod Med (1977) 18, 275-277.

Edwards R G, Steptoe P C, Abraham G E, Walters E, Purdy J M, and Fotherby K. Steroid assays and pre-ovulatory follicular development in human ovaries primed with gonadotrophins. Lancet (1972) ii 612-615.

Einer-Jensen N. Endometrial blood flow in rats during early pregnancy. J Reprod Fertil (1977) 49, 377-379.

Fanger H and Barker B E. Capillaries and arterioles in normal endometrium. Obstet Gynecol (1961) 17, 543-550.

Farrer-Brown G, Beilby J O W and Tarbit M H. The blood supply of the uterus. I Arterial vasculative. J Obstet Brit Cwlth (1970a)77, 673-681.

Farrer-Brown G, Beilby J O W and Tarbit M H. The vascular patterns in myomatous uteri. J Obstet Gynaecol Brit Cwlth (1970b)967-971.

Ferenczy A. Studies on the cytodynamics of human endometrial regeneration. 1 Scanning electron microscopy. Amer J Obstet Gynecol (1976) 124, 64-76.

Finn C A. The implantation reaction. Biology of the Uterus (Wynn R M. editor) Plenum Press New York (1977) 245-308.

Flowers C E, Willborn W H. New observations on the physiology of menstruation. Obstet Gynecol (1978) 51, 16-24.

Foley M E, Griffin D B, Zuzel M, Aparicio S R, Bradbury K, Bird C C, Clayton J K, Jenkins D M, Scott J S, Rajah S M and McNicol G P. Heparin-like activity in uterine fluid. Brit Med J (1978) 2, 322-324.

Forssman L. Xenon-133 clearance from the human non-pregnant uterus. Acta Obstet Gynecol Scand (1973) 52, 327-334.

Forssman L. Xenon-133 clearance from the human non-pregnant uterus; A methodological and clinical study. M D Thesis presented to the University of Gothenberg (1974).

Forssman L. Methods of calculating uterine blood flow from the washout curves of intra-arterial and local injections of Xenon-133. Acta Obstet Gynecol Scand (1975) 54 479-484.

Forsyth R P and Hoffbrand B J. Redistribution of cardiac output after sodium pentobarbital anaesthesia in the monkey, Amer J of Physiol (1970) 218, 214-217.

Fowler W M, Barer P A. Rate of hemoglobin regeneration in blood donors. J A M A (1942) 118, 421-423.

Fox H. The aetiology and pathology of endometrial cancer. Clin Obstet Gynecol (1976) 3, 371-386.

Fraser I S. Danazol-a steroid with a unique combination of actions. Scot Med J (1979) 24, 147-150.

Fraser I S. Discussion following paper by B L Sheppard and J Bonnar. Endometrial bleeding and steroidal contraception (Diczfalusy E, Fraser I S and Webb F T G, editors). Pitman Press Bath (1980) p 79.

Fraser I S. Abnormal uterine bleeding due to hormonal steroids and intrauterine devices. Recent Advances in Fertility Regulation (chang C F, Griffin D and Woolman A. editors) Atlar Press, Geneva (1981) 265-300).

Fraser I S. The dysfunctional uterus = dysmenorrhoea and dysfunctional uterine bleeding. Clinical Reproductive Endocrinology (Shearman R P. editor)Churchill Livingstone, Edinburgh (1985), in press.

Fraser I S and Baird D T. Endometrial cystic glandular hyperplasia in adolescent girls. J Obstet Gynaecol Brit Cwlth (1972) 79, 1009-1015.

Fraser I S and Baird D T. Blood production and ovarian secretion of estradiol-17β and estrone in women with dysfunctional uterine bleeding. J Clin Endocrinol Metab (1974)39, 564-570.

Fraser I S, Baird D T and Cockburn F. Ovarian venous blood PO_2, PCO_2 and pH in women. J Reprod Fert (1973) 33, 11-17.

Fraser I S, Brown B W and Mattner P E. Endometrial blood flow in anaesthetized sheep as measured with Krypton-85 clearance and microsphere techniques. Quart J Exp Physiol (1980)65,19-26.

Fraser I S, Brown B W and Mattner P E. Measurements of endometrial blood flow in anaesthetized ewes by Xenon-133 clearance and microsphere techniques. Quart J Exp Physiol (1982) 67,531-535.

Fraser I S and Macey D J. Methods for measuring uterine blood flow in non-pregnant women. Proceedings of Symposium on Biomedical Engineering in Obstetrics and Gynaecology, University of Sussex. January 1976 (1976) 150-152.

Fraser I S, Michie E A, Wide L, Baird D T. Pituitary gonadotropins and ovarian function in adolescent dysfunctional uterine bleeding. J Clin Endocrinol Metab (1973) 37, 407-414.

Fraser I S, McCarron G, Markham R. A preliminary study of factors influencing perception of menstrual blood loss volume. Amer J Obstet Gynecol (1984a) 149, 788-793.

Fraser I S, McCarron G, Markham R and Resta T. Total blood and fluid loss in the menstrual discharge. Obstet Gynecol (1984b), in press.

Fraser I S, McCarron G, Markham R, Robinson M, Smyth E. Long term treatment of menorrhagia with mefenamic acid. Obstet Gynecol (1983) 61, 109-112.

Fraser I S, Pearse C, Shearman R P, Elliott P M, McIlveen J, Markham R. Efficacy of mefenamic acid in patients with a complaint of menorrhagia. Obstet Gynecol (1981) 58, 543-551.

Fraser R C, Cudmore D C, Melanson J and Morse W I. The metabolism and production rate of estradiol 17β in premenopausal women with cervical carcinoma. Amer J Obstet Gynecol (1967) 98, 509-519.

Gao J, Ma L-Y, Zeng S, Fan H-M, Han L-H. Menstrual blood loss in healthy Chinese women. Contraception (1981) 23, 591-601.

Ghalioungui P. Magic and medical science in Ancient Egypt. Hodder and Stoughton London (1963).

Giorgi E P. Steroids in cyst fluid from ovaries from normally menstruating women and women with functional uterine bleeding. J Reprod Fertil (1965) 10, 309-320.

Giorgi E P, Addis M and Colombo G. The fate of free and conjugated oestrogens injected into the Graafian follicle of equines. J Endocrinol (1969) 45, 37-49.

Goding J R, McCracken J A and Baird D T. A study of ovarian function in the ewe by means of a vascular autotransplantation technique. J Endocrinol (1967) 39, 37-52.

Goldenberg R L, Vaitukaitis J L and Ross G T. Oestrogen and follicle stimulating hormone interactions on follicle growth in rats. Endocrinol (1972) 90, 1492-1497.

Goldrath M H, Fuller T A and Segal S. Laser photovaporisation of the endometrium for the treatment of menorrhagia. Amer J Obstet Gynecol (1981) 140, 14-19.

Goldzieher J W. Polycystic ovarian disease. Fertil Steril (1981) 35, 371-394.

Gray R H. Patterns of bleeding associated with the use of steroidal contraceptives. In: (Diczfalusy E, Fraser I S and Webb F T G. editors) Endometrial Bleeding and Steroidal Contraception Bath Pitman Press (1980) 14-49.

Greenberg M. Hysterectomy, hormones and behaviour. Lancet (1981) 1, 449.

Greenberg M. The meaning of menorrhagia: an investigation into the association between the complaint of menorrhagia and depression. J Psychosomatic Res (1983) 27, 209-214.

Guillebaud J, Anderson A B M, Turnbull A C. Reduction by mefenamic acid of increased menstrual blood loss associated with intrauterine contraception. Brit J Obstet Gynaecol (1978) 85, 53-62.

Guillebaud J, Bonnar J, Morehead J and Matthews A. Menstrual blood loss with intrauterine devices. Lancet (1976) 1, 387-390.

Guillebaud J and Bonnar J. Longer though lighter menstrual and intermenstrual bleeding with copper as compared to most intrauterine devices. Brit J Obstet Gynaecol (1978) 85, 707-715.

Gurpide E, Mann J and Lieberman S. Analysis of open systems of multiple pools of administration of tracers at a constant rate or as a single dose as illustrated by involving steroid hormones. J Clin Endocrinol Metab (1963) 23, 1155-1162.

Hahn L. Composition of Menstrual Blood. In: Endometrial Bleeding and Steroidal Contraception. (Diczfalusy E, Fraser I S, Webb F T G. editors) Bath England Pitman Press (1980) 107-131.

Hales J R S. Radioactive microsphere measurement of cardiac output and regional tissue blood flow in sheep. Pflügers Archiv (1973) 344, 119-132.

Hales J R S and Cliff W J. Direct observations of the behaviour of microspheres in microvasculature. Bibliotheca Anatomica (1977) 15, 87-91.

Hallberg L, Hogdahl A M, Nilsson L, Rybo G. Menstrual blood loss - a population study. Acta Obstet Gynecol Scand (1966) 45, 320-351.

Hallberg L, Nilsson L. Determination of menstrual blood loss, Scand J Clin Lab Invest (1964a) 16, 244-248.

Hallberg L, Nilsson L. Constancy of individual menstrual blood loss. Acta Obstet Gynecol Scand (1964b) 43, 352-361.

Handbook of Biological Data. (Spector W S. editor) Saunders, Philadelphia (1956).

Harrison R F, Campbell S. Double-blind trial of ethamsylate in the treatment of primary and intrauterine device menorrhagia. Lancet (1976) 2, 283-285.

Haynes P J, Anderson A B M, Turnbull A C. Endocrine studies in unexplained menorrhagia. Proceedings of 22nd British Congress of Obstetrics and Gynaecology. Royal College of Obstetricians and Gynaecologists London. (1980).

Haynes P J, Hodgson H, Anderson A B M, Turnbull A C. Measurement of menstrual blood loss in patients complaining of menorrhagia. Brit J Obstet Gynaecol (1977) 84, 763-768.

Hefnawi F, El-Zayat A F, Yacout M M. Physiologic studies of menstrual blood loss. Int J Gynaecol Obstet (1980) 17, 343-352.

Hembree W, Bardin C W and Lipsett M B. A study of oestrogen metabolic clearance rates and transfer factors. J Clin Invest (1969) 48, 1809-1815.

Henzl M R, Smith R C, Boost G, Tyler E T. Lysosomal concepts of menstrual bleeding in humans. J Clin Endocrinol Metab (1972) 34, 860-875.

Hodgen G D. The dominant ovarian follicle. Fertil Steril (1982) 38, (3) 281-300.

Hoedt-Rasmussen K and Veall N. Studies of cerebral blood flow. Radio isotopes in Medical Diagnosis (Belcher E H and Vetter H. editors) Butterworths London (1971) 500-517.

Hoffman G E, Rao C V, Barrows G H and Sanfillipo J S. Topography of human uterine prostaglandin E and F_2 receptors and their profiles during pathological states J Clin Endocrinol Metab (1983) 57, 360-366.

Hunter W M, Edmond P, Watson G S and Mclean N. Plasma LH and FSH levels in subfertile men. J Clin Endocrinol Metab (1974) 39, 740-745.

Hytten F E, Cheyne G A and Klopper A I. Iron loss at menstruation. J Obstet Gynaecol Brit Cwlth (1964) 71, 255-262.

Internation Commission on Radiological Protection. Protection of the Patient in Radionuclide Investigations. I C R P Publication 17, Pergammon Press (1971).

Israel S L. Diagnosis and treatment of menstrual disorders and sterility. Harper and Row New York (1967).

Jacobs A, Butler E B. Menstrual blood loss in iron-deficiency anaemia. Lancet (1965) 2, 407-409.

Jacobs H S. Failure of components of the negative feedback system. Clinics in Obstet Gynaecol (1976) 515-534.

Jacobs J B. Selective gonadal venography. Radiology (1969) 92, 885-896.

Jacubowicz D L and Wood C. The use of the prostaglandin synthetase inhibitor mefenamic acid in the treatment of menorrhagia. Aust NZ J Obstet Gynaecol (1979) 18, 135-142.

Jansson I. Xenon-133 clearance in the myometrium of pregnant and non-pregnant women. Acta Obstet Gynecol Scand (1969) 48, 302-321.

Jeffcoate T N A. Treatment of functional uterine haemorrhage by means of gonadotropic and ovarian hormones. J Obstet Gynaecol Brit Emp (1937) 44, 31-85.

Johnson I R. Renin substrate, active and acid-activatable renin concentration in human plasma and endometrium during the normal menstrual cycle. Brit J Obstet Gynaecol (1980) 87, 875-882.

Jones H B. Medical Physics (Glasser O. editor) (1950) Vol II p 855.

Judd H L and Yen S S C. Serum androstenedione and testosterone levels during the menstrual cycle. J Clin Endocrinol Metab (1973) 36, 475-481.

Karsch F J, Weick R F, Butler W R, Dierschke D J, Krey L C, Weiss G, Hotchkiss J, Yamaji T and Knobil. Induced LH surges in the rhesus monkey: strength-duration characteristics of the estrogen stimulus, Endocrinol (1973) 92, 1740-1744.

Kasonde J, Bonnar J. Aminocaproic acid and menstrual blood loss in women using intrauterine devices. Brit Med J (1975a) 4, 17-19.

Kasonde J, Bonnar J. Effect of ethamsylate and aminocaproic acid on menstrual blood loss in women using intrauterine devices. Brit Med J (1975b) 4, 21-22.

Kasonde J, Bonnar J. The effect of sterilisation on menstrual blood loss. Brit J Obstet Gynaecol (1976) 83, 572-580.

Kelly R W. Prostaglandin synthesis in the male and female reproductive tract. J Reprod Fertil (1981)62, 293-304.

Kety S S. The theory and applications of the exchange of inert gas at the lungs and tissues. Pharmacol Rev (1951) 3, 1-41.

Killam A P, Rosenfeld C R, Battaglia F C, Makowski E L and Meschia G. Effect of estrogens on uterine blood flow in oophorectomized ewes. Amer J Obstet Gynecol (1973) 115, 1045-1052.

Kirschner M A and Taylor J P. Urinary estrogen production rates and normal and endocrine-ablated subjects. J Clin Endocrinol Metab (1972) 35, 513-519.

Kirschner M A and Jacobs J R. Combined ovarian and adrenal vein catheterization to determine the sites of androgen overproduction in hirsute women. J Clin Endocrinol Metab (1971) 33, 199-209.

Klingenberg I. Measurement of blood flow in human myometrium by local hydrogen clearance. Acta Obstet Gynecol Scand (1969) 48, 470-478.

Klingenberg I. Measurement of uterine blood flow in non-pregnant women by electro-magnetic flowmeter. Acta Obstet Gynecol Scand (1973) 52, 317-326.

Klingenberg I. The effect of oxytocin on local uterine blood flow in women with secondary amenorrhoea and early pregnancy measured by local hydrogen clearance. Acta Obstet Gynecol Scand (1974) 53, 191-196.

Klopper A, Michie E A and Brown J B. A method for the determination of urinary pregnanediol. J Endocrinol (1955) 12, 209-218.

Koetsawang S. Present management of abnormal bleeding associated with steroidal contraceptives. Endometrial bleeding and steroidal contraception (Diczfalusy E, Fraser I S and Webb F T G, editors). Pitman Press Bath (1980) 50-58.

Korenman S G and Sherman B R. Further studies of gonadotropin and estradiol secretion during the preovulatory phase of the menstrual cycle. J Clin Endocrinol Metab (1973) 36, 1205-1209.

Kovacs L, Annus J. Effectiveness of ethamsylate in intra-uterine device menorrhagia. Gynecol Invest (1978) 9, 161-165.

Ladefoged J and Andersen A M. Solubility of Xenon-133 at 37°C in water, saline, olive oil, liquid paraffin, solutions of albumin and blood. Physics in Med Biol (1967) 12, 353-358.

Landgren B M, Diczfalusy E. Hormonal effects of the 300µg Norethisterone minipill. Contraception (1980) 21, 87-99.

Lassen N A. Studies of peripheral circulation. Radioisotopes in Medical Diagnosis (Belcher E H and Vetter H. editors). Butterworths London (1971) 474-499.

Lawson S, Cole R A, Templeton A A. The effect of laparoscopic sterilisation by diathermy or silastic bands on post-operative pain, menstrual symptoms and sexuality. Brit J Obstet Gynaecol (1979) 86, 659-663.

Lieberman B A, Belsey E, Gordon A G, Wright C S W, Letchworth A T, Noble A D, Niven P A R. Menstrual patterns after laparoscopic sterilisation using a spring-loaded clip. Brit J Obstet Gynaecol (1978) 85, 376-380.

Lloyd C W, Lobotsky J, Weisz J, Baird D T, MacCracken J A, Pupkin M, Zanartu J and Puga J. Concentration of unconjugated estrogens, androgens and gestogens in ovarian and peripheral venous plasma of women. J Clin Endocrinol Metab (1971) 32, 155-164.

Loeser A A. The action of intravenously injected sex hormones and other substances on blood flow in the human endometrium. J Obstet Gynaecol Brit Emp (1948) 55, 17-23.

Longcope C, Layne D S and Tait J F. Metabolic clearance rates and interconvesions of estrone and estradiol in normal males and females. J Clin Invest (1968) 47, 93-98.

Longcope C and Tait J F. Validity of metabolic clearance rates and interconversion rates of estrone and estradiol in normal adults. J Clin Endocrinol Metab (1971) 32, 481-489.

Louvert J P and Vaitukaitis J L. Induction of FSH receptors in rat ovaries by estrogen treatment. Endocrinol (1976) 99, 758-764.

Lundstrom V, Green K. Endogenous levels of PGF_{2_a} and its main metabolites in plasma and endometrium of normal and dysmenorrhoeic women. Amer J Obstet Gynecol (1978) 130, 640-649.

MacDonald P C, Rombaut R P and Siitteri P K. Plasma precursors of estrogen. I. extent of conversion of plasma Δ^4 and-rostenedione to estrone in normal males and non pregnant normal castrate and adrenalectomized females. J Clin Endocrinol Metab (1967) 27, 1103-1109.

MacDonald P C, Grodin J M and Siitteri P K. Dynamics of androgen and oestrogen secretion. Control of gonadal steroid secretion (Baird D T and Strong J. editors)

Edinburgh University Press (1971) 157-174.

March C M. Dysfunctional uterine bleeding. Reproductive endocrinology, infertility and contraception (Mishell D R and Davajan V. editors) F A Davis Co Philadelphia (1979) 299-309.

Markee J E. Menstruation in intraocular endometrial transplants in the rhesus monkey. Contrib Embryol (Carnegie Institution of Washington) (1940) 28, 219-308.

Markee J E. The relation of blood flow to endometrial growth and the inception of menstruation. In: Engle E T. editor) Menstruation and its disorders, Thomas C C Illinois (1950) 165-185.

Maslar I A, Riddick D H. Prolactin production by human endometrium during the normal menstrual cycle. Amer J Obstet Gynecol (1979) 135, 751-754.

Mattner P E and Thorburn G D. Ovarian blood flow in sheep during the oestrous cycle. J Reprod Fertil (1969) 19, 547-549.

Mattner P E, Brown B W and Hales J S R. Evidence for functional arterio-venous anastomoses in the ovaries of sheep. J Reprod Fertil (1981) 63, 279-284.

Menzer D, Morris T, Gates P, Sabbath J, Robey H, Plant T, Sturgis S H. Patterns of emotional recovery from hysterectomy. Psychosomatic Med (1957) 19, 379-388.

Mikhail G. Hormone secretion by the human ovaries. Gynecol Invest (1970) 1, 5-16.

Millis J. The iron losses of healthy women during consecutive menstrual cycles. Med J Aust (1951) 2, 874-876.

Moor R M. The ovarian follicle of the sheep: inhibition of oestrogen secretion by luteinizing hormone. J Endocrinol (1974) 61, 455-464.

Muldoon M J. Gynaecological illness after sterilisation. Brit Med J (1972) 1, 84-88.

Munck O, Lysgaard H, Pontonnier G, Lefevre H and Lassen N A. Measurement of blood flow through uterine muscle by local injection of Xenon-133. Lancet (1964) 1, 1421-1424.

McLennan C E, Rydell A H. Extent of endometrial shedding during normal menstruation (1965) 26, 605-621.

McNatty K P and Baird D T. Relationship between FSH, androstenedione and oestradiol in human follicular fluid. J Endocrinol (1978) 76, 527-531.

McNatty K P, Hunter W M, McNeilly A S and Sawers R S. Changes in the concentration of pituitary and steroid hormones in the follicular fluid of human Graafian follicles throughout the menstrual cycle. J Endocrinol (1975) 64, 555-571.

McNatty K P, Sawers R S and Baird D T. Concentration of oestrogens and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle. J Endocrinol (1976) 71, 77-85.

McNatty K P, Smith D M, Makris A, Osathanondh R and Ryan K J. The microenvironment of the human antral follicle : Interrelationships among the steroid levels in antral fluid, the population of grannulosa cells and the status of the oocyte in vivo and in vitro. J Clin Endocrinol Metab (1979) 49, 851-860.

National Opinion Poll Research Survey. Menstrual Symptoms in the Community. Commissioned by Parke-Davis Company, United Kingdom (1980).

Nedoss B R. Dysfunctional uterine bleeding: relation of endometrial histology to outcome. Amer J Obstet Gynecol (1971) 109, 103-107.

Neil J R, Hammond G T, Noble A D, Rushton L, Letchworth A T. Late complications of sterilisation by laparoscopy and tubal ligation. Lancet (1975) 2, 699-700.

Newton J R. A new dynamic test of pituitary function. Postgrad med J (1972) 48, 5-14.

Newton J, Barnard G, Collins W. A rapid method for measuring menstrual loss using automatic extraction. Contraception (1977) 16, 269-282.

Newton J R and Dixon P. Site of action of clomiphene and its use as a test of pituitary function. J Obstet Gynaecol Brit Cwlth (1971) 78, 812-821.

Newton J R, Snowden S A, Parsons V. Control of menstrual bleeding during haemodialysis. Brit Med J (1976) 1, 1016-1017.

Nillius S and Wide L. Induction of a midcycle-like peak of luteinizing hormone in young women with exogenous oestradiol-17β, J Obstet Gynaecol Brit Cwlth (1971) 78, 822-827.

Nilsson L, Rybo G. Treatment of menorrhagia. Amer J Obstet Gynecol (1971) 110, 713-720.

Nogales-Ortiz F, Puerta J and Nogales F F. The normal menstrual cycle. Chronology and mechanism of endometrial desquamation. Obstet Gynecol (1978) 51, 259-268.

Noyes R W, Hertig A T and Rock J. Dating the endometrial biopsy. Fertil Steril (1950) 1, 3-25.

Paschkiss A and Rakoff A E. Some aspects of the physiology of estrogenic hormones. Rec Prog Horm Res (1950) 5, 115-141.

Paton R C, Tindall H, Zuzel M, McNicol G P. Haemostatic mechanisms in the normal endometrium and endometrium exposed to contraceptive steroids. In: (Diczfalusy E, Fraser I S, Webb F T G. editors) Endometrial bleeding and steroidal contraception Pitman Press Bath (1980) 325-341.

Pavlin V, Flynn M J, Mulder J L, Cort J H. The treatment of uterine bleeding with vasopressin hormonogen (Glypressin) - a pilot study. Brit J Obstet Gynaecol (1978) 85, 801-805.

Peterson W F and Novak E R. Endometrial polyps. Obstet Gynecol (1956) 8, 40-56.

Powell M J D. An efficient method for finding the minimum of a function of several variables without calculating derivatives. Computer J (1964) 7, 155-162.

Prill H J and Götz F. Blood flow in the myometrium and endometrium of the uterus. Amer J Obstet Gynecol (1961) 82, 102-108.

Purchas S. Microcosmus: or, The Historie of Man. London (1619) p 481.

Quick A J. Menstruation in hereditary bleeding disorders. Obstet Gynecol (1966) 28, 37-40.

Rathmacher R P and Anderson L L. Blood flow and progesterone levels in the ovary of cycling and pregnant pigs. Amer J Physiol (1968) 214, 1014-1018.

Reiter E O, Kulin H E and Hamwood S M. The absence of positive feedback between estrogen and luteinizing hormone in sexually immature girls. Pediatr Res (1974) 8, 740-751.

Rekonen A, Luotola H, Pitkänen M, Kuikka J and Pyörälä T. Measurement of intervillous and myometrial blood flow by an intravenous Xe-133 method. Brit J Obstet Gynaecol (1976) 83, 723-728.

Reyniak J V and Lauersen N H. Danazol-a versatile pharmacologic agent. Fertil Steril (1982) 37, 475-477.

Richards D H. A general practice view of functional disorders associated with menstruation. Res Clin Forums (1979) 1, 39-45.

Roos P. Isolation and purification of gonadotrophins - Immunoassay of gonadotrophins (Diczfalusy E. editor) Acta Endocrinol (1969) Suppl 131, 1-14.

Ross G T, Cargille C M, Lipsett M B, Rayford P L, Marshall J R, Strott C A and Rodbard D. Pituitary and gonadal hormones in women during spontaneous and induced ovulatory cycles. Rec Prog Horm Res (1970) 26, 1-62.

Royal College of General Practitioners. Oral contraception and health: an interim report from the Oral Contraceptive Study of the Royal College of General Practitioners. Pitman Medical New York (1974).

Rumbolz W L, Moon C F, Norelli J C. Use of protamine sulphate and toluidine blue for abnormal uterine bleeding. Amer J Obstet Gynecol (1952) 63, 1029-1036.

Ryan K J and Smith O W. Biogenesis of steroid hormones in the human ovary. Rec Prog Horm Res (1965) 21, 367-386.

Rybo G. Plasminogen activator in the endometrium. Acta Obstet Gynecol Scand (1966) 45, 411-450.

Salvatore C A. Arteriole alteration in normal and hyperplastic endometrium. Amer J Obstet Gynecol (1968) 102, 493-500.

Sanyal M K, Berger M J, Thompson I E, Taymor M L and Horne H W. Development of Graafian follicles in adult human ovary. I. Correlation of oestrogen and progesterone concentration in antral follicles with growth of follicles. J Clin Endocrinol Metab (1974) 38, 828-836.

Sapire K E, Davey D A. The effect of sterilisation by bipolar cautery and falope ring on menstrual bleeding patterns. S Afr Med J (1980) 58, 275-277.

Sasaki Y, Wagner H N, Iio M, Murao S, Takayanagi S and Kobayashi T. Catheter semiconductor detector and its application to clinical experimental medicine. In: Semiconductor detectors in the future of nuclear medicine. (Hoffer P B, Beck R N and Gottschalk A. editors)New York: Society of Nuclear Medicine Inc. (1971) 144-156.

Schild W. Untersuchingen über die Kozentration von Ostron Ostradiol un Ostriol in Ovarialvenenblut. Geburtsch Frauenkielk (1966) 26, 607-616.

Schröder R. Endometrial hyperplasia in relation to genital function. Amer J Obstet Gynecol (1954) 68, 294-309.

Scott J C, Mussey E. Menstrual patterns in myxoedema. Amer J Obstet Gynecol (1964) 90, 161-164.

Secher N J, Einer-Jensen N and Juhl B. Blood flow through the myometrium and endometrium in standing and supine women measured by intrauterine Xenon application. Amer J Obstet Gynecol (1973) 117, 386-388.

Selwood T, Wood C. Incidence of hysterectomy in Australia. Med J Aust (1978) 2, 201-203.

Sensky T E, Liu D T. Endometriosis: associations with menorrhagia, infertility and oral contraceptives. Int J Gynecol Obstet (1980) 17, 573-576.

Setchell B P. Discussion following paper: The study of ovarian function by means of transplantation of the ovary in the ewe. The Gonads (McKerns K W. editor). Appleton-Century-Crofts New York (1969) 203.

Shaw S T. Quantitation of menstrual blood loss. Postgrad Med (1973) 53, 204-209.

Shaw T. On quantifying menstrual blood loss. Contraception (1977) 16, 283-284.

Short R V and London D R. Defective biosynthesis of ovarian steroid hormones in the Stein-Leventhal syndrome. Brit Med J (1961) i, 1724-1727.

Short R V. The evolution of human reproduction. Proc Roy Soc Med (1976) B 195, 3-24.

Shutt D A, Shearman R P, Lyneham R C, Clarke A H, McMahon G R and Goh P L. Radioimmunoassay of progesterone, 17-hydroxy progesterone, estradiol 17β and prostaglandin F in human corpus luteum. Steroids (1975) 26, 299-306.

Singh E J, Baccarini I M and Zuspan F. Levels of prostaglandins $F_{2\alpha}$ and E_2 in human endometrium during the normal menstrual cycle. Amer J Obstet Gynecol (1975) 121, 1003-1006.

Sixma J J, Christiaens G C M L, Haspels A A. The sequence of haemostatic events in the endometrium during normal menstruation. In: (Diczfalusy E et al. editor) Endometrial bleeding and steroidal contraception, Pitman Press Bath (1980) 86-96.

Smith I D, Temple D M and Shearman R P. The antagonism by anti-inflammatory analgesics of $PGF_{2\alpha}$-induced contractions of human and rabbit myometrium in vitro. Prostaglandins (1975) 10, 41-57.

Smith O W. Oestrogens in ovarian fluids of normally menstruating women. Endocrinol (1960) 67, 698-704.

Smith S K, Abel M H, Kelly R W and Baird D T. Prostaglandin synthesis in the endometrium of women with ovular dysfunctional uterine bleeding. Brit J Obstet Gynaecol (1981a) 88, 434-442.

Smith S K, Abel M H, Kelly R W and Baird D T. A role for prostacyclin in excessive menstrual bleeding. Lancet (1981b) 1, 522-524.

Smith S K, Abel M H, Kelly R W, Baird D T. The synthesis of prostaglandins from persistent proliferative endometrium. J Clin Endocrinol Metab (1982) 55, 284-289.

Smith S K, Abel M H, Kelly R W, Baird D T. Prostaglandins and dysfunctional uterine bleeding. Res Clin Forums (1982) 4, 39-45.

Snowden R and Christian B. Patterns and perceptions of menstruation. World Health Organisation, Geneva; Croom Helm, London and St Martins Press New York (1983).

Southam A L. The natural history of menstrual disorders. Ann NY Acad Sci (1959) 75, 840-854.

Southam A L, Richart R M. The prognosis for adolescents with menstrual abnormalities. Amer J Obstet Gynecol (1966) 94, 637-643.

Stormshak F, Inskeep E K, Lynn J E, Pope A L and Casida L E. Progesterone levels in corpora lutea and ovarian venous effluent of the ewe. J Anim Sci (1963) 22, 1021-1026.

Sundaram S K and Stob M. Effect of unilateral ovariectomy on reproduction and induced ovulation in the ewe. J Anim Sci (1967) 26, 374-376.

Sutherland A M. The histology of the endometrium in functional uterine haemorrhage. Glasgow Med J (1949) 30, 1-28.

Sutherland A M. Functional uterine haemorrhage in puberty and adolescence. Glasgow Med J (1953) 34, 496-514.

Szego C, Seeler B J, Steadman R A, Hill D F, Kimura A K and Roberts J A. The lysosomal membrane complex: focal point of primary steroid hormone action. Biochem J (1971) 123, 523-538.

Tait J F. A review: The use of isotopic steroids for the measurement of production rates in vivo. J Clin Endocrinol Metab (1963) 23, 1285-1291.

Tait J F, Little B, Tait S A S and Flood C. The metabolic clearance rate of aldosterone in pregnant and non-pregnant subjects estimated by both single injection and constant-infusion methods. J Clin Invest (1962) 41, 2093-2098.

Takayanagi S, and Iio M. Miniature semiconductor radiation detectors. In: Instrumentation in Nuclear Medicine Vol 2 (Hine G J and Sorenson J A. editors) New York Academic Press (1974) 499.

Takikawa H. Binding of steroids to follicular fluid proteins. Steroid Dynamics (Pincus G, Nakao T and Tait J F. editors) Academic Press New York (1966) 217.

Tauber P F, Wolf A S, Herting W, Zaneveld J J. Hemorrhage induced by intrauterine devices: control by local proteinase inhibition (1977) 28, 1375-1377.

Taw R L. Review of menstrual disorders in which a secretory endometrium was found. Amer J Obstet Gynecol (1975) 122, 490-496.

Taylor E S. Essentials of Gynecology. 3rd Edition. Lea and Ferbiger Philadelphia (1965) p 429.

Taymor M L, Sturgis S H, Vonica C. The etiological role of chronic iron deficiency in the production of menorrhagia. J A M A (1964) 187, 323-325.

Thorburn G D, Casey B H and Molyneux G S. Distribution of blood flow within the skin of the rabbit with particular reference to hair growth. Circ Res (1966) 18, 650-659.

Ueda H, Sasaki Y, Iio M, Kaihara S, Machida K, Ito I, Takayanagi S, Kobayashi T and Sugita T. Catheter semi-conductor radiation detector for continuous measurement of cardiac output. J Nuclear Med (1969) 10, 713-721.

Vande Wiele R. Urinary production rates of estrogens measured by isotope dilution. Estrogen assays in clinical medicine (Paulsen C A. editor) University of Washington Press Seattle (1965) 148-164.

Vande Wiele R, Bogumil J, Dyrenfurth I, Ferin M, Jewelewicz R, Warren M, Rizkallah T and Mikhail G. Mechanisms regulating the menstrual cycle in women. Rec Prog Horm Res (1970) 26, 63-95.

Vande Wiele R, Dyrenfurth I and Gurpide E. Estrogens. Clinical Endocrinology II (Astwood E B and Cassidy C E. editors) Grune and Stratton New York (1968) 603-620.

Van Look P F A, Hunter W M, Corker C S, and Baird D T. Failure of positive feedback in normal men and subjects with testicular feminization. Clin Endocrinol (1977) 7, 353-366.

Van Look P F A, Hunter W M, Fraser I S, Baird D T. Impaired estrogen-induced luteinizing hormone release in young women with anovulatory dysfunctional uterine bleeding. J Clin Endocrinol Metab (1978) 46, 816-823.

Van Look P F A, Lothian H, Hunter W M, Michie E A, Baird D T. Hypothalamic-pituitary-ovarian function in perimenopausal women. Clin Endocrinol (1977) 7, 13-22.

Vollman R F. The menstrual cycle. Major problems in Obstetrics and Gynecology Vol 7 W B Saunders Philadelphia (1977).

Vora I M, Raizada R M, Rawall M Y, Chadda J S. Adenomyosis. J Postgrad Med (1981) 27, 7-11.

Wagner G and Ottesen B. Vaginal blood flow during sexual stimulation. Obstet Gynecol (1980) 56, 621-624.

Wang C F, Lasley B L, Lein A and Yen S S C. The functional changes of the pituitary gonadotrophs during the menstrual cycle. J Clin Endocrinol Metab (1976) 42, 718-727.

Wehefritz E. Der ovarien. Zeitschr Konsitlehre (1923) 9, 161-175.

Wilson E W. Lysosome function in normal endometrium and endometrium exposed to contraceptive steroids. In: Diczfalusy E et al (editors) Endometrial Bleeding and Steroidal Contraception. Pitman Press Bath (1980) pp 201-218.

Westrom L, Bengtsson L P. Effect of tranexamic acid (AMCA) in menorrhagia with intrauterine contraceptive devices. A double-blind study. J Reprod Med (1970) 5, 41-48.

Wide L. Extraction and separation of gonadotrophins. Immunoassay of gonadotrophins (Diczfalusy E. editor) Acta Endocrinol (1969) Suppl 131, 207-219.

Wide L and Porath J. Measurement of gonadotrophins in plasma by a radioimmunosorbent technique. Biochim Biophys Acta (1966) 130, 257-265.

Willman E A, Collins W P, Clayton S G. Studies in the involvement of prostaglandins in uterine symptomatology and pathology. Brit J Obstet Gynaecol (1976) 83, 337-345.

Wood C, Larsen L, Williams R. Menstrual characteristics of 2,343 women attending the Shepherd Foundation. Aust NZ J Obstet Gynaecol (1979) 19, 107-110.

World Health Organization, Special Programme of Research, Development and Research Training in Human Reproduction (1979) Eighth Annual Report Geneva.

Yen S S C, Vela P and Rankin J. Inappropriate secretion of follicle stimulating hormone and luteinizing hormone in polycystic ovarian disease. J Clin Endocrinol Metab (1970) 30, 435-442.

Yki-Jarvinen H, Wahlström T and Seppälä M. Immunohistochemical demonstration of relaxin in the genital tract of pregnant and non-pregnant women. J Clin Endocrinol Metab (1983) 57, 451-454.

Younglai E V and Short R V. Pathways of steroid biosynthesis in the intact Graafian follicle of mares in oestrus. J Endocrinol (1970) 47, 321-330.

ACKNOWLEDGMENTS

I have been extremely fortunate to have had the opportunity to work with some of the most stimulating, charming and encouraging clinicians, scientists, technical and secretarial staff one could hope to meet. To all of these I am deeply grateful.

I am especially grateful to Dr David Baird, now Professor of Obstetrics and Gynaecology at the University of Edinburgh, who first introduced me to the excitement, thoroughness, tediousness, anxiety, inner motivation, integrity and the many other facets which go to make up good scientific research. I will never forget the superb grounding in clinical and scientific medicine which I received in the University of Edinburgh, the Simpson Memorial Maternity Pavilion and the Royal Infirmary of Edinburgh.

I also owe a debt of gratitude to my other supervisors and heads of departments for their ready advice, encouragement and chiding to complete the work embodied in this thesis:

- Professor Robert Kellar, University of Edinburgh
- Professor Geoffrey Thornburn, now at Monash University
- Professor Alec Turnbull, University of Oxford
- Professor Geoffrey Dawes, Nuffield Institute for Medical Research
- Professor Chris Hudson, University of Sydney
- and in particular to my present head of department, Professor Rodney Shearman, University of Sydney.

I have received advice, encouragement and collaboration from numerous scientists and clinicians, including Dr Egon Diczfalusy, Dr John Parboosingh, Professor Melville Kerr, Dr Phil Mattner, Dr John Challis, Dr Jeffrey Robinson, Dr Dan Macey, Dr Paul Van Look, Dr Bill Hunter, Dr Leif Wide, Dr Norman Veale, Mrs Eileen Michie, Mr Brian Hutton, Mr John Cormack and Mr Bruce Brown.

This work would never have been completed without the ready and highly competent assistance of technical staff in Edingburgh, Oxford and Sydney, including Miss Helen Lothian, Mr Alan Galbraith, Mr Leslie MacKenzie, Miss Michelle Robinson, Miss Tanya Resta and a very special mention of my present senior professional officer, Mr Robert Markham. I have been privileged to work with three excellent research sisters in Sydney, who have made the clinical research work a pleasure and never a trial: Sister Jan Pigott; Sister Jan McIlveen; and for the past 5 years I have received unswerving support from Sister Gay McCarron.

Mention must also be made of the large number of highly cooperative women who have willingly agreed to participate in these research studies even after full and informative explanation.

The research described in this thesis was supported at different times by the Medical Research Council (U.K.) --

when I was a holder of a Junior Research fellowship and later a Clinical Research fellowship -, the National Health and Medical Research Council (Australia), the Commonwealth Scientific and Industrial Research Organisation of Australia and a number of smaller grants including two from the Parke-Davis Company. Continuous support was also provided by the three institutions in which the work was carried out - the Universities of Edinburgh, Oxford and Sydney.

I am indebted to the secretarial staff of the Department of Obstetrics and Gynaecology, University of Sydney who have cheerfully tolerated my whims and translated them into intelligible script - Mrs Judy Shade, Miss Robyn Carrier and especially to Miss Donna Codey who has typed the major part of this manuscript.

The last, most important, most heartfelt acknowledgement goes to my wonderful family - Dorothy, Lindsay, Gael and Rowan - for their love, help, encouragement, understanding and extraordinary forebearance in the face of interrupted nights, weekends and family occasions - and to my parents, Stewart and Ellis, whose example enabled me to find my own way into this exciting specialty, and whose advice ensured that I appreciated the disadvantages of obstetrics and gynaecology before I discovered the challenges.

SECTION 9

PUBLICATIONS ARISING FROM THE CANDIDATE'S RESEARCH

PUBLICATIONS ARISING WHOLLY OR PARTLY FROM RESEARCH REPORTED IN THIS THESIS:

Section 2.

Baird D T and Fraser I S. Blood production and ovarian secretion rates of estradiol-17β and estrone in women throughout the menstrual cycle. J Clin Endocrinol Metab (1974) 38, 1009-1017.

Fraser I S and Baird D T. Blood production and ovarian secretion rates of estradiol-17β and estrone in women with dysfunctional uterine bleeding. J Clin Endocrinol Metab (1974) 39, 564-570.

Baird D T and Fraser I S. Concentration of oestrone and oestradiol in follicular fluid and ovarian venous blood of women. Clin Endocrinol (1975) 4, 259-266.

Section 3.

Fraser I S and Baird D T. Endometrial cystic glandular hyperplasia in adolescent girls. J Obstet Gynaecol Brit Cwlth (1972) 79, 1009-1015.

Fraser I S, Michie E A, Wide L and Baird D T. Pituitary gonadotropins and ovarian function in adolescent dysfunctional uterine bleeding. J Clin Endocrinol Metab (1973) 37, 407-414.

Van Look P F A, Hunter W M, Fraser I S and Baird D T. Impaired estrogen-induced luteinizing hormone release in young women with anovulatory dysfunctional uterine bleeding. J Clin Endocrinol Metab (1978) 46, 816-823.

Section 4.

Fraser I S, McCarron G, Markham R and Resta T. Total blood and fluid loss in the menstrual discharge. Obstet Gynecol (1984), in press.

Fraser I S, McCarron G and Markham R. A preliminary study of factors influencing perception of menstrual blood loss volume. Amer J Obstet Gynecol (1984) 149, 788-793.

Section 5.

Fraser I S and Macey D J. Methods for measuring uterine blood flow in non-pregnant women. Biomedical Engineering in Obstetrics and Gynaecology. Proceedings of a Symposium. University of Sussex (1976) pp 150-152.

Fraser I S, Brown B W and Mattner P. Endometrial blood flow in anaesthetised sheep as measured with Krypton-85 clearance and microsphere techniques. Quart J Exp Physiol (1980) 65, 19-26.

Fraser I S, Brown B W, Hutton B and Mattner P. Measurements of endometrial blood flow in anaesthetised ewes by Xenon-133 clearance and microsphere techniques. Quart J Exp Physiol (1982) 67, 531-535.

Brown B W, Fraser I S and Mattner P. Changes in reproductive tract blood flow during the oestrone cycle of the ewe and effects of arterial cannulation. Aust J Biol Sci (1984), in press.

Other Publications related to the topic of this thesis.

REVIEWS:

Baird D T and Fraser I S. Disorders of the hypothalamic-pituitary-ovarian axis. Clinics in Endocrinology and Metabolism (1973) 2, 469-487.

Fraser I S. Clinical manifestations and end organ effects associated with abnormal gonadotrophin release. Clinics in Obstetrics and Gynaecology (1976) 3, 591-614.

Fraser I S. Adolescent Menstrual Disroders. Postgrad Med Bull Univ Syd (1977) 76-81.

Fraser I S. Ovarian function and its control. Human Reproductive Physiology (Shearman R P. editor) Second Edition (1979), Blackwells, Oxford. 40-72.

Diczfalusy E, Fraser I S and Webb F T G (editors). Endometrial Bleeding and Steroidal Contraception. Pitman Press, Bath (1980).

Fraser I S and Diczfalusy E. A perspective of steroidal contraception and abnormal bleeding: What are the prospects for improvement? Endometrial Bleeding and Steroidal Contraception (Diczfalusy E, Fraser I S and Webb FTG. editors), Pitman Press, Bath (1980) 384-409.

Fraser I S. Abnormal uterine bleeding due to hormonal steroids and intrauterine devices. Recent Advances in Fertility Regulation (Chang C F, Griffin D and Woolman A. editors) Atar Press, Geneva (1981) 265-300.

Fraser I S. Update on the physiology of menstruation and the menstrual cycle. Healthright (1982) 2, 13-16.

Fraser I S. The treatment of menorrhagia with mefenamic acid. Research and Clinical Forums (1983) 5, 93-99.

Fraser I S. Progestogen therapy and disturbances of menstrual bleeding. Progestogens in Therapy (Benagiano G, Diczfalusy E and Zulli P. editors) Raven Press, New York (1983) 179-190.

Fraser I S. The Dysfunctional Uterus - dysmenorrhoea and dysfunctional uterine bleeding. Clinical Reproductive Endocrinology (Shearman R P. editor). Churchill Livingstone, Edinburgh (1985), in press.

ORIGINAL RESEARCH PAPERS:

Fraser I S, Baird D T and Cockburn F. Ovarian venous and follicular fluid PO_2, PCO_2 and pH in women. J Reprod Fertil (1973) 33, 11-17.

Fraser I S, Pearse C, Shearman R P, Elliott P M, McIlveen J and Markham R. Efficacy of mefenamic acid in patients with a complaint of menorrhagia. Obstet Gynecol (1981) 58, 543-551.

Fraser I S, McCarron G, Markham R, Robinson M and Smyth E. Longterm treatment of menorrhagia with mefenamic acid. Obstet Gynecol (1983) 61, 109-112.

SECTION 10

APPENDIX : ABBREVIATIONS

APPENDIX 1

ABBREVIATIONS USED IN MORE THAN ONE CHAPTER

AMCA	tranexamic acid
^{14}C	carbon-14
$C_{BB}^{E_1E_2}$	conversion ratio of oestrone to oestradiol in blood
Ci	curie
	{mCi: millicurie; µCi: microcurie}
CGH	cystic glandular hyperplasia of the endometrium
cpm	counts per minute
DMPA	depotmedroxyprogesterone acetate
dpm	disintegrations per minute
DUB	dysfunctional uterine bleeding
E_1	oestrone
E_2	oestradiol
EACA	epsi-amino-caproic acid
EBF	endometrial blood flow
FSH	follicle stimulating hormone
g	gram
	{mg: milligram; µg: microgram; ng: nanogram; pg: picogram}
GnRH	gonadotrophin-releasing hormone
^{3}H	tritium
HCG	human chorionic gonadotrophin
^{125}I	iodine-125
IRP-HMG	international reference preparation of human menopausal gonadotrophin
IUCD/IUD	intrauterine contraceptive device
^{85}Kr/Kr-85	Krypton-85
l	litre
	{ml: millilitre}
LH	luteinising hormone
m	metre
	{cm: centimetre; mm: millimetre}
MCR	metabolic clearance rate
	{MCR^{E_1}:MCR of oestrone; MCR^{E_2}: MCR of oestradiol}
mol	mole
	{mmol: millimole}
MPA	medroxyprogesterone acetate
n	number of observations
NET	norethisterone
$P_B^{E_2}$	blood production rate of oestradiol
PCO_2	partial pressure of carbon dioxide
PGE	prostaglandins of the E series
PGE_2	prostaglandin E_2
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
PGI_2	prostacyclin
pH	hydrogen ion concentration
PO_2	partial pressure of oxygen
PRL	prolactin
rpm	revolutions per minute
^{35}S	Sulphur-35
SD	standard deviation
SEM	standard error of the mean

Sov^{E_1}	ovarian secretion rate of oestrone
$t_{½}$	half time of exponential clearance
TLC	thin layer chromatography
TXA_2	thromboxane A_2
U	unit
	{mU: milliunit}
^{133}Xe/Xe-133	Xenon-133