# SUMMARY

## EPIDEMIOLOGICAL AND HORMONE STUDIES IN EARLY HUMAN PREGNANCY - NORMAL AND ABNORMAL

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Chapter 1. Introduction to the Thesis.

Chapter 2. Epidemiological studies in abortion, and subsequent reproductive performance.

1. Women who start childbearing with 1 or 2 consecutive abortions are compared with those who start with 1 or 2 normal pregnancies.
2. Women who start childbearing with 2 abortions are older, shorter and of lower socio-economic status than the other 3 groups.
3. In the first continuing pregnancy these women have a higher incidence of threatened abortion and premature labour. Their perinatal mortality is increased due mainly to 'prematurity' and 'foetal deformity'. They also have an increased incidence of operative delivery.
4. The group with 2 previous abortions have a tendency to poor foetal growth and this is

associated with premature labour.

5. Women who abort in their first pregnancy have an increased risk of abortion in their subsequent reproductive life.

6. The recurrent abortion risk increases with successive consecutive abortions from 25% after 1 abortion to 58% after 3 abortions.

7. One third of abortions occur before 2 months gestation and 50% by 3 months.

8. In abortion studies the 'primary recurrent aborter' with 2 or more consecutive abortions should be studied. Observations must start before 8 weeks of gestation or earlier if a previous abortion has occurred before this time.

Chapter 3. Hormone assays in normal early pregnancy, in abortion and in hydatidiform mole.

1. Urinary pregnanediol and oestriol assays are made in 3 groups of women (1) Normal pregnancy (2) Women with at least 2 previous abortions and no successful pregnancies and (3) Women who aborted in the pregnancy studied.

2. There is no significant difference in oestriol and pregnanediol excretion between the women who

aborted and those in which the pregnancy continued successfully, until after 16 weeks of gestation.

3. These 2 assays are of no value in forecasting abortion until after 16 weeks of pregnancy.

4. The assays were performed in 5 cases of hydatidiform mole. In these cases the urinary pregnanediol and oestriol levels may be normal but are more likely to be low. There may be some change in steroid metabolism in this type of case.

Chapter 4. Conversion of progesterone to pregnanediol.

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3. There was no difference in conversion in the same subject, non-pregnant and pregnant.

Chapter 5. Progesterone metabolism in the human previable foetus.

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2. The foetal liver is the chief site of progesterone metabolism and this organ produces mainly reduced metabolites. The adrenal metabolises progesterone to corticosteroids.
3. Progesterone from the placenta is used by the foetus to produce corticosteroids for its own homeostasis.

Chapter 6. Steroid studies in a case of hydatidiform mole.

1. The urinary pregnanediol:pregnanetriol ratio in molar pregnancy is 2:1 compared with 20:1 in normal pregnancy.

2. Mole tissue was incubated with [4-$^{14}$C] pregnenolone as precursor, and 17α hydroxypregnenolone, progesterone, 16α hydroxyprogesterone and 16β hydroxyprogesterone were isolated.

3. Cholesterol, pregnenolone, 17α hydroxypregnenolone, pregnanediol, pregnanetriol and androstenedione were isolated from extracts of mole tissue and theca lutein cyst fluid: 17α hydroxyprogesterone was isolated from cyst fluid but not from mole tissue.

4. Mole tissue is capable of steroidogenesis but there was less synthesis of progesterone than normal.

5. The increase of 17α hydroxylation indicated by the elevation of urinary pregnanetriol excretion is ovarian in origin.

Chapter 7. Urinary steroid excretion after gonadotrophin therapy.

1. There is a significant increase in pregnanediol excretion in the luteal phase of the menstrual cycle due to the ovarian production of 17α hydroxyprogesterone.

2. Urinary pregnanetriol excretion also rises in the luteal phase of gonadotrophin stimulated cycles and in gonadotrophin induced pregnancies until 6 - 8 weeks.

3. Measurement of this metabolite may be a valuable parameter of corpus luteum function in the menstrual cycle, and in early pregnancy.

4. This assay may be of value in detecting hyperstimulation of the ovaries by gonadotrophins. It may also indicate when the corpus luteum of pregnancy is deficient and be of help in forecasting early abortion.

# EPIDEMIOLOGICAL AND HORMONE STUDIES IN EARLY HUMAN PREGNANCY - NORMAL AND ABNORMAL

A THESIS

SUBMITTED FOR THE DEGREE OF DOCTOR OF MEDICINE

by

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# CHAPTER 1.

## Introduction to the Thesis

The theme of this thesis is "early pregnancy", that is from conception until about 20 weeks of gestation. This period includes implantation, placentation, the early development of the foetus itself, and of the foeto-placental unit.

The writer's original interest, which led to this study, was concerned with abortion and, in particular, with the study of recurrent abortion. Much had been written, about methods whereby abortion could be forecast but the results were so inconsistent that further investigation seemed necessary. Although much research had been devoted to the study of abortion, and a great volume of writings on the subject had accumulated, understanding of the process had expanded little. There are a number of reasons for this, not least of which, is the fact that much of the study had had, of necessity, to be conducted within the context and limitations of clinical medicine. The scientific principles of exact definition of material, and of controls, have been largely ignored. Although widely

accepted definitions of 'threatened' abortion, 'inevitable' abortion, 'missed' abortion and 'recurrent' abortion have existed for decades these categories have not been rigidly separated, and, in writings on the subject there has been insufficient discrimination between these entities. For example, the meaning of recurrent abortion is not clear from the literature, and it seemed probable that limitation of study of recurrent abortion material to patients who have had three (rather than two) consecutive abortions, was an unnecessarily severe restriction which was based on a tacit acceptance of Malpas' (1936) formula.

It seemed therefore, important, in the first instance to investigate the general incidence of abortion, and then to determine the reproductive history of women who began childbearing with an abortion. This would give factual information about the number who aborted again and again, indicate whether there was generally poor reproduction in these women, and the particular aspects of their subsequent pregnancies to which attention should be paid during the period of antenatal care. It would, in particular, help to define the type of woman most liable to abort

again and also give information about the gestation period at which abortion occurred. It was felt by the writer, that frequently, in therapeutic trials of drugs in recurrent abortion, the drugs were given long past the gestation period at which abortion was likely and that the pregnancies were already highly selected by virtue of having already progressed passed this period.

The results of an investigation into these problems are reported in Chapter 2. In this chapter some description of the characteristics of the women concerned, such as age, height and socio-economic group are discussed, and the problems associated with their next pregnancy are assessed with particular reference to perinatal mortality. Their later reproductive pattern is reviewed, and the number of subsequent abortions is compared with that in a normal group of women. The question of the gestation period at which abortion and especially recurrent abortions take place is surveyed, and the chapter concludes with a dissertation on the implication of the results to the design of studies on abortion with particular relation to the gestation period at which therapy, if any, should be started and the women who should be studied.

The design evolved in Chapter 2 is then applied to the study of a group of abortion prone women and a study is made to discover if the measurement of two steroid hormones, oestriol and pregnanediol, in the maternal urine in pregnancy, will give any helpful information about the likelihood of abortion occurring in these women. The reason why these two steroids are most likely to be of value is discussed along with information about their derivation and the metabolism of their precursors. The chapter concludes with some observations on another abnormality of early pregnancy - hydatidiform mole - which is of particular interest due to the absence of the foetus in these cases.

The results obtained in Chapter 3 pose a number of questions concerning progesterone metabolism and two of these are selected for further investigation in Chapters 4 and 5.

In Chapter 4 the conversion of progesterone to pregnanediol is examined. The work in Chapter 3 showed a great variation in pregnanediol excretion, and one of the possible explanations of this was that

there was a variability in conversion from the parent steroid to the urinary metabolite. The results of previous work on this aspect were confusing. The chapter begins with a review of progesterone metabolism in early human pregnancy. A description of the methodology involved is then given and the results are discussed.

The other aspect selected for intensive study is discussed in Chapter 5 on progesterone metabolism in the human previable foetus. On a previous occasion the writer and Dr. Klopper (Klopper & Macnaughton 1959) had found pregnanediol in the liquor amnii, and the question of the metabolism of progesterone and the production of pregnanediol by the foetal compartment was another possible factor affecting maternal excretion. There was little information available at the time on this aspect, and perfusion experiments of previable foetuses with radioactive progesterone were undertaken to elucidate the problem. The technique of the experiments and the results are discussed in this chapter.

As a result of the work on conversion of progester-

one to pregnanediol described in Chapter 4, women with hydatidiform mole were found to have lower conversions than normal women. The question of a different pathway of steroidogenesis in these cases was thought to be a possible explanation. Steroid studies in hydatidiform mole were then undertaken and are reported in Chapter 6. In these studies molar tissue was incubated with precursor pregnenolone to determine the steroidogenic pathways in this tissue and steroids were extracted directly from the molar tissue itself. The steroid content of the theca lutein ovarian cysts which are frequently present in cases of hydatidiform mole was examined and the origin of certain steroids such as 17α hydroxyprogesterone ascertained. The results here do show a change in emphasis of the steroid pathway in these women.

The findings of Chapter 6 are then applied to the measurement of the ovarian response in amenorrhoeic women being treated with human menopausal gonadotrophins. Chapter 7 discusses an investigation into urinary steroid excretion after gonadotrophin therapy. The stimulation of the ovary caused by gonadotrophins is not unlike that found in some cases of hydatidiform

mole and the particular use of pregnanetriol excretion in measuring corpus luteum function in the menstrual cycle, and also in early pregnancy is evaluated. The chapter concludes with a reference to the possible use of this assay in forecasting abortion, the original starting point of this thesis.

In each chapter the relevant literature is reviewed and the relation of the work to that in the literature is shown. Each chapter discusses a different aspect of the overall theme and the relevant literature and discussion have been included in each chapter rather than in an overall introduction. In this way repetition is avoided and the thoughts on each aspect are kept together.

The purpose of this introduction is to show the relationship of the chapters to each other and to the overall theme of early pregnancy. This theme is illustrated by plates I - VI, which are taken from 'Dr. Granville's Graphic Illustrations of Abortion and The Diseases of Menstruation' (1833).

# CHAPTER 2.

## Epidemiological studies on abortion and subsequent reproductive performance

### Introduction

Much has been written on the problem of abortion and its incidence (Malpas 1938; Devis 1951; etc.) and there is confusion in the literature on the probability of abortion occurring in a subsequent pregnancy particularly in women who have experienced recurrant abortions. The theoretical calculations made first by Malpas (1938) and later revised by Eastman (1956) estimate the risk of spontaneous abortion occurring in women with a history of a given number of abortions. Malpas considered that, after one previous abortion, the risk was 12%, after 2 previous abortions 38% and after 3 previous abortions 73%. These were revised by Eastman to 13, 37 and 84% respectively. These figures have been subjected to much criticism on the grounds that certain assumptions on which the calculations were based were faulty (Warburton & Fraser 1959; Goldzieher & Benigno 1958). Malpas (1938) for example, assumed that spontaneous abortions occur

either for a 'recurrent cause' or for a 'non-recurrent' cause i.e. that causes of abortion can be divided into a group with 100% chance of recurrance and a group with a chance of recurrance equal to the incidence of abortion (excluding habitual abortion) in the general population.

The gestation process depends upon such a complex interaction between maternal and foetal factors, both genetic and environmental, that it must be susceptible to interference by a very large number of agents and therefore Malpas' assumption is not valid. Results from these calculations have been widely quoted, and have been used as controls in studies concerned with the prevention of habitual abortion. James (1962) suggested that these calculations should be abandoned as they have been shown to be based on unreliable estimates of the parameters used.

It is the purpose of this chapter to discuss the question of the incidence of abortion and to describe an investigation into the characteristics of women who abort and their subsequent reproductive performance. This investigation describes some characteristics of

women who are more liable to abortion and indicates the important points in their subsequent reproductive life. As a result women at particular risk can be identified and attention directed to prevention of the special risks of their subsequent pregnancies such as, threatened abortion or premature labour. The chapter concludes with a discussion on the relationship of the results to the design of studies on abortion and the particular pitfalls which should be avoided in such studies.

## Incidence of abortion

It is difficult, if not impossible, to obtain accurate information about the incidence of abortion for a number of reasons -

a). In early abortions, before the placenta is well defined, the products of conception may easily be passed with little or no discomfort especially in a multiparous patient, and any bleeding may be mistaken for a menstrual period. There are considerable numbers of women who come to the gynaecological department with menorrhagia, discharge or staining, and are shown to have a 'missed' or incomplete abortion

by the presence of villi and/or decidual reaction in the endometrium when material recovered at curettage is examined histologically.

b). A number of women, particularly, it is presumed, multiparae, have an abortion, usually early, which they recognise as such; but they are not ill and do not seek advice.

c). A number of women, with abortions, are treated at home by their own doctors and no record or notification of the event is made.

d). There may be deliberate concealment in a number of cases where the woman is not married or where the abortion is criminally induced.

It is thought, in the series described here, where the patients' first pregnancies end in abortion, and who have recurrent abortions with no live children, that the information concerning the abortions is likely to be more accurate than abortion information in general because the women presumably want children. The exception to this may be the abortions which occur in later pregnancy numbers in which the question of induced abortions cannot always be ruled out.

Some data on the range of abortion incidence from the literature is given in Table 1. Here the lowest incidence is 7.0% (Tietze et al, 1950) and the highest 18.0% (Malpas, 1938). Hertig and Livingstone (1944) give a proportion of 10.6% ending in abortion of 1150 pregnancies in private practice. Tietze and Martin (1957) found a rate of 17% spontaneous and 19% induced abortions in a sample of white married women of rather superior education, in the United States. Baird (1957) says that 'the incidence of abortion relative to births at term is probably 10% if criminal abortions are excluded' and Stallworthy (1955) gives a similar figure. Whitehouse (1929) in an investigation of 3000 hospital and private patients attending for gynaecological complaints found that 1972 of 11,430 patients (17.2%) had ended in abortion and the Interdepartmental Committee on Abortion (1939) estimated that for every 600,000 live births in the 5 years preceeding their report between 110,000 and 150,000 abortions occurred; of these the Committee estimated that 40% were induced.

Probably the most accurate incidence is that reported by Stevenson et al, (1959) from

Belfast. In this study total hospital abortion admissions were obtained and to this was added the numbers treated at home, ascertained from information from midwives and health visitors, and from the claims of doctors for fees for treatment of abortions under the Northern Ireland Health Services Scheme. A most comprehensive sample was therefore obtained and an estimate of 11.8% of all pregnancies terminating in identifiable abortions was arrived at. The writers, however, thought that the real frequency was probably somewhat greater than this. This magnitude of abortion frequency is somewhere about the middle of the ranges shown in Table 1. It is clear that there will be a wide fluctuation of rates depending on how a sample is obtained.

## Epidemiological characteristics and obstetric performance of women who abort

### Sources of Data

In this study a number of different sources of data have been used and these have been collated to make the resultant information as comprehensive as possible. While it is recognised that the main

difficulties discussed previously obtain, it is thought that the results given here are as accurate as possible. By considering abortions occurring in the early pregnancies of women with no live children the points mentioned above are greatly obviated since the women are anxious to have a successful pregnancy. In the following series, however, where abortions are noted, it is possible that some of these may be induced and not spontaneous.

Five main sources of data have been used:-

Group 1. Maternity Records of women having first (7391) or second (5370) pregnancies have been scrutinised. This group has been used as controls. Their obstetric performance has been compared with women who began their obstetric life with one or more abortions.

Group 2. A series of women (911), obtained from Maternity Records, whose childbearing life began with an abortion. The performance of this group in the second pregnancy has been compared with that of groups 1 and 5.

Group 3. A series of women (104) taken from

gynaecological records, where the first pregnancy ended in abortion. These women have been followed up for at least 5 years to determine their subsequent obstetric history and to compare it with group 4.

Group 4. A series of women (83), taken from obstetric records, who began childbearing with a normal pregnancy and who were followed up for a period of 10 years. This group has been used as a normal control for comparison with group 3.

Group 5. A series of women (129), having their 3rd. pregnancy where the first two ended in abortion. This group has been used to illustrate any changes in obstetric performance compared with groups 1 and 2.

In the tables, it will be seen that the numbers in some of the groups are not always complete. This is due to certain aspects of the data being incomplete in individual cases. These are relatively few in number and this does not alter the main trend of the results.

## Epidemiological Characteristics

In order to compare women who start childbearing with abortions and those who start with normal pregnancies the factors of age, height and husband's Social Class are compared first.

Age - Table 2.

The group of women whose first 2 pregnancies ended in abortion contain a higher proportion of women over 30 years than any of the other groups. This group and that with 2 normal pregnancies resemble each other most closely. The risk of abortion is said to be greater among older than among younger women (Tietze et al 1950). Foetal loss in general tends to increase with increasing age of the mother after 20 years (Shapiro et al 1962) and these workers also showed that women whose last pregnancy ended in a foetal death have twice as high a loss rate in their next pregnancy as other women. When the incidence of abortion is related to marriage duration and age an increase is found in women over 25 years (Table 3), and the National Statistics for England and Wales show a steady increase of childlessness with rising age at

marriage. Childlessness in this sense includes women who have both abortions and foetal deaths and who have no living children. The classical studies of Louis Henry (1953) showed that, in a society not using modern contraceptive methods - nineteenth century England - the probability of having another child was related to the age of the woman and not to the number of pregnancies she had already had. More recently McKeown and Record (1957) have shown that the proportion of women becoming pregnant again within 2 years of their first baby diminished with increasing age. There is much accumulated data showing the dilaterious effect of age on the reproductive process and this effect is again shown here in connection with women who start their childbearing with 2 abortions (Baird et al 1958). The association of age and abortion was recognised many years ago by Burns (1843) who stated that "advancement in life, before marriage, is another cause of frequent abortion, the uterus being then somewhat imperfect in its action.

Height - Table 4.

The features of this table are the differences in

stature between the women who have 2 abortions and those in the other 3 groups, one third of women with 2 initial abortions are under 5'1" compared with one fifth in the other 3 groups. At the other end of the scale 20% of these women are over 5'4" compared with at least 26% in the other 3 groups.

Husbands Social Class - Table 5.

This table shows that women in the 2 abortion group have a higher proportion in social class 4 & 5 than in the other groups.

Height and social class taken together are a well recognised measure of physique and general health. A person's height is determined by both genetic and environmental factors; to attain one's potential, adequate nourishment is required at all stages of growth including that in utero. Maternal stature has a strong association with social class and Table 6 taken from the Perinatal Mortality Survey shows the distribution of maternal height within socio-economic groups. The proportion of women under 62" is greater in social class IV and V and least in social class I and II so that there are more short

women as one goes down the social scale. The effect of height and socio-economic group on perinatal mortality is also well known and Table 7 from the 2nd. Report of the Perinatal Mortality Survey (1969) shows this clearly. The lowest perinatal mortality is in tall women in Social Class I and the highest in small women in Social Class IV and V.

The results of this study indicate that women starting their childbearing with 2 abortions are likely to be smaller and of lower socio-economic status than those in the other 3 groups shown.

Duration of Marriage and number of children

Duration of Marriage and number of childrenures for women having their first normal pregnancy and a series of women whose first pregnancy ended in abortion, taken from group 5, are compared by duration of marriage (Table 8 ).

Almost half (49%) the women who begin with an abortion complete this pregnancy within one year of marriage compared with 42% of women who have a legitimate first birth. The cumulative total shows

that there is little difference between the two groups at the 5 year period. These findings are not surprising. The majority of women, whatever their social class conceive within 1 year of marriage (Illsley 1956) and if abortion should occur it will be completed within the year, whereas if the pregnancy continues the delivery will not occur within the year from marriage.

Obstetric performance in first continuing pregnancy

The next aspect to be considered is how women, who have started their childbearing with an abortion behave in their first continuing pregnancy. In Table 9 the two groups of women who begin with 1 or 2 abortions are compared with 2 normal groups. The particular aspects considered are, threatened abortion, antepartum haemorrhage, prematurity and perinatal mortality.

Threatened Abortion.

The table shows that there is more threatened abortion in these continuing pregnancies in the abortion groups than in the normal groups. This is particularly marked in women whose first two pregnancies both end in abortion suggesting that in these women

there is a particular tendency to early pregnancy bleeding.

Antepartum Haemorrhage.

There is little difference in the incidence of antepartum haemorrhage in the groups. The figure of 3.1% found in women having a third pregnancy after two previous abortions is very close to that of 3.0% found in a survey of 30,383 single pregnancies by Paintin (1962).

Prematurity.

There is also a tendency for the number of premature births to be greater in the women with 1 previous abortion and this trend is more marked in women having their third pregnancy where the first two ended in abortion. Prematurity is therefore commoner in pregnancy when abortion has occurred in the previous pregnancy. To distinguish between prematurity due to the "small for dates" or poorly grown baby, and the baby that is normally grown for the gestation period, but is delivered early, the weights of babies at various gestation periods in the four groups of cases were examined (Table 10). Babies born to women in

both previous abortion groups up to 35 weeks of gestation seem to be smaller than in the other two groups. This may mean that pregnancies in these groups terminate at an earlier stage of gestation. However, women in their third pregnancy with 2 previous abortions have babies at least half a pound lighter than any of the other groups at the 36 - 37 week period.

The proportion of deliveries occurring before 36 weeks of gestation is considered in Table 11. There are more deliveries before 36 weeks in both abortion groups than in the normal groups and this is particularly so in the group with 2 previous abortions where it is double that found in normal primigravidae.

These results show that women who begin their childbearing life with two abortions have an increased risk of premature labour. The women who have premature labour also have small babies for the gestation period and these babies are therefore 'small for dates'. There are a number of possible actiological factors. In some cases a uterine factor may operate. This may be a uterine abnormality which can be easily detected

on hysterography. Another possible explanation is that of inadequate uterine accommodation which is well documented in animals by Reynolds (1959) but very difficult to prove in the human subject. There is a very constant relation between the uterus, on one hand, and the size of its contents on the other. The uterus affects the size of the offspring and the size of the offspring affects the uterus. Reynolds (1959) noted that, as the last phase of uterine accommodation becomes established the contents of the uterus outgrow the confines of an increasingly unfavourable uterine environment and either parturition or foetal death is the inevitable climax. It seems possible that in this group of women there may be a fault in uterine accommodation leading to inadequate foetal growth and premature labour.

It has also been shown (Baird 1953) that the incidence of prematurity is related to social class being highest in the lower socio-economic groups. This seems to be a factor in women having two initial abortions. It is not the whole answer since a number of women with a good socio-economic history also have premature labours and poorly grown babies.

Here the effect of age on the reproductive process may be important since these women are on the whole older than those in the lower socio-economic groups. Reid (1961) has shown that some mothers repeatedly have premature babies. The tendency to repeat unsuccessful pregnancies is also higher in women who begin childbearing with one or two abortions. It is of prime importance to monitor foetal growth with care during these pregnancies. Although poor foetal growth can be detected clinically the use of urinary steroid analysis (Macnaughton 1967) or ultrasonic techniques (Willocks et al 1967) are particularly helpful in management of these cases. If deficient foetal growth is detected it is these women who are also more likely to have premature labour.

Perinatal Mortality

The main difference between the abortion and normal groups in Table 9 is in perinatal mortality. In the abortion groups, the rates for one previous abortion and two previous abortions are 43.2/1000 and 38.8/1000 respectively compared with 30.7 and 20.9 in the first and second normal pregnancy group.

The causes of these deaths have been analysed and classified by the method of Baird et al (1954) and the results are shown in Table 12. In the case of the group of third pregnancies with 2 previous abortions, numbers only are given since these are too small to make percentages meaningful. There are two main causes for the increased perinatal mortality. The number of deaths due to prematurity and foetal deformity are considerably greater in both the previous abortion groups. Seven of 26 deaths in the 1 previous abortion group and 1 of 5 in the 2 previous abortion groups were due to prematurity and 7 of 26 deaths in the former group were due to deformity. In the latter group 3 of 5 deaths were due to foetal deformity - a high proportion.

The cause of most foetal abnormalities is obscure. Indirect evidence suggests that foetal defects may be brought about by inadequate intra uterine environment. Abnormalities of implantation are associated with a high incidence of malformation (Hertig 1967) and it is well known that in many early abortions the foetus or the fertilized ovum are abnormal (Hertig and Rock 1944).

There is an increased incidence of foetal abnormality if bleeding has occurred in the early months of pregnancy (Turnbull and Walker 1956). It seems likely that the faulty intrauterine environment which leads to deficient implantation and development with resultant abortion in the first one or two pregnancies in the abortion groups may be present to a lesser extent in the next pregnancy. It may be insufficient to precipitate abortion but sufficient to interfere with normal foetal development thus resulting in foetal abnormality.

Performance in Labour

The four groups are considered by incidence of Caesarean Section, forceps delivery, assisted delivery and spontaneous delivery (Table 13 ). There is a slight increase in the number of Caesarean sections in the 1 previous abortion group and this rate of 5.9% is doubled in the 2 previous abortion group. More forceps deliveries (19.6%) were done in the 1 previous abortion group than in the normal group but this was not so in the 2 previous abortion group where the main difference was in the number of Caesarean sections

performed. In Table 14 the indications for Caesarean section in the third pregnancy of the 2 previous abortion group are given. Fourteen women were delivered by Caesarean section. In 7 of these (50%) the operation was elective and 6 were over 30 years of age. Other reasons for section were placenta praevia (1), Breech presentation (1), Foetal distress (3), Incoordinate uterine action (1) and Disproportion (1).

These increased operative delivery rates reflect the desire of the attendant to minimise delay in labour and not to 'take any chances' with the baby. In most cases where the obstetric history is poor there is an increased incidence of operative delivery.

In the Caesarean Section group the factor of age is particularly evident and 8 out of the total of 14 women were over the age of 30 years. In all but one of these women the Caesarean section was elective because of the combination of bad obstetric history and age. The other interesting feature of these women is that only 2 of the total were over 5'4" in height. They were almost all medium (5'1" - 5'4") or small (under 5'1").

This is a highly selected group with a large proportion of short women. The combination of small stature, relatively old age and a history of previous abortion make the likelihood of Caesarean Section very great in these women.

There are still, in spite of the adverse history, a relatively large number of women in both abortion groups who had a spontaneous delivery although the number is lower than in the normals.

Fertility following first pregnancy

In Tables 15 and 16 the fertility of women following a normal first pregnancy is compared with that of women whose first pregnancy ended in abortion. In the first table (15) the number of subsequent children, the number of unsuccessful pregnancies over 28 weeks and the number of women who had abortions only following the initial pregnancy are shown. The detailed data about abortions are shown in the second table (16).

Nearly 8% of women who aborted in their first pregnancy had abortions in all their subsequent pregnancies, whereas no woman whose first pregnancy was

normal had abortions in all their subsequent pregnancies. Two women in the first group did carry pregnancies past 28 weeks but these were unsuccessful. Therefore 10% of women in the abortion group had no subsequent successful pregnancy compared with none in the normal group. The number of women in the normal group who had no further pregnancies after the first one was double that in the abortion group. The total number of women having abortions during subsequent childbearing in the abortion group is 36.6% compared with 20.5% in the normal group.

There seems little doubt that when a woman begins childbearing with an abortion a greater number of her subsequent pregnancies will end in abortion than when her initial pregnancy ended with a live child. Very little information is available on the subsequent pregnancies of women who initially miscarried. According to the data of Whelpton and Kiser (1948) approximately 75-80% of women having an initial miscarriage will eventually have a potentially viable foetus of 20 or more weeks gestation. This is not very different from the figure of 83% obtained in this

study. The remaining 17% consists of approximately 7% who had no further pregnancies, 8% who had nothing but abortions, and 2% whose pregnancies continued past 28 weeks, but where the baby did not survive.

Eighty-five percent of women whose first pregnancy was normal had further live children but the remainder had no more pregnancies. None of the 83 patients in this group had any subsequent abortions or premature labours. The fact that 14.5% had no further pregnancies is unusual. The reasons are not clear. It is not solely an age phenomenon since of the 12 women, 6 were over 30 years and 6 were under 30 years at the time of their first pregnancy. This 50:50 ratio does show that an unusually large number were in fact older at the time of their first pregnancy and may not have desired or been able to have another child. These women were all followed up for 10 years so it is unlikely that they would have another pregnancy. It is of interest here to recall the views of Matthews Duncan (1871) who enunciated what he called his 'Law of relative sterility'. This was, of course, in an age of no contraception but it may be still partially true. The law states that 'a wife, who, having had

children, has ceased for 3 years to exhibit fertility, has probably become relatively sterile; that is will probably bear no more children, and the probability increases as time elapses'.

The woman with repeated abortions does not have difficulty in becoming pregnant but in 'holding on' to the pregnancy. The findings in this study support the view of Javert (1957) who also said that fertility was not reduced in these women. They do not bear out a commonly held opinion that women with a history of repeated or spontaneous abortion have a low overall fertility (Donnelly and Locke 1953). The repeated abortion rate in sterility patients (16%) reported by Birnberg et al (1952) is not very much above that in 'non-sterile' patients.

It does not seem that there is a general close relationship between abortion and sterility.

Recurrent Abortion

The number in the abortion group who aborted again in successive pregnancies is shown in Table 17. Analysis of these results shows that there is an

increasing abortion rate in this type of woman. In the second pregnancy with one previous abortion the rate was 25% which is not so very much greater than the incidence of abortion for first pregnancies. In the third pregnancy with two previous abortions the rate is almost doubled to 40% and more than half (58.3%) of those women who have aborted three times aborted again in their fourth pregnancy.

The results show that the main increase in abortion probability occurs after 2 successive abortions and therefore, if abortion prone women are being selected for study it is unwise to restrict the study to women who have had 3 or more recurrent abortions as suggested by Malpas (1938). This view is also expressed by the Council on Pharmacy and Chemistry of the American Medical Association (1940), Goldzieher and Benigno (1958) and Roberts (1964). There is, however, a wide scatter of probabilities in the literature (Table 18). This wide range of recurrent abortion frequency suggests that the published material is quite heterogeneous and that for more accurate studies individual control groups must be

used. James (1963) noted the high variances seen in the observed distributions and suggested that the abortion probability should remain relatively constant within each woman; if it had varied this would have reduced the variance (Edwards 1960). It seems likely that abortion probabilities do, in fact, vary from one woman to another although not appreciably within a given woman. This, of course, does not deny that in a few cases abortions are associated with blood group incompatibility and that the probability of iso-immunisation increases with gravidity (Glass 1949).

Gestation Period of Abortion

The results of an analysis of the gestation periods at which abortion occurs are shown in Tables 19, 20 and 21. About one third of abortions take place before 2 months gestation and 50% have occurred by 3 months. It seems likely that many of these early abortions may have a chromosome anomaly. Carr (1967) found that the mean duration of pregnancy for 44 abortion specimens with chromosome anomalies was 85.9 days from the first day of the last menstrual period to the day of abortion. The mean gestational

age for 144 abortions with normal chromosomes was 106.7 days. It is probable that women who abort early in pregnancy are more likely to do so due to a foetal or ovular abnormality and those abortions which occur later are more likely to be due to some uterine or environmental factor. When therapy is considered it should be remembered that 50% of women abort after the third month and that the danger of abortion is not over by this time.

Women who abort in successive pregnancies show the same pattern except that those having a third successive abortion tend to continue their pregnancy for slightly longer. It is possible that these women are subjected to more care during this pregnancy in view of their previous obstetric history, and that continuation of their pregnancy for a longer period may be due to this care.

If the gestation period of the second abortion is compared with the first (Table 22) in the same woman, between one half and two thirds of the women will abort at the same time in the second pregnancy as in the first. This means that there is a strong

tendency for women who are abortion prone to abort at the same gestation period in a subsequent pregnancy.

The relevance of these results to the design of studies and the use of therapy in recurrent or habitual abortion will be discussed later.

Interval between pregnancies

The interval between pregnancies in women whose first pregnancy is normal compared with women whose first pregnancy ends in abortion is shown in Tables 23 and 24. Half of the women (50%) in the abortion group have a second pregnancy within a year from the time of the abortion and 80% have a third pregnancy within 2 years compared with 17% and 38% respectively for the normal women. In the abortion group 92% of women have had a second pregnancy within 5 years whereas only 73% of women starting with a normal pregnancy have a second child within this time. The picture is the same when the interval between second and third pregnancies is considered (Table 24).

This bears out the previous finding that women who abort are not infertile, indeed their fertility is, if anything, increased. It is not that they are unable to become pregnant as suggested by Guttmacher (1956) but that when they do achieve a pregnancy it does not continue to viability.

Age and Duration of Marriage

The incidence of first abortion is greater over the age of 25 years than under 25 years for all marriage durations (Table 3 ), but particularly so after marriage has lasted for over 5 years when the rate is more than double that under 25 years for the same marriage duration. Guttmacher (1956) showed that when contraception is not practised "There is a detrimental effect of increasing age on fertility". These findings substantiate the results discussed on page 16showing the deleterious effect of age on the reproductive process. Guttmacher (1956) has also shown, that age and a prolonged state of matrimony were separate anti-fertile factors. He was surprised to find that the time required for a first conception increased by seven hundredths of a month for each year of the wife's

age and almost two fifths of a month for each year of marriage. The explanation of the effect of age and duration of marriage on abortion and fertility are no doubt complex and certainly obscure.

Relation of results to the design of abortion studies

There is so much variation in the design of studies on abortion that the results are seldom comparable. One common error is that 'Primary habitual aborters', women who have had 2 or 3 consecutive spontaneous abortions beginning with the first pregnancy are confused with 'Secondary habitual aborters', a term which designates those women who have had three or more consecutive spontaneous abortions following delivery of one or more immature, premature or full-term infants (Javert et al 1949).

The two main points brought out by the present work are -

(1) The type of patient that should be studied and

(2) The gestation period which should be studied.

1). Type of patient

The classification of patients liable to abortion

has given rise to much discussion (Goldzieher and Benigno 1958). The term 'recurrent abortion' is frequently used to describe women who have had three or more successive abortions (e.g. Malpas 1938, Speert 1954, and others). It is, however, apparent from the present work that women who have had two abortions are likely to abort again in 40% of cases and that these women should be termed 'recurrent aborters' and placed in the 'abortion prone' group (James 1963). It is important to exclude women who have had only one abortion. Many first pregnancies end in abortion after which further pregnancies are successful (over 80%). Subjects with only one previous abortion are therefore a heterogeneous group in which the recurrent element is minimally represented. Subjects who have had one or more viable pregnancies interspersed with abortions (secondary recurrent abortion) are likewise a mixed group and unsuitable for comparative study. Ideally subjects with four or more abortions are the most suitable group to study as the abortion rates in this group are considerably higher than those with two or three previous abortions and the results of therapy would therefore be easier to evaluate. The numbers of

these patients, however, which present at any one clinic are very small.

Threatened abortion may, in practice, be very difficult to distinguish from inevitable or missed abortion. Studies on threatened abortion (Colvin et al 1950; Goldzieher and Benigno 1958) have not proved very useful in discriminating between successful or indifferent treatment or supplying information as to the cause of abortion. Much the more promising group in this respect would appear to be the primary recurrent abortion group with two or more previous abortions.

2). Gestation period to be studied

Little attention has been paid to defining this aspect of the problem. In his monograph on 'Spontaneous and habitual abortion' Javert (1957) barely mentions gestation period even although the question of therapy in habitual abortion is widely discussed.

Most workers have commenced studying patients at varying stages of gestation without any reference being

made to the gestation period at which previous abortions had occurred. It has been shown here that one third of abortions take place before two months of gestation and 50% have occurred by the end of the third month. Furthermore between one half and two thirds of women abort at the same gestation period in subsequent pregnancies. This suggests that initial observations in abortion studies must be made at the latest by 8 weeks, and any therapy being used should have started by this time. If a previous pregnancy has ended in abortion at an earlier stage than 8 weeks, this earlier period should be taken as the time by which initial observations should be made. If, for instance, observations, and/or therapy, are started as late as the 14th week of gestation (Goldzieher 1964) it is highly probable that these subjects are already naturally selected against abortion and this tends to vitiate the trial of therapy.

A further point which has to be noted is the time at which women normally present themselves at the antenatal clinic for the first visit. Scott et al (1956) have given some information on this aspect. They found that, in Aberdeen, 35% of women with two living children

attended for the first time after the 20th week of pregnancy as compared with 21% of women with one child and 12% of childless women. Women with the same number of children attended earlier if their previous pregnancies had ended in abortion or stillbirth showing that patients who have reason to fear complications consult a doctor earlier in pregnancy. This tendency was found to be more pronounced among women who had had previous pregnancies ending in abortion than in women who had stillbirths or whose children had died subsequently. In spite of previous abortion, however, the average week of first attendance in multigravidae with no living children was 12.5 weeks. It is quite evident therefore, that special arrangements must be made to see cases for abortion studies early in pregnancy before they normally attend the antenatal clinic.

This study shows that when a woman starts child-bearing with an abortion her subsequent reproductive behaviour is different, with more complicated pregnancies and a higher rate of foetal loss than a woman who starts with a normal pregnancy. These women therefore, form a group requiring strict antenatal supervision in all

subsequent pregnancies so that the foetal loss can be lessened.

# CHAPTER 3.

## Hormone assays in normal early pregnancy, an abortion and in hydatidiform mole

### 1. Normal early pregnancy and abortion

In chapter 2 it was suggested that once a woman had had two abortions without any live children she should be placed in an abortion-prone group. This chapter which deals with steroid assays in abnormal early pregnancy, stems directly from this work. The object here was to discover the value of certain steroid assays in the management of recurrent abortion and to ascertain what happens to the hormone levels in this type of case.

The two main steroids to be considered in the first instance are (1) pregnanediol (5β-pregnane-3α-20α-diol) and (2) oestriol, one of the three classical oestrogens (oestradiol 17β, oestrone and oestriol).

Both these substances are excreted in the urine in pregnancy and some initial observations will be made on their derivation and metabolism.

## 1. Pregnanediol

This compound is a characteristic metabolite of progesterone and was isolated from late pregnancy urine by Marrian in 1929. In 1934 Butenandt and Schmidt demonstrated a connection between pregnanediol and the active hormone progesterone by synthesising the latter from pregnanediol. In 1936 Odell and Marrian extracted a combined form of pregnanediol from pregnancy urine. They noted that the free steroid could be obtained by hydrolysis of this compound with hot acid. In the same year Venning and Browne (1936) isolated the water soluble combined form of pregnanediol from butanol extracts of late pregnancy urine. They were able to show that it was present as the monosodium salt of pregnanediol glucuronic acid.

In 1937 Venning et al demonstrated a physiological connection between progesterone and pregnanediol by isolating the latter from the urine of subjects injected with progesterone and of normal women in the luteal phase of their menstrual cycle.

The relationship of progesterone to pregnanediol in the human and the recovery of urinary pregnanediol

after the injection of progesterone will be discussed in detail in chapter 4.

Organs producing pregnanediol precursor in pregnancy.

Organs producing pregnanediol precursor in pregnancy.

A. Ovary. Progesterone is produced by the corpus luteum in the ovary and has been isolated from this tissue. (Butenandt et al 1934; Short 1962).

B. Placenta. Progesterone has been isolated from human placentas (Pearlman and Cereee, 1952b). Tissue culture experiments suggest that it is produced by the syncytiotrophoblast and most of the pregnanediol found in late pregnancy urine originates from placental progesterone. Some metabolism of progesterone occurs in the foetus (see Chapter 5), and pregnanediol has been isolated from the foetal liver. That the foetus might excrete pregnanediol in the urine seemed likely following the finding of this compound in the liquor amni by Klopper and Macnaughton (1958). Some of the pregnanediol in the maternal urine in late pregnancy is almost certainly of foetal origin but since foetal death results in very little if any reduction in pregnanediol excretion in the

in the maternal urine the amount must be small (Solomon 1968 - personal communication).

C. Adrenal. A significant fraction of the pregnanediol normally present in the urine has been shown to come from the adrenal gland, and there is an association between the levels of urinary pregnanediol and the physiological activity of the adrenal (Klopper et al 1957). In fact most of the pregnanediol present in the urine of post menopausal women comes from the adrenal gland.

Pregnanediol is therefore derived from precursor synthesised in these three sites and the quantitative relationship between progesterone production and metabolite excretion will be discussed in Chapter 4 together with further notes on the metabolism of progesterone itself.

Pregnanediol excretion in normal pregnancy

Table[25] from the paper by Klopper & Billewicz (1963) shows the steady rise in pregnanediol excretion throughout normal pregnancy. In the present context only the levels up to 16 weeks gestation have been

given as this is the period of pregnancy being particularly studied. These results agree well with those of Shearman (1959) using the same assay technique.

## 2. Oestriol

During pregnancy, increasing amounts of oestrogens are excreted in the maternal urine and it has been established that the placenta is the main source of urinary oestrogen in the pregnant woman. The evidence for this view is adequately summarised by Diczfalusy & Troen (1961). Although a large number of different oestrogens are found in pregnancy urine (Brouer 1964), not all of these appear to be manufactured in the placenta and consideration is usually centred on the so called classical oestrogens - oestrone - oestradiol 17β and oestriol. These substances, and particularly oestriol, have been most intensively studied in practice.

### Biogenesis of Oestrogens in pregnancy

It was assumed at first that the human placenta, like the ovary, could synthesise oestrogens from

acetate, since the conversion of acetate to urinary oestrogen had been demonstrated in the pregnant mare (Herbin et al 1957) and the conversion of radioactive cholesterol to urinary oestrogen in a pregnant woman had also been reported (Levitz et al 1962). It is not possible, however, to demonstrate *in vitro* the placental conversion of acetate to oestrogen, and the disproportionately large amounts of oestriol found in late pregnancy cannot be explained by the placental metabolism of oestrone or oestradiol because the necessary 16α hydroxylation reaction is inefficient in the placenta (Levitz et al 1962). Furthermore it has become evident from the studies of Frandsen and Stakeman (1963) and Caesmer (1959) that the foetus is essential for maximum oestrogen production.

It seems true however, that the placenta does secrete oestriol since the concentration of total oestriol is higher in uterine venous blood than in maternal arm vein blood (Roy 1962). The increased urinary excretion of oestrogens during pregnancy closely parallels the increase in placental weight and, after removal of the placenta the oestrogen excretion

diminishes rapidly (Brown 1967). The placenta produces the three 'classical' oestrogens, oestrone, oestradiol 17β and oestriol - the last compound being manufactured in the largest amount.

The placenta appears to be capable of producing oestriol *in vivo* and *in vitro* (Ryan 1960), but not by synthesis from acetate and cholesterol. Placental perfusion or incubation with labelled acetate and cholesterol (Levitz et al 1962) did not show the formation of oestrogens; but after perfusion of term placentas *in vitro* (Warren & Timberlake, 1964; Cedard et al 1962) and midterm placentas *in situ* (Bolte et al 1964a & b), testosterone and other neutral C-19 steroids have been transformed into the corresponding oestrogens. The combined activities of foetus and placenta convert non oestrogenic steroids to oestrogens and this is an important source of oestrogen in pregnancy, (Mitchell, 1967).

Early in normal pregnancy a large fraction of the small total amount of oestriol present is derived from sources such as ovarian oestradiol. As pregnancy proceeds this fraction decreases and at term amounts

to only about 10% of total oestriol production (Siiteri and MacDonald 1966).

Frandsen and Stakeman (1961, 1963) first suggested that the foetal adrenal might supply an oestrogen precursor to the placenta. The work of Baulieu and Dray (1963), Siiteri and MacDonald (1963) and Bolte et al (1964b) showed that dehydroepiandrosterone sulphate (DHAS) which is circulating in pregnant women is converted to urinary oestrogens. High concentrations of DHAS have been found in cord blood (Simmer et al 1964; Eberlein 1965), supporting the suggestion that this oestrogen precursor may be produced in the foetal adrenals. The DHAS which reaches the placenta via the umbilical circulation is converted to urinary oestrogen (Bolte et al, 1964b); it is reduced at C-17 and 16α hydroxylated mainly in the foetal liver, (little 16α hydroxylation takes place in the placenta).

Once oestriol has been formed by the placenta some reaches the foetus, where it is converted into the corresponding 3-sulphate. The oestriol-3-sulphate formed is returned to the placenta, where it is, in part, hydrolysed by the arylsulphatase and

liberated oestriol is returned to the foetal and maternal compartments. In the mother this oestriol is conjugated with the 3-sulphate and glucosiduronate and excreted. The metabolism of oestriol in both maternal and foetal compartments is characterised mainly by changes in conjugation and not in the steroid moiety (Diczfalusy & Benagiano 1966).

Haynes et al (1964) studied the metabolism of oestradiol-4-$C^{14}$ in perfused foetuses and found that the major metabolites were oestradiol-3-sulphate and oestrone-3-sulphate. They were not able to demonstrate the formation of oestriol from oestradiol in the foetus. Furthermore, the perfusion of the foeto-placental unit in situ did not reveal the presence of any significant amount of oestriol. Oestradiol, therefore, which reaches the foetus via the umbilical circulation, is not metabolised to oestriol to any extent. There is also little conversion of oestrone to oestriol since, when the isolated foetus was perfused with oestrone-3-sulphate, only a small amount of oestriol was isolated (Emerman et al 1965).

Oestriol is manufactured by the placenta from both

foetal and maternal precursors. A foeto-placental, oestriol-3-sulphate cycle is established consisting of foetal sulphurylation and placental desulphurylation (Goebelsmann et al, 1966). Some oestriol-3-sulphate is excreted by the foetus in the amniotic sac (Troen 1961; Diczfalusy et al 1963; Katz et al 1965); it reaches the membranes where it is hydrolysed, the oestriol is liberated, and transferred to the maternal and foetal organisms. The metabolic pathways of oestrogen metabolism which occur in the foeto-placental unit are basically due to differences in enzyme distribution between the foetus and placenta.

## Clinical Significance

Clinical Significance

As a result of the part played by the foetal adrenal glands in supplying precursor 16α hydroxy-dehydroepiandrosterone sulphate (DHAS) for the placental elaboration of oestriol, much of which is excreted in increasing amounts throughout pregnancy in the maternal urine, the measurement of oestriol in the urine has provided a useful tool for measuring foetal vitality. Maternal DHAS contributes a relatively minor proportion of the total oestriol throughout pregnancy via both

neutral and phenolic pathways.

Early in normal pregnancy a large proportion of the total amount of oestriol is derived from other sources - possibly ovarian. With lengthening pregnancy this source decreases in importance, and at term 90% of the oestriol is formed in the placenta, mainly from neutral precursors arising in the foetus (Siiteri & MacDonald 1966). If, therefore, there is any abnormality of the foetus, this may be reflected in the oestriol excretion in the maternal urine.

This is supported by the finding that all the oestriol formed by a woman with an anencephalic foetus appears to be derived from maternal DHAS primarily via oestradiol (Siiteri and MacDonald 1966). When the foetus is anencephalic, the maternal urinary oestriol excretion is one-tenth of that found in normal pregnancy (Frandsen and Stakeman 1963). In these foetuses the foetal zone of the adrenal gland is small or absent.

Maternal oestriol excretion has also been shown to correlate with baby weight (Coyle & Brown 1963; Frandsen & Stakeman 1960).

## Urinary Oestriol excretion in normal pregnancy

The main urinary end product of the oestrogens is oestriol and many workers have been interested in the urinary output of this steroid in pregnancy (Brown 1956; Zondek & Goldberg 1957; Lantero 1958; Keller et al 1959; Frandsen & Stakeman 1960; Hobkirk et al 1960; Kaiser 1960; Borth 1960; Klopper et al 1961; Greene et al 1961; Borth 1961; Banerjea 1962; Hobkirk & Nilsen 1962; Boling 1963; Wray & Russell 1963; Coyle & Brown 1963). It is difficult to compare the values obtained by different workers as their assay methods, their criteria for normal pregnancy and their experimental designs have been different. The papers quoted report mean values during the last month of pregnancy varying from 11mg (Zondek & Goldberg 1957) to 32mg. (Keller et al 1959) per 24 hrs. It is not possible on the basis of these publications to specify with any exactitude the changes in oestriol excretion during pregnancy but only the extent of variation to be expected. As with pregnanediol the swings of excretion from day to day in the same patient may be wide (Klopper 1962).

## Present Study

### Method of assay of urinary pregnanediol

The assay method used in the present investigation is that of Klopper et al (1955). All assays were made on 24 hr. urine collections which were performed at weekly intervals.

### Method of assay of urinary oestriol

In the present investigations two methods of assay were used. For very early pregnancy the classical method of Brown (1956) was used but after 12 weeks of gestation the shorter method of Klopper & Wilson (1962) was preferred. In the latter method the first four steps of the Brown (1956) method, acid hydrolysis, extraction, solvent partition, and hot alkali treatment are used and the oestriol content of the residue is determined by the Kober reaction as in the Brown method. Because in early pregnancy more than 5 ml. of urine has to be used in order to get enough oestriol for accurate spectrophotometric measurement sufficient impurity remains in the oestriol fraction to produce an appreciable increase in the background colour. A further purification step is, therefore, necessary with early

pregnancy urine. This is achieved by methylating the oestriol fraction from the chromatographic column and then recovering the 3-methyl ether by a second chromatographic step.

Subjects

Urinary oestriol was studied in three groups of women (1) Normally pregnant women (2) Women with no successful pregnancy and at least two abortions none of which had exceeded 24 weeks gestation. The pregnancy studied ended successfully. (3) Women who aborted in the pregnancy studied.

Urinary pregnanediol excretion was also studied in groups 2 and 3 and the levels compared with those reported for normal pregnancy by Klopper & Billewicz (1963). Urine collections from women in groups 2 and 3 were always started before the gestation time at which any previous abortion had occurred. This is important, since as pointed out in Chapter 2, abortion tends to occur at the same time in successive pregnancies, and trends of excretion might be missed if collections are begun after this time, furthermore the individuals would then be selected toward continuation of the pregnancy.

## Results

### Pregnanediol excretion

Tables 25, 26 & 27 show the mean excretion of pregnanediol with standard deviation in the three groups from 5 - 16 weeks of pregnancy. There is no statistically significant difference between the three groups at similar stages of gestation. The mean of the "successful two previous abortion" group tends to be slightly higher at each stage than the normal group which was composed mainly of primigravidae. This suggested that they were behaving, as far as pregnanediol is concerned, like multiparous women. Klopper and Billewicz (1963) showed that primigravid women excreted significantly less pregnanediol than multigravidae during pregnancy and these findings support this observation. Shearman (1959) did not give sufficient data in his paper but (Personal communication 1968) did say that other data he had confirmed this view.

### Special Cases

Two individual cases where pregnanediol excretion had been measured in the luteal phase of the menstrual

cycle before a pregnancy had occurred have also been studied. Figure 1. In one woman the pregnanediol level rose to 14mg/24hr. at 11 weeks of pregnancy, well within the normal range. It then dropped to 4mg/24hr. at 16 weeks, the normal non pregnant luteal level for this individual. Thereafter it rose steadily until term when the patient was delivered of a normal child. At no time during the low level of pregnanediol were any signs of abortion present.

The second subject had a rise in urinary pregnanediol level to 19mg/24hr. at 16 weeks. The level then fell to 2.5mg/24hr. at 25 weeks, the normal luteal level for this patient. Spontaneous abortion occurred shortly after. These serial results indicate that even this type of measurement in individual women may give little indication of the outcome of the pregnancy.

Urinary oestriol excretion

Tables 28, 29 & 30 show the urinary oestriol excretion in the 3 groups from 5 - 16 weeks of pregnancy. Again no significant difference in excretion is evident. Figure 2 shows that up until 16 weeks some

women who are going to abort show a remarkably normal oestriol excretion. Serial readings in these women give little indication of impending abortion.

The gestation period studied in these subjects was between 5 and 16 weeks of pregnancy and little indication of impending abortion can be gained from these studies. However, in later abortions some information may be gained by the measurement of oestriol excretion in a similar way in which it is used to give information in late pregnancy. Oestriol excretion in the urine in late pregnancy has given information as to the wellbeing of the foetus. After 20 weeks the excretion of oestriol in the maternal urine gives information about the growth of the baby. Coyle and Brown (1963) studied the excretion of oestriol in 31 women who were delivered of babies weighing under 3000g. after a gestational period of more than 37 weeks. In this series the oestriol values were normal until about 18 weeks but thereafter the rise was two thirds of the normal level after 30 weeks. Oestriol excretion appears to correlate with the weight of the baby, a finding also reported by Frandsen and Stakeman (1960).

In a patient being studied as a case of normal pregnancy, oestriol excretion remained low for 3 months before abortion finally occurred at 26 weeks (Fig. 3), possibly reflecting the decline in foetal vitality and eventual foetal death. Pregnanediol excretion did not fall until shortly before the abortion and presumably remained at normal levels until placental death irrespective of the state of the foetus. These findings therefore support the contention of Klopper and Billewicz (1963) that up to 20 weeks of pregnancy or so the urinary oestriol excretion represents oestrogen production by the trophoblast from maternal precursors and perhaps the ovary, while later in pregnancy the effects of the foetal production of precursors, previously discussed, become important.

Pregnanediol/oestriol ratio

It has been suggested that differences in hormonal concentration between oestrogen and progesterone influence myometrial contractility in the human (Bengtsson 1962). This suggestion is based on investigations of the hormonal control of the rabbit myometrium and Csapo (1961b) was able to produce in

this animal conditions resembling missed abortion by means of progesterone injections after removal of the ovary and separation of the placenta. Csapo (1961a) suggested that progesterone, by affecting the membrane potential of the myometrial cell might block the propagation of contraction waves through the uterus. Kuriyama and Csapo (1961) claimed that the myometrial block was most pronounced in the immediate vicinity of the placenta, supporting the view that the restraining factor was derived from the placenta. A second hormonal factor concerned in myometrial activity may be oestrogen. Corner and Csapo (1953) in a study of the contractile system of uterine muscle found that the concentration of the contractile protein actomysin, could be increased by oestrogen.

Klopper and Billewicz (1963) studied oestriol excretion during pregnancy and suggested that this particular oestrogen might be concerned in the onset of labour.

If, therefore, one measures the excretion of a progesterone metabolite (pregnanediol) and oestriol in women who abort, the ratio of these hormones present at the time of abortion may be obtained.

Few workers have in fact measured the pregnanediol/oestriol ratio. Kaiser (1960) considered this aspect but made monthly measurements in only one woman. Klopper and Billewicz (1963) made some observations on this subject (Fig. 4). They found that in early pregnancy about 100 times as much pregnanediol as oestriol is being excreted. By 20 weeks the ratio has fallen to about 3:1. They also observed that subsequent to 20 weeks the ratio continued to fall although, more slowly, and by term the amounts of the two metabolites were approximately equal.

Pregnanediol/oestriol ratio in the present study

Tables 31, 32 and 33 show the ratio of excretion of urinary pregnanediol and oestriol in the three groups. There are great variations within the groups and this may be due to the small numbers at some weeks of gestation. However, there is no significant difference between the groups.

The results shown here suggest that there is no significant difference in urinary excretion of oestriol and pregnanediol in abortion and in normal pregnancy.

## Discussion

There are many reports of urinary pregnanediol levels in threatened and habitual abortion and results vary greatly. Some reports suggest that a decreasing or constantly low excretion is followed by abortion (Borglin 1956; Rawlings & Krieger 1959; and others). Other investigators have found pregnanediol determinations of little prognostic value (Russell et al 1957). Measurement of urinary pregnanediol in cases of threatened and recurrent abortion has sometimes been used as a criteria for progestin therapy. In some reports therapy has been based on the results of single assays (Kupperman et al 1960; Morgan et al 1960). It is evident from the series reported here that this is not justifiable.

It is interesting that great variations occur in pregnanediol excretion in the same patient. This is very evident in the case shown in Fig. 1, where the pregnanediol excretion fell to luteal levels at 17 weeks and then rose normally to term without any clinical evidence of abnormality in the pregnancy. It could be suggested that the fall was due to the failing corpus

luteum. However, it has been shown by Diczfalusy and Borell (1961) that the ovarian contribution to the excretion of both oestrogen and pregnanediol at this stage of pregnancy is very small if indeed there is any at all. In pregnancy variable amounts of administered progesterone are converted to pregnanediol (see Chapter 4) and, furthermore, pregnanediol has been isolated from the faeces during pregnancy as mentioned already (Klopper and Macnaughton 1959). It is clear, therefore, that changes in pregnanediol excretion may be due to alterations in metabolism and may not entirely reflect progesterone production. It is plain from the results that pregnanediol excretion gives little indication of the prognosis.

The same is true of oestriol measurements up to 16 weeks of pregnancy. Thereafter when the production of foetal precursor becomes evident oestriol results may be more meaningful, as in the case mentioned when oestriol output was depressed for 3 months before abortion occurred at 26 weeks. The findings are in agreement with those of Klopper and Billewicz (1963). A similar picture is reported by Coyle and Brown (1963)

when measurement of oestriol excretion proved of great value in assessing the viability of the foetus in cases with complications such as pre-eclampsia and poor foetal growth syndrome. Hähnel and Martin (1964) have also found that the oestrogen excretion in cases of threatened abortion continuing to term was not significantly different from normal but that in cases of inevitable abortion the urinary excretion of oestradiol 17β and oestrone was significantly lower than in the normal groups probably due to the trophoblastic and foetal production being cut off by this time.

The ratio of pregnanediol to oestriol in normal pregnancy has been considered by Kaiser (1960) and Klopper and Billewicz (1963). It is clear from the results of this study that there is no significant change in this ratio before abortion occurs. Here it should also be remembered that both oestriol and pregnanediol are the end results of a series of metabolic processes and may not reflect events occurring at the cellular level. Short and Moore (1950) and Walker (1963) found no change in blood progesterone levels at the onset of labour and Eton and Short (1960) could find no constant relationship between blood

progesterone levels and the outcome of threatened abortion. Fig. 5 shows the relationship of blood progesterone levels in pregnancy (Eton and Short 1960) and urinary excretion of pregnanediol (Klopper and Billewicz 1963, and Shearman 1959).

## 2. Urinary excretion of Oestrogen and Pregnanediol in Hydatidiform Mole

### Introduction

It is well established that the urine of patients with hydatidiform mole contains high concentrations of chorionic gonadotrophin (Hamburger 1943) but there are few reports on the excretion of other hormones. Pigeaud and Burthiault (1951) found that some patients with hydatidiform mole excreted increased amounts of pregnanediol, some normal and some diminished amounts. Zander and von Munstermann (1956) reported that mole tissue had almost the same concentration of progesterone as normal placental tissue. Payne (1941) found a normal excretion of oestrogens in two cases of hydatidiform mole and Hinglais and Hinglais (1949) reported a similar finding in 16 cases. In both these investigations a biological assay method was used.

In five cases, however, Smith and Smith (1936) found excretion of oestrogen which corresponded to non-pregnant levels. Erb et al (1961) reported on two cases of hydatidiform mole and found that one patient excreted normal amounts of the three classical oestrogens, oestrone, oestradiol and oestriol but low amounts of pregnanediol whereas the other excreted small amounts of oestrogen - the lower limit of the normal range - but normal amounts of pregnanediol.

Frandson and Stakeman (1964) have reviewed some of the information concerning the excretion of these hormones in hydatidiform mole and have described their findings in 10 cases. They concluded that, as far as pregnanediol excretion was concerned only one out of five cases had an excretion level similar to that found in normal pregnancy, whereas all the others had a lower excretion level like that found in the non-pregnant subject.

As far as oestriol excretion was concerned they found that this was, in general, much lower than in normal pregnancy of the same gestation period. In addition the ratio of excretion of oestriol to oestradiol

and oestrone corresponded to that found in the non-pregnant woman. These workers concluded that oestrogen metabolism in cases of hydatidiform mole resembled that found in the non-pregnant woman.

It was thought that, since there was some confusion in the literature about the excretion of oestrogens and pregnanediol in cases of hydatidiform mole further information was desirable as this would help to elucidate the hormone picture in early pregnancy there being no foetal component in cases of hydatidiform mole.

A small number of suitable cases became available and were studied from this aspect.

Methods of Assay of the Hormones

In cases 1 and 2 oestrone, oestradiol and oestriol were measured by the method of Brown (1955) and in cases 3, 4 and 5 oestriol was measured by the method of Brown and Coyle (1963).

Pregnanediol was measured by the method of Klopper et al (1955).

Clinical Summaries

Case 1

The patient was a primigravida aged 21, who was admitted with vaginal bleeding at the 10th week of pregnancy. The Hogben test was positive in a dilution of 1:100 but negative in 1:200. The uterus was enlarged to the size expected at 14 weeks of gestation and continued to grow more rapidly than normal. Intermittent bleeding continued and a mole was passed at 17 weeks.

Case 2

The patient was a primigravida aged 24 years, who was admitted at the 11th week of pregnancy with vaginal bleeding. The uterus was of normal size for the gestation period. The Hogben test was positive in a dilution of 1:100 and negative 1:200. Intermittent bleeding continued and a mole was passed at 19 weeks. Three weeks later the patient was re-admitted to hospital with severe vaginal bleeding. The uterus was bulky and soft and a blue vascular nodule was present in the lower vagina. Frozen sections of curettings and of the nodule suggested chorio-carcinoma so

hysterectomy and excision of the nodule were performed. At operation two theca lutein cysts were found measuring 7 x 5 x 4cm. and 8 x 7 x 1cm. respectively. Histology of the uterus and vaginal nodule suggested that the condition was chorio-adenoma destruens and not chorio-carcinoma. Follow up over the next two years showed no evidence of recurrance and the titre of gonadotrophins in the urine remained normal.

Case 3

The patient was aged 26, para 2, admitted at 15 weeks gestation with vaginal bleeding. The uterine size was normal for the gestation period. The pregnancy continued with intermittent bleeding until the 19th week when the blood pressure rose to 200/120mmHg and the urine contained 12g/l of albumen. Spontaneous delivery of a hydatidiform mole followed the acute rise in blood pressure. The patient has since had a normal pregnancy.

Case 4

The patient was a primigravida aged 19 years, who was admitted at 16 weeks of pregnancy with vaginal bleeding. The uterus was small for the gestation period.

The Hogben test was weakly positive 1:10 dilution. Intermittent bleeding continued and a mole was passed at 20 weeks.

Case 5

The patient was aged 26, para 2, and was admitted with intermittent vaginal bleeding at 11 weeks gestation. The uterine size was normal for the gestation period. The Hogben test was positive 1:10 dilution but negative 1:100 dilution. Intermittent bleeding continued and a mole was passed at 16 weeks.

## Results

Table 34 shows the urinary excretion of hormones in the 5 cases. Pregnanediol excretion and oestriol excretion were measured in all cases and the three 'classical' oestrogens and pregnanediol in two subjects.

Pregnanediol

In Cases 1 and 3 the levels are higher than in normal pregnancy whereas in Cases 2, 4 and 5 the levels are much lower than normal and indeed approximate more to those found in the non-pregnant state.

Oestriol

Case 1. The levels of oestriol excretion remained normal until 16 weeks thereafter flattening out in a manner found in intra uterine death already mentioned.

Case 3. Only a single reading at 19 weeks was available. This was lower than normal but considerably higher than those found in Cases 2 and 4 where results were available at 19 weeks.

Cases 2, 4 and 5. The levels of oestriol excretion in these subjects were much below the normal for the gestation period and were similar to the levels reported by Frandsen and Stakeman (1964) for hydatidiform mole.

Oestrone and Oestradiol

The levels of these steroids tended to follow those of oestriol, being high in Case 1 and low in Case 2. The oestriol:oestrone plus oestradiol ratio varied from 1 to 4.7.

Discussion of mole results

As has been mentioned before it seems almost certain that the placenta produces progesterone and

oestrogen.

In Case 1 in this series, the levels of oestriol remained high until the 16th week of pregnancy, while those of pregnanediol were above those of normal subjects at the same gestation period. In this subject at least until the 16th week, the oestrogen production was within normal limits in spite of the absence of a foetus.

It seems possible, therefore to have normal oestrogen production in the first 5 months of pregnancy without a foeto-placental circulation.

The oestrogen level might be due to increased production by the ovaries, which may be excessively stimulated by the large amounts of gonadotrophin present. This seems doubtful, however, since from the data in Table 1 of the paper of Frandsen & Stakeman (1964) Table 35) the level of gonadotrophin was lowest in the patient with the highest level of oestriol. In the present cases there was no correlation between the level of gonadotrophin, as measured by the Hogben test and the hormone excretion, or the presence of theca

lutein cysts and the excretion level. The hormone may therefore, either be produced by the ovary or by the trophoblast, possibly by a different metabolic pathway from that found in normal pregnancy, and further work to try to elucidate this problem is reported in Chapter 6.

# CHAPTER 4.

## The Conversion of Progesterone to Pregnanediol

In chapter 3, when the origin of pregnanediol was discussed the relationship of the amount of progesterone represented by urinary pregnanediol was mentioned. Almost invariably estimates of urinary metabolites are made to obtain information about the production of the original hormone. For this purpose it is useful to know the ratio between active hormone and inert metabolite and it is essential that some fairly constant relationship should exist between the two to make results meaningful. Hytten & Leitch (1964) rightly remark in their book 'The Physiology of Human Pregnancy' that "It is curious that so much research has been expended on polishing the technique for estimating pregnanediol and so little on discovering how faithfully it is likely to represent its parent hormone". It was with this admonition in view and also because of the rather varied results reported in chapter 2 that it was decided to investigate further the relationship of progesterone to urinary pregnanediol.

Percentage recovery of injected progesterone as urinary pregnanediol

This figure has varied from report to report. The following are some figures from the literature, 7.9% Quilligan and Rothchild (1957); 9-16% Sommerville and Marrian (1950); 20% Klopper and Michie (1956); approx. 20% Guterman (1953); 20-40% Davis and Plotz (1957) and 30-35% Davis and Fugo (1947). Some workers have also reported that the percentage recovery of administered progesterone as urinary pregnanediol is higher in pregnancy than in the non-pregnant state (Venning and Browne (1940); Sommerville and Marrian (1950); Guterman (1953).

In contrast Pearlman (1957) using tritiated progesterone found that the conversion of administrated progesterone to pregnanediol was lower in the pregnant subject (6-15%) than in the oophorectomised/ hysterectomised woman (14-27%). This latter finding seems more logical since some of the administered progesterone could be taken by the foetus and/or the mother whereas in the non-pregnant woman it would overload the system giving rise to increased conversion

and excretion of pregnanediol. It also seems true that the exogenous progesterone can be converted to pregnanediol in the absence of a uterus and/or ovaries, Buxton (1940) and Guterman (1953).

## Present Study

In view of the varying results obtained and relative lack of information on the conversion of progesterone to pregnanediol it was decided to investigate this problem further using tritiated progesterone to study any difference in the conversion between normal and abnormal pregnancy and to determine whether the conversion values were related to abnormalities of pregnancy, especially early pregnancy. Guterman (1953) had suggested that there was a higher conversion of progesterone to pregnanediol when a viable foetus was present.

## Metabolism of progesterone

Pregnanediol is considered to be the principal urinary metabolite of progesterone, Weist et al (1958) and in no report has more than 40% (average 20%) of administered progesterone been converted to pregnanediol. When 4-$C^{14}$ progesterone was injected Davis et al (1956)

recovered 28.54% from the faeces in the next 10 days. Negligable amounts are secreted via the skin (Davis and Plotz 1957) and although no $C^{14}O_2$ could be detected following administration of 4-$C^{14}$ progesterone $C^{14}O_2$ was found in expired air after administration of 21-$C^{14}$ progesterone (18-19% in 31 hr.) Davis and Plotz (1958). This final report illustrates that the side chain can be broken off in metabolism with the possible formation of androgens e.g. androstenedione, androsterone and/or etiocholanolone.

It has been reported that a large amount of progesterone is stored in maternal fat (Kaufman and Zander, 1956) and Plotz and Davis (1957). The latter workers showed that after 12, 24 and 48 hr. 17.7, 33.7 and 19.6% respectively of injected progesterone was found in maternal fat either as progesterone or its metabolites.

Until radioactive progesterone could be administered in the form of tritiated progesterone other urinary metabolites of progesterone could be detected only with difficulty. The use of labelled progesterone in humans has shown that this hormone can also be metabolised to

compounds which are more polar than pregnanediol. Chang et al (1960) found that 2% of the administered dose appeared in the polar fraction and Contractor and Pearlman (1960) found 0.5 - 2% of the administered dose to appear in the ketonic polar fraction. Since these latter workers used progesterone labelled with tritium at C-16 and also hydrolysed the urine with acid it is likely that many of the polar compounds were destroyed and the figure they quote is therefore much smaller than the true conversion. Greater conversions have, in fact, been reported by Harkness and Fotherby (1963) who found about 6% of the administered dose in the polar fraction and by Romanoff et al (1963) who found a value of 6 - 7%. The results of Harkness and Fotherby showed the ketonic fraction to contain a greater proportion of these polar metabolites than the non-ketonic fraction.

It seems likely that a large proportion of the polar fraction is composed of metabolites with an oxygen function at position 6, Kamyab and Fotherby, (1963), and James and Fotherby (1965) have shown that the administration of progesterone is also associated with an increased excretion of these 6-oxygenated metabolites.

A number of compounds with a hydroxyl group at position 16 have also been isolated from urine. These compounds arise partly from 16α-hydroxyprogesterone. This compound has been isolated from corpora lutea and placental blood after normal delivery (Zander et al 1962), and also from the peripheral metabolism of progesterone or its metabolites (Fotherby 1964).

Therefore, although conversion of progesterone to a number of other metabolites occurs in the human and many of these metabolites can be isolated from human urine following administration of labelled progesterone, pregnanediol is the most characteristic metabolite and has been most commonly used as an index of progesterone production. The radioactivity recovered as urinary pregnanediol following intravenous injection of tritiated progesterone gives a measure of the conversion rate of progesterone to pregnanediol.

Assay of urinary pregnanediol

The method used for the estimation of pregnanediol was that of Klopper et al (1955). It is considered specific for 5β-pregnane-3α-20α-diol since the final purified diacetate is only slightly less pure

than the diacetate of the pure compound (Klopper et al 1955; Coyle et al (1956)). The method involves acid hydrolysis, toluene extraction, a permanganate oxidation step to remove the decomposition products of pregnanediol following acid hydrolysis, chromatography on an alumina column to isolate the free pregnanediol, acetylation and further chromatography to isolate pregnanediol diacetate. The final assay is by colour development with sulphuric acid. No correction was made for extraction loss, all studies were comparative and losses occurring during the procedure were known to be constant within the overall error of the method.

Measurement of the radioactivity present in the urinary pregnanediol following the intravenous injection of $H^3$ progesterone

The radioactivity contained in the pregnanediol diacetate produced was measured in a Nuclear Enterprises single channel liquid scintillation counter. The efficiency recorded when tritium was measured was 22.5%. The radioactivity was measured in counting vials of 15 ml. capacity.

The scintillation fluid used consisted of:-

4g. 2,5 Diphenyloxazole (PPO) and 0.05g. p-bis[2-(5-phenyloxazolyl)] benzene (POPOP) per litre of toluene (Scintillation grade).

The components of the scintillation fluid were all purchased from Nuclear Enterprises.

To remove any errors in radioactivity measurement due to the background urinary material extracted, a sample of urine obtained prior to the radioactive injection was also extracted. Since the daily percentage recovery of injected progesterone as pregnanediol falls off markedly after the first two days, larger volumes (150 ml.) from the 3rd., 4th and 5th days had to be extracted to give sufficient counts for accurate measurement.

Duplicate analyses were performed in all cases and duplicate samples were also extracted for direct measurement of the pregnanediol present.

The overall findings are expressed as results under the following headings -

1). The absolute amount of pregnanediol excreted each day in mg/24 hr. pregnanediol diacetate.

2). The amount of radioactivity recovered each day and hence the percentage of tritiated progesterone injected which is converted to pregnanediol and excreted on each of the 5 days.

The amount of radioactivity left in the syringes and ampoules after injection was measured in a number of cases. The average amount remaining in these was 1.19μc (SD ± 0.20) in 14 cases. This means that an average amount of 98.81μc was injected. In view of the very small error involved results were calculated on a basis of 100% injection of the progesterone.

## Results

### The following intravenous injection of tritiated progesterone

following intravenous injection of tritiated progesterone

The patients studied were separated into four main groups: non-pregnant, early pregnancy, late pregnancy and women with abnormal pregnancies. One hundred microcuries of tritiated progesterone dissolved in ethanol were given by intravenous injection into the antecubital vein and urine collected in 24 hour periods for 5 days after the injection.

The daily percentages recovered as pregnanediol on

the first 5 days are tabulated in Tables 36, 37, 38 & 39.

It is clear that the majority of the radioactive pregnanediol was recovered in the first 24 hour urine specimen after injection of the labelled steroid. The excretion of radioactivity fell to very low levels by Day 5. Table 36 illustrates the recoveries in the non-pregnant subject. In Case No. 3, it should be pointed out that the probable reason for high recovery on Day 2 was due to post operative renal retention. On Day 1, urine volume, creatinine excretion, and pregnanediol excretion were all sub-normal. The percentage recovered as pregnanediol in the group of non-pregnant subjects varied from 5.69 - 14.21% - a fairly wide scatter.

Table 37 illustrates recoveries for subjects in early pregnancy.

Case 11 had tritiated progesterone injected into the uterine muscle just before hysterectomy and the first 48 hour urine sample was pooled. In the 11 cases of apparently normal early pregnancy studied it can be seen from the table that the total percent recovered as urinary pregnanediol varied from 6.81 - 15.14, There

appeared to be no difference from the 7th to 19th week, the amount recovered depending on the individual. In Case 11 where an intrauterine injection was performed the percentage recovered was 10.79 and fell within the range reported after intra venous injection.

Table 38 shows the conversion in the late pregnancy subjects (all three are in the last 2 weeks of pregnancy). The total percentage recovered in these women varied from 5.74 - 16.35 and again it appeared that any difference seemed to depend on the individual rather than on the stage of gestation.

Table 39 includes a number of clinically abnormal cases showing their conversion over the five day period. In this group the percentage recovered as urinary pregnanediol ranged from 1.42 to 9.90 (Case 22 had only a three day recovery period but assuming that she had a similar fall in percentage recovery as is present in all other cases the total recovery should hardly be influenced). Although there is a wide range in the total percentage activity recovered as pregnanediol the average in the abnormal cases appeared lower.

Table 40 illustrates the variation between the four groups. Testing for significance by the 't' test there was no significant difference between the non-pregnant, early pregnant and the late pregnant subjects. However, there was a significant difference between abnormal pregnancy and the other three groups and it seems likely that there may be some altered metabolism of progesterone to urinary pregnanediol in these abnormal cases. Further work on this subject will be described in chapter 6.

Specific Activity of the pregnanediol isolated

The specific activity of the pregnanediol diacetate isolated from the urine was calculated for each of 5 urines. The pregnanediol excretion in mg/24 hr. was determined as pregnanediol diacetate.

$$\text{Specific Activity} = \frac{\text{radioactivity in pregnanediol diacetate mμc/24hr.} \times 1000}{\text{mg. pregnanediol diacetate/24hr.}}$$

Table 41 illustrates the daily specific activity of the isolated pregnanediol diacetate. The results may be separated into two main groups. The first group contains all the patients whose specific activity of

pregnanediol diacetate is above 450mμc/mg on the first day and above 100mμc/mg on the second day. The second group contains the remainder although there is possibly a small third group present which does not fit either category.

The non-pregnant cases all fell into the first group together with 5 out of 11 of the early pregnant cases. No late pregnancy or abnormal cases were in this group. In the second group, all but one of the late pregnancy cases were present. The third group contained a mixture of early pregnancy and abnormal cases.

Since the radioactivity excreted in the urine as pregnanediol was almost constant, i.e. there were no significant differences in the percentage recovery between the early, late and non-pregnant subjects, when the pregnanediol was low - the non-pregnant and early pregnant women - the specific activity was high. Conversely, in late pregnancy, when there was an increase in the pregnanediol excretion the specific activity was low.

These results do not suggest that specific activity

per se gave any particularly useful additional information about the various types of pregnancy studied.

Urinary pregnanediol excretion, and percentage conversion of progesterone to pregnanediol in a hysterectomised woman

The results are shown in Table 42.

The percentage conversion to pregnanediol fell within the usual range for a non-pregnant subject but the specific activity was extremely high due to the very low absolute amount of pregnanediol excreted daily.

Excretion by same patients non-pregnant and pregnant

Two patients were studied in these two states to determine whether pregnancy changed the conversion in the same individual.

Cases 5 and 21 were the same patient in the non-pregnant and then the pregnant state - before abortion. Similarly Case 4 and Case 8 were the same patient in the non-pregnant and then the pregnant state. Direct comparison of these results is shown in Table 43.

When both women became pregnant the percentage radioactivity recovered as pregnanediol dropped slightly and, since the absolute amount of pregnanediol rose with pregnancy, in both cases the specific activity decreased.

## Discussion

The average amount of radioactivity recovered as urinary pregnanediol over 6 days after intravenous injection of tritiated progesterone in normal non-pregnant and pregnant subjects was 10% with a range of 5.32% and 19.59%. This result compared favourably with previous reports Quilligan & Rothchild (1957), Sommerville & Marrian (1950) and Gutorman (1963) although not as high as Davis & Plotz (1957). However, contrary to Venning and Browne (1940) and Sommerville and Marrian (1950) who found higher levels of recovery in pregnancy than in non-pregnancy, and to Fenrlman (1957), who, conversely, found higher levels of recovery in non-pregnant women, this work suggests that there is no difference in the percentage recovery between the pregnant, (early or late) and the non-pregnant women. Klopper and Michie (1966) also found no difference in

recovery. Hence it would appear that the presence of a foetus does not make any difference to the percentage of progesterone that is converted to pregnanediol. In one case, in the present study, in which abortion occurred the percentage recovered as pregnanediol was low. This result is in accordance with the view of Guterman (1953) who suggested that the percentage recovered as pregnanediol was higher when a viable foetus was present. It is clear that this matter is not finally resolved and further work is required to clarify this point. In contrast to this, patients with abnormal pregnancies, for example hydatidiform mole and pre-eclamptic toxaemia, tend to have a lower percentage conversion (average 5.32%). The production of progesterone in these cases is lower than normal since the absolute urinary pregnanediol levels tend to be low also. In the hysterectomised patient, (Table 42), the percentage recovered as pregnanediol was within the normal range and this confirmed previous reports that the uterus and/or ovaries were not necessarily required for the normal conversion of progesterone to pregnanediol. Romanoff (1962) in fact, found 14% of administered progesterone

converted to urinary pregnanediol in men.

Although a viable foetus was delivered in Case 30, an extremely low conversion to urinary pregnanediol (0.49%), and absolute urinary pregnanediol excretion (2.5mg/24hr.) were found, neither apparently inconsistent with a successful pregnancy. It seems likely that the metabolism or excretion in this case must be different. The total urinary radioactivity in this case was only 1.5% on day 1 suggesting - (1) alternative excretion route (2) some alteration in metabolism or (3) increased maternal fat storage or of 'bound' progesterone. **(Table 44).**

The first suggestion is quite possible and is supported by the work of Klopper and Macnaughton (1959) who isolated pregnanediol from the faeces in late pregnancy. This pregnanediol was in the free form and these workers suggested that the alimentary excretion of pregnanediol might depend on hydrolysis of its conjugate in the gut. They also suggested that the possible alimentary loss of a variable proportion of the pregnanediol production of the body might explain the considerable variation in urinary output of pregnanediol

as in the case cited.

An alteration in metabolism may be found in liver disease when disappearance of progesterone from blood plasma takes much longer (Patrini 1964), probably due to interference with the conjugation reaction of pregnanediol (Rogers 1956). Since the urinary pregnanediol excretion was low, the formation of progesterone was probably low and hence the increased requirement for exogenous progesterone which may be 'bound' to protein for transport and readily available, Hooker & Forbes (1940), or stored in the fat, Kaufman & Zander (1956) and Plotz and Davis (1957).

The first 24 hour urine specimen after the intravenous injection of progesterone contained most of the labelled pregnanediol but over the next 4 days measurable amounts were still present. These findings are similar to those of Rothchild (1953), Harkness & Fotherby (1963), Davis & Plotz (1958) who showed that after a sharp rise there was a levelling off in the cumulative pregnanediol recovered 48 hr. after intravenous injection. Van de Wiele et al (1960) reported that most of the pregnanediol was excreted in the first

12 hours with some throughout the next 36 hr. and Quilligan & Rothchild (1957) found that the majority was excreted as early as 4 - 12 hr. showing a very rapid metabolism to pregnanediol glucosiduronate.

Sommerville & Marrian (1950) detected a 'priming effect' i.e. a stepwise rise in excretion of pregnanediol following administration of progesterone, which required the presence of a uterus. However, the results of Rothchild (1953) gave no indication of this priming effect in the post menopausal female and this was later confirmed by Marrian et al (1954) and Klopper & Michie (1956). In the present study only the first 5 days urine were collected and therefore this effect was not studied.

The results discussed in this chapter are generally rather inconclusive and a much larger series of cases would be required to obtain more concrete results. The change in conversion of most interest is perhaps that obtained in hydatidiform mole. It was decided to examine this situation further and the results of this investigation are reported in Chapter 6.

## CHAPTER 5.

## Progesterone metabolism in the human previable foetus

When the metabolic pathways to and origins of pregnanediol were being discussed earlier in this thesis allusion was made to the foetal metabolism of progesterone, and foetal production of pregnanediol as a possible factor in altering pregnanediol excretion in the mother. This chapter describes work undertaken to ascertain the role of the foetus in progesterone metabolism and the foetal production of pregnanediol.

### Introduction

There is good evidence that the foetus receives progesterone from the placenta and metabolises this hormone. It had formerly been thought that the foetus itself was a source of progesterone when Forbes (1955) found that the level of the hormone in the umbilical artery was higher than in the umbilical vein. However, the method used (Hooker and Forbes 1947) was non-specific and it was later shown that 20α-dihydro-progesterone (20α-hydroxypregn-4-en-3-one) had a higher potency in this test than progesterone (Zander et al 1958).

Runnebaum and Zander (1962) showed that the level of progesterone in the umbilical arteries was considerably lower than that in the umbilical vein, whereas the concentrations of 17α-hydroxyprogesterone, 20α and 20β-dihydroprogesterone were higher in the arteries. This was confirmed by Greig et al (1962) who showed that the ratio of progesterone in the arterial to that in the venous umbilical vessels was 1:1.8 and this finding was substantiated by Van der Molen (1963) and Harbet et al (1964).

In view of the differences in progesterone levels in the umbilical vessels (see Fig. 6) it seemed therefore likely that the foetus metabolised some of the progesterone it received from the placental circulation.

Solomon et al (1965) perfused foetuses of gestational age 17 - 21 weeks with progesterone-4-$^{14}$C and isolated pregnanediol from the liver. This compound contained approximately 25% of the radioactivity perfused into the foetus. It was concluded from this and later work, by the same group (Bird et al 1965), showing the pregnanediol was in the form of the sulphate as well as the glucosiduronate, that the function of

the foetal liver was to produce reduced metabolites while the adrenal gland of the foetus at mid term utilized circulating progesterone for the production of corticosteroids.

## Present Study

### Materials and Methods

Several experiments were performed. The results of two, indicating the short and long term metabolism of progesterone are described.

1). A foetus of 16 weeks gestational age was obtained at therapeutic termination of pregnancy. A formalin soaked wad of cotton wool was placed over the umbilical cord to prevent spasm of the vessels during cannulation. [4-$^{14}$C] Progesterone (2μc) dissolved in two drops of alcohol and 1ml. of 0.9% Sodium Chloride solution was injected through the umbilical vein. The heart stopped 14 minutes after the injection. The foetus was dissected at once, the organs removed, weighed and stored at -20$^{0}$ until extracted.

2). A foetus of 18 weeks gestational age was obtained at therapeutic abortion as in experiment 1. Group O, Rh positive blood diluted 3:1 (v/v) with 5% dextrose in Ringer solution and oxygenated by bubbling oxygen through it, was used to perfuse the foetus by a modification of the method of Westin et al (1956). The composition of the perfusion fluid (pH 6.6) was: glucose, 400mg/100ml; Oxygen 95% saturation; $Cl^-$ 89m-equiv/l. and $CO_2$ content, 10.6m-equiv/l. After cannulation of the umbilical vessels the foetus was immersed in 5% dextrose in Ringer solution in a perfusion chamber completely filled with solution. In this way pressure changes due to increase or decrease in blood volume could be detected on a manometer. The blood dripped into the umbilical vein at a rate of 5 - 20 drops/min. [4-$^{14}$C] Progesterone (10μc) was injected and the arterial outflow collected for 3 x 15 min. periods. The blood was centrifuged and stored as in experiment 1. The accompanying diagram, Fig. 7, illustrates the appearance of the apparatus used in the perfusions.

The identification of radioactive metabolites and extraction procedures used have already been published (Greig and Macnaughton 1967) and will not be described here in detail. A flow sheet is appended, Fig. 8 which shows the outline of the procedures used.

## Results

### Radioactivity in foetal tissues 14 min. after injection of [4-$^{14}$C] Progesterone

After 14 min. 40% of the radioactivity was present in the liver and 3.4% in the adrenals. In both the liver and adrenals the radioactive material extracted was mainly 'free' steroid, 91% & 82.5% respectively. After hydrolysis with the enzyme preparation from Patella Vulgata, which contains mainly B-glucuronidase, 8.5% of the conjugated material in the liver and 65% of that in the adrenal was extractable.

The identification techniques showed that the radioactivity was present mainly as 20α-dihydro-progesterone in the liver, whereas almost half of that in the adrenals was present in the form of polar material, (Table 45). A similar distribution of

compounds was found in the 'conjugated' fractions (see Table 46); the principal conjugated steroid in the liver was pregnanediol whereas the adrenals contained mainly polar compounds. Table 47 compares the percentage of free and conjugated steroids in the liver and adrenals 14 min. after injection of [4-$^{14}$C] progesterone.

Radioactivity present in foetal tissues after perfusion Radiwith [4-$^{14}$C] progesterone for 45 min.

with [4-$^{14}$C] progesterone for 45 min.

The total radioactivity recovered from the foetus was 15.3%.

The compounds detected in the free fraction are shown in Table 48. The main compound in the liver was pregnanediol and this was confirmed after recrystallisation of the diacetate to constant specific activity. The Table shows that in many cases, progesterone was still the major 'free' steroid, particularly in the blood, where it accounted for 66.5% of the radioactivity in the first 15 min. sample and 51% in the 30 - 45 min. period. Pregnanediol was found both in intestine and liver and a little radioactivity was present as progesterone in the liver. In the plasma samples,

as the amounts of progesterone and polar material decreased the amount of 20α-dihydroprogesterone, the principal initial metabolite of progesterone, rose. In the liver and plasma, the polar fraction separated into several compounds one of which was probably 16α hydroxyprogesterone and another probably 6β hydroxyprogesterone.

Therefore the main 'free' steroid in the liver in the first experiment was 20α-dihydroprogesterone and in the second experiment pregnanediol - see Tables 45 & 46 In the adrenals most of the radioactivity was present as polar material in both experiments.

## Discussion

These experiments show that a large proportion of administered progesterone was metabolised in the foetal liver and this accords with the results of other workers (Solomon et al 1967). Liver deactivation of progesterone has been reported *in vivo* (Forbes & Hooker 1949) although not *in vitro*, Engel (1944). It is not really surprising that the liver is one of the main sites of metabolism in the foetus since, in the adult a large proportion of the metabolites of progesterone have been

detected in the bile (Sandberg & Slaunwhite 1958; Weist et al 1958). Zander (1961) also injected 4-$^{14}$C progesterone into the umbilical vein at termination of pregnancy and found that the majority of radioactivity was present in the liver.

In both foetuses investigated the percentage of conjugated steroids was much less than that of free steroids (liver expt. 1, 91% free; expt. 2, 93.5% free). In experiment 1, of the conjugated material in the liver and adrenals 80% and 74% was hydrolysed by the enzyme preparations used. In experiment 2, all the radioactivity was released by enzymic hydrolysis except for small amounts in the liver. Enzyme hydrolysis was performed using Patella Vulgata. Since this enzyme contains greater β-glucuronidase activity than sulphatase activity (Leon et al 1960) it suggests that the conjugates were principally glucosiduronates particularly in the case of the liver. This seems reasonable since conjugated pregnanediol in the urine is composed almost entirely of pregnanediol glucosiduronate (Crepy et al 1962) although Zander (1964) has found principally sulphate conjugation. Solomon et al (1967) isolated both pregnanediol glucosiduronate and the sulphate from

the foetal liver after perfusion with labelled progesterone. The liver has been known to have a principal role in progesterone metabolism. In the rabbit it was shown to have a major role in the deactivation of progesterone (Masson & Hoffman 1945). In women, it has been shown that when liver disease was present, the disappearance of added progesterone from the plasma took much longer and this was attributed to slower intrahepatic metabolism of the liver (Patrini, 1964). In the liver of the foetal and newborn guinea pig Pulkinen et al (1961) found that half the progesterone was metabolised and about one tenth could be identified as a pregnanediol-type metabolite.

In the present study, 14 minutes after injection of labelled progesterone the principal steroid identified was 20α-dihydroprogesterone (36.5%) with approximately equal amounts of polar compounds, pregnanediol, 20β-dihydroprogesterone and unchanged progesterone. A small amount (3%) of pregnanolone was also detected.

In the second experiment 45 minutes after injection of the progesterone 69% was present as pregnanediol and almost equal amounts of polar compounds and progesterone.

The presence of 20α-dihydroprogesterone and other 3α, 20α diols was expected after the finding by Villee and Loring (1963) of the presence of 20α hydroxy-dehydrogenase and 3α hydroxy-dehydrogenase in the human foetal liver. They had found 20α-dihydroprogesterone and pregnanediol as products of progesterone; a finding supported by Zander (1961). The latter, however, also detected 6β hydroxyprogesterone, 17α hydroxyprogesterone and 20β dihydroprogesterone as well as a large amount (26%) of polar steroids. Solomon et al (1965) detected progesterone, pregnanolone, 20α-dihydroprogesterone and chiefly pregnanediol present in the liver. There was no radioactivity in the 17α hydroxyprogesterone area in the present study or in that of Solomon et al (1965) but since it was detected by Zander (1961) 1 min. after injection it may be produced in the very early stages of the metabolism of progesterone.

In the adrenals there seemed to be more active metabolism and conjugation of the radioactive progesterone. This view is supported by the results of Solomon et al (1967). After 14 min., 47% of the free radioactivity extracted was polar material although pregnanediol,

20α-dihydroprogesterone and unchanged progesterone were also present with a small amount (6.7%) of 20β-dihydroprogesterone. In experiment 2, after 45 min. 60% was present in the polar steroid fraction with the rest of the radioactivity as unchanged progesterone. Solomon et al (1958) after incubating human foetal adrenals with progesterone, isolated 17α-hydroxy-progesterone and androstenedione, and Weliky and Engel (1963) found 16α-hydroxyprogesterone on incubation of human hyperplastic adrenocortical slices with progesterone. In a study of the metabolism of progesterone by foetal testes in vitro, Acevedo et al (1963), detected 16α-hydroxyprogesterone, 17α-hydroxy-progesterone, 20α and 20β dihydroprogesterone and deoxycorticosterone.

It is therefore evident that extensive metabolism of progesterone occurs in the foetus. In the liver reduction products form the main compounds and pregnane-diol is a major metabolite in this situation. In the adrenal glands polar compounds are formed and these have been shown (Solomon et al 1967) to be mainly corticoids. The foetus therefore uses the progesterone it obtains from the placenta to manufacture its own corticosteroids.

Since pregnanediol has been shown to be present in liquor amnii (Klopper & Macnaughton 1959) it seems very likely that this comes from foetal urine and is therefore a result of the foetal metabolism of progesterone.

It is probable that foetal pregnanediol passes to the maternal circulation either across the amniotic sac or via the placental circulation so that a proportion of the pregnanediol excreted in the maternal urine originates in the foetus. It is not known what proportion of maternal pregnanediol this foetal component forms. In early pregnancy it must be very small since there is little change when foetal death occurs at abortion unlike oestriol excretion which falls very steeply.

Some information, however, is given on this aspect by Klopper et al (1960) who found that, when saline abortion was performed, pregnanediol output did not fall greatly. In seven patients the average only fell to 78% of the pre-injection levels with a range of 44 - 105%. Two patients in fact showed no fall in average pregnanediol output at all during the injection-abortion period.

Changes in pregnanediol excretion are not easy to interpret. It may be that the foetus is responsible for some 20% of the pregnanediol which appears in the mothers urine. The fact that oestriol output falls much more steeply than pregnanediol output in the first 40 hr. after saline abortion (Klopper et al 1966) makes it unlikely that foetal metabolism of precursors is as important a step in the production of pregnanediol in the mothers urine as it is with the production of oestriol.

It seems therefore that the foetal metabolism of progesterone and the foetal production of pregnanediol may be relatively unimportant as far as the maternal excretion of pregnanediol is concerned. The changes in conversion of progesterone to pregnanediol discussed in Chapter 4 are probably of more importance. It was found in these investigations that particularly low conversions accompanied the presence of a hydatidiform mole and it was decided to look at steroidogenesis in a case of hydatidiform mole in detail. Some observations on this subject are reported and discussed in the next chapter.

# CHAPTER 6.

## Steroid Studies in a case of hydatidiform mole

It has been already shown in Chapter 4, that, in certain abnormal pregnancies, of which one variety is hydatidiform mole, the conversion of progesterone to pregnanediol is lower than in normal pregnancy. The question then arises as to what other pathways of metabolism could be occurring in these women. Stitch et al (1966) observed a considerable increase in the production of urinary pregnanetriol in a case of hydatidiform mole suggesting that an alternative pathway might be favoured in this type of case.
It was therefore decided to investigate this problem by -

(1) determining the urinary steroids in hydatidiform mole and a suitable case of molar pregnancy was used for this purpose,

(2) investigating the steroids present in mole tissue and theca lutein cyst fluid,

(3) incubation of a homogenate of the mole tissue with [4-$^{14}$C] pregnenolone, to determine its steroidogenic capacity.

Clinical History:

A woman 4 months pregnant in her second pregnancy was admitted to hospital with persistent vaginal bleeding. At examination, the uterus was found to be considerably larger than expected for the gestation period. A Hogben test was performed and found to be positive in a dilution of 1/200. A diagnosis of hydatidiform mole was made and laparotomy performed. At this operation a typical hydatidiform mole was evacuated from the uterus; bilateral ovarian luteal cysts were found and 200 ml. of cyst fluid was aspirated and stored at $-20^{0}$ for later examination.

Histological examination of the mole confirmed the diagnosis and examination of a biopsy specimen of ovarian tissue showed that the cysts were of the theca lutein type.

Determination of Urinary Steroids

Determination of Urinary Steroids

The urinary levels in mg/24hr. of pregnanediol and pregnanetriol were determined by the methods of Klopper et al (1955) and Stern (1957).

Injection of Radioactivity

Conversion of injected progesterone to pregnanediol and pregnanetriol was measured by using the technique described earlier in Chapter 4. Fifty microcuries of [7α-$^{3}$H] progesterone were injected into the anteecubital vein of the mother and the steroids were isolated from the maternal urine for 3 days after the injection. The tritium content of these fractions was then determined. These determinations were made before laparotomy and evacuation of the uterus and after a provisional diagnosis of molar pregnancy had been made.

Determination of steroids in mole tissue and ovarian cyst fluid.

Fifteen grams of mole tissue were incubated with [4-$^{14}$C] pregnenolone as precursor. Steroids were extracted from the incubation mixture the remainder of the mole tissue and the ovarian cyst fluid once with ethanol and a further three times with 80% ethanol; the combined extracts were then evaporated to dryness.

The dried extracts were partitioned between ether and water and the ether fraction from the extracts was examined for neutral steroids. Preliminary fractionation

was performed by column adsorption and column partition chromatography and zones of individual steroids were isolated by thin layer chromatography. Spots on thin layer chromatograms were identified by spray colour reagents, ultra-violet absorption and gas liquid chromatography after elution.

The individual metabolites from each of the extracts were chromatographed until radio-chemically pure and characterised by isotope dilution or reverse isotope dilution. All metabolites and derivatives were crystallised to constant specific activity through at least three crystallisation steps. Where no high specific activity radioactive standards were available identification of steroids isolated from the mole tissue and cyst fluid was performed using some or all of the following criteria: chromatographic mobility, derivative formation, ultraviolet absorption, colour reactions (Zimmerman chromogens, sulphuric acid chromogens), and gas liquid chromatography. Quantitive estimations of the amounts of each material isolated from the mole tissue and cyst fluid were made on the basis of crystalline weights, isotope dilution studies, colour chromogens and ultraviolet absorption and are

expressed as µg/100ml. (cyst fluid) and µg/100g wet wt. (mole tissue). Radioactive zones on thin-layer plates were located using a Nuclear-Chicago Actigraph III Strip Scanner with a thin layer attachment. Samples for quantitation of radioactivity were counted in benzene solution in a Nuclear-Chicago Unilux Mark I Liquid Scintillation Spectrometer in a scintillation fluid containing 5g. 2,5-diphenyloxazole (PPO) and 50mg of 2,2-paraphenyl bis 5-phenyloxazole (POPOP)/litre toluene.

The above is a summary of the main steps of the methodology involved and the full details of the extraction procedures, isolation and characterisation of steroids, quantitative determination of isolated steroids and determination of radioactivity are given in the paper by Coutts et al (1969).

Urinary pregnanetriol excretion

The urinary excretion of pregnanetriol, the excretion product of 17α-hydroxyprogesterone is only slightly elevated in normal pregnancy (Fotherby et al 1965) measured from 11 weeks until term. The whole subject of pregnanetriol excretion in pregnancy is discussed fully in Chapter 7.

## Results

The levels of urinary pregnanediol and pregnanetriol were 16.5 and 10.7mg/24hr. when determined by colorimetric methods; 6.43 and 2.84% conversions respectively from injected [7α-$^{3}$H] progesterone were found.

By both methods the ratio pregnanediol:pregnanetriol in this subject was approximately 2:1 whereas that in normal pregnancy is 20:1 (Harkness & Love 1966). Since the pregnanediol levels are within the normal range (Coyle et al 1956) this ratio signifies a greater excretion of pregnanetriol than occurs normally, indicating increased secretion of 17-hydroxylated steroids.

Table 49 shows the metabolites isolated after incubation of [4-$^{14}$C] pregnenolone with mole tissue. As well as unchanged precursor, 17α hydroxypregnenolone, progesterone, 16α hydroxyprogesterone and 16β hydroxy-progesterone were isolated.

Table 50 shows concentrations of steroids and steroid precursors found in the extracts of mole tissue and cyst fluid.

Cholesterol, pregnenolone, 17α hydroxypregnenolone,

pregnanediol, pregnanetriol and androstenedione were isolated from both extracts. Progesterone and its 17α hydroxylated derivative were isolated from cyst fluid but not from the mole tissue in measureable amounts.

Detectable quantities of oestrogens were not observed in either of the extracts but colour reactions suggested the presence of traces of them in the cyst fluid extract. The maternal urinary excretion of oestriol was low (250μg/24hr.) in this patient before laparotomy determined by the method of Brown & Coyle (1963).

## Discussion

In a case of hydatidiform mole accompanied by ovarian theca lutein cysts a greatly increased urinary excretion of pregnanetriol was observed. Stitch et al (1966) made the same observation in a similar case and characterised this pregnanetriol as 5β-pregnane-3α,17α, 20α-triol, the excretion product of 17α hydroxyprogesterone.

### Incubation of mole tissue

On incubation of the mole tissue with [4-$^{14}$C] pregnenolone under conditions suitable for steroid-

ogenesis 17α hydroxypregnenolone, progesterone, 16α hydroxyprogesterone and 16β hydroxyprogesterone were formed. Although a search was made for 17α hydroxy-progesterone, none was found. It would appear however, that since mole tissue was able to form progesterone and 17α hydroxypregnenolone it should have the capacity for synthesising 17α hydroxyprogesterone. Perhaps on production 17α hydroxyprogesterone is metabolised at a similar rate to its synthesis thus preventing isolation.

It therefore seems evident from the lack of accumulation of 17α hydroxyprogesterone that the increased excretion of pregnanetriol is unlikely to be a function of the mole tissue.

16α hydroxylation is an important step in the biosynthetic pathway to oestriol. Current views on the foeto-placental steroid relationships at mid term (Solomon et al 1967) indicate that 16α-hydroxylation is a function of the foetal liver and adrenal and that this hydroxylated progesterone is then transported to the placenta. However, this abnormal placenta is capable of forming the precursor of oestriol. This hydroxylation may only occur in the abnormal tissue because larger

quantities of precursor than normal are being formed or possibly as an alternative to normal pathways which are absent - no 20-dihydroprogesterones were formed.

The results of this incubation shows that hydatidiform mole tissue is active in steroidogenesis; it is able to form progesterone and to perform hydroxylations of steroids.

Steroids of the mole tissue

Levitz et al (1962) showed that the human placenta could not form the steroid nucleus from acetate but depended upon preformed precursors for the synthesis of progesterone. In the placenta both cholesterol (Solomon 1960) and pregnenolone (Palmer et al 1966) can act as progesterone precursors. It is postulated that these substances are transferred to the placenta in the maternal blood and are retained by that organ for synthesis of progesterone and related hormones. From the levels of cholesterol and pregnenolone found in maternal blood, cholesterol would appear to be the preferred precursor. In this case the synthesis of progesterone will proceed by way of pregnenolone (Solomon 1960).

As well as forming progesterone the placenta has been shown by studies *in vitro*, to contain enzyme systems capable of metabolising progesterone to 20α-dihydroprogesterone and 17α-hydroxyprogesterone (Little et al 1959; Little & Shaw 1961), 16α-hydroxy-progesterone and androstenedione (Little et al 1963; Warren & Cheatum 1964) and 6β-hydroxyprogesterone (Berliner & Salhanick 1956).

From the abnormal trophoblastic mole tissue cholesterol, pregnenolone, 17α hydroxypregnenolone, pregnanediol, pregnanetriol, androstenedione and a trace of progesterone were isolated. Although Pearlman and Cereco (1952b) observed that successful extraction of progesterone from placental tissue required a high pH, it is not felt that poor extraction was responsible for the low level of progesterone found, since Greig and Macnaughton (1967) - see chapter 5 - used a similar extraction method to that used here and also because progesterone was isolated from the ovarian cyst fluid by the same methods. Failure to extract a substance from a tissue does not necessarily indicate lack of synthesis of it but in the light of the amounts of progesterone isolated from placental tissue in previous

studies (Chamberlain et al 1968a) it is concluded that in this hydatidiform mole there was a decreased synthesis of placental progesterone. This might reflect a lowered placental function or could mean that part of the placenta had regressed or separated from the uterine wall and that only the remainder was capable of producing steroids. The lowered progesterone production must be equated with the normal pregnanediol levels found in this patient and this will be discussed later. 20α-Dihydroprogesterone was not found in the mole tissue and neither was 17α-hydroxyprogesterone although 17α-hydroxypregnenolone was identified. Pregnanediol and pregnanetriol, the saturated reduction products of progesterone and 17α-hydroxyprogesterone respectively, were both identified. Pregnanediol is a normal constituent of placental tissue (Pearlman and Cerceo 1952a) and the presence of pregnanetriol was not surprising since the patient excreted large quantities of this substance in her urine.

It is concluded from these studies that the mole tissue was capable of steroidogenesis but there was less synthesis of progesterone than is normal; there

was also an accompanying lack of the normal hydroxylated derivatives of progesterone. Chamberlain et al (1968b) using gas-liquid chromatography have studied extracts from three separate moles. Although these workers found more progesterone than observed in this study they also concluded that the abnormal tissue produced less progesterone than normal. No 17α-hydroxyprogesterone was extracted from the mole tissue and only a small amount of 17α-hydroxypregnenolone. Although a small amount of pregnanetriol was isolated these findings lend support to the conclusion drawn from the incubation experiments that the increased urinary excretion of pregnanetriol in this case of hydatidiform mole did not originate from the mole tissue.

Steroids in ovarian cyst fluid

Cholesterol, pregnenolone, 17α-hydroxypregnenolone, progesterone, 17α-hydroxyprogesterone, pregnanediol, pregnanetriol and androstenedione were isolated from the ovarian cyst fluid. The compounds isolated from the cyst fluid were the same as those found in the mole tissue with the addition of measureable amounts of

progesterone and 17α-hydroxyprogesterone. Apart from the precursors cholesterol and pregnenolone, all other substances isolated were found in much larger quantities than in the mole tissue, (Table 50). These results suggest that the increase in 17-hydroxylation which was signified by an increased urinary excretion of pregnanetriol, is ovarian in origin. This conclusion would be in agreement with the findings of Stitch et al (1966) who observed that pregnanetriol excretion remained high after evacuation of the uterus until regression of the cysts.

In hydatidiform mole the trophoblast produces large quantities of human chorionic gonadotrophin (H.C.G.) - the Hogben test was positive in a dilution of 1:200 - and it is probably as a result of over-stimulation by this hormone that the ovaries became cystic. These ovaries were stimulated to produce 17α-hydroxyprogesterone. Evidence for the secretion of a pregnanetriol precursor by the ovary is given by Fotherby (1962) and this will be discussed in the next chapter when the ovarian production of hormones is considered following stimulation with human menopausal gonadotrophin.

Cholesterol from the maternal blood or ovary is probably used as the precursor of 17-hydroxy steroids and overstimulation would result in increased production of all the intermediates in this pathway - pregnenolone, 17α-hydroxypregnenolone and progesterone - as well as the excretory product, pregnanetriol. - Fig. 9.

The polycystic ovaries produced small quantities of androstenedione but no trace of testosterone was found in the cyst fluid. The fact that the ovaries are not stimulated to produce oestriol is in agreement with the findings of Frandsen (1965) and the lowered urinary excretion of oestriol frequently found in many cases of hydatidiform mole, also observed in this case and described in some detail in chapter 3. This is a further factor which supports the hypothesis that the presence of foetal precursors of oestriol are necessary for the increased production of oestriol in pregnancy.

The normal pregnanediol levels found in such a case may be misleading since they may reflect normal placental function, or may indicate lowered placental function at the same time as an increased ovarian production of progesterone which may explain the

findings reported in chapter 3, where the pregnanediol levels in cases of hydatidiform mole vary from low to normal.

## Conclusions

From these investigations the following conclusions may be drawn (1) there was an increased urinary excretion of pregnanetriol (2) the mole tissue was active in steroid metabolism producing 17α-hydroxy-pregnenolone, progesterone, 16α-hydroxyprogesterone, and 16β-hydroxyprogesterone from pregnenolone, but apparently less progesterone than a normal placenta. The increased urinary excretion of pregnanetriol is not a function of the mole tissue (3) the high urinary level of pregnanetriol is probably of ovarian origin (possibly as a result of overstimulation by the high levels of H.C.G. present).

The effect on the ovary of the high levels of H.C.G. produced by the molar tissue is of particular interest. In the therapy of some amenorrhoeic women combinations of follicle stimulating hormone and H.C.G. are now in regular use for the induction of ovulation. One of the most troublesome complications of this

therapy is hyperstimulation of the ovary. The syndrome, as far as the ovary is concerned, is analogous to that produced by the H.C.G. from a hydatidiform mole. The results of this molar investigation are now applied, in the next chapter, to women being treated with human menopausal gonadotrophin and their relevance to the measurement of corpus luteum function in these women is discussed.

# CHAPTER 7.

## Urinary Steroid excretion after Gonadotrophin Therapy

In the previous chapter it was suggested that the high level of pregnanetriol in the urine of women with hydatidiform mole has its origin in the ovary. It is produced in the theca lutein cysts of the ovary which are caused by the large quantities of H.C.G. present in the circulation of these women and does not result from metabolism occurring in the trophoblast itself. These findings are relevant to early pregnancy induced by human menopausal or pituitary gonadotrophins where hyperstimulation of the ovary frequently occurs, due to excessive dosage of gonadotrophin. This is a very similar situation to that found in the hyperstimulated ovaries in hydatidiform mole.

The information obtained in the previous chapter has been applied to the study of a small number of women in whom ovulation was induced by human menopausal gonadotrophin and this work forms the basis of this

chapter. These results are as yet rather preliminary but are of considerable interest and it is felt they are worthwhile discussing in this thesis. They both help to round off the work reported and at the same time pose a number of problems for future work. To put the position of pregnanetriol into perspective some aspects of steroidogenesis in the human ovary will first be discussed.

Steroidogenesis in the human ovary

The human ovary is capable of synthesising cholesterol from acetate probably by the same route as that established for hepatic cholesterol synthesis (Popjak and Cornforth 1960). Pregnenolone is formed by splitting off the side chain and progesterone is formed from pregnenolone by removal of 2 hydrogen atoms and the shift of the double bond from the delta 5 to the delta 4 position. The steps of the biosynthetic pathway to oestrogens are shown in the diagram (Fig. 9 ). The relative importance of the several alternative routes from pregnenolone to oestradiol are not known in any detail. There is some evidence that in the follicle, oestradiol may be formed by a pathway not

involving progesterone, whereas in the corpus luteum a pathway involving progesterone may predominate (Ryan and Petro 1966).

Oestriol: As has been said earlier in this thesis oestriol is present in large quantities in human urine and if oestradiol is given to a woman a considerable proportion of this material is excreted in the urine as oestriol. The conversion of oestradiol into oestriol occurs in the maternal liver and oestriol has usually been considered to be a catabolic product of oestrone or oestradiol. It has also been thought to be a detoxication product because its oestrogenicity is lower and it is more soluble in water than the other two classical oestrogens. There is, however, evidence that the ovary does secrete oestriol, especially during the luteal phase of the cycle (Barlow and Logan 1966) and the ovary has been shown to contain a 16α hydroxylase. Oestriol may therefore be considered an ovarian oestrogen in its own right. It is not yet clear whether, as in the liver this ovarian oestriol is formed from oestrone and oestradiol or whether, as in the placenta from 16α hydroxy dehydroepiandrosterone.

Control of ovarian biogenesis

The pituitary gland secretes trophic hormones which stimulate the endocrine glands to produce their respective hormones. Follicle stimulating hormone (F.S.H.) stimulates follicular growth and results in increased oestrogen excretion. In this sense F.S.H. stimulates oestrogen production but it is not clear that it has any specific effect on steroidogenesis. At the menopause, for example, high levels of F.S.H. are associated with low oestrogen levels because of the absence of growing follicles.

In vitro preparations of F.S.H. have been reported to stimulate the rate of conversion of cholesterol to pregnenolone in non pregnant bovine corpora lutea (Ichii et al 1963), but these results could not be reproduced (Yago et al 1967). It is also impossible to obtain pure F.S.H. i.e. not containing a proportion of Luteinising hormone (LH) and it cannot therefore be said that any action due to this F.S.H. is due to this material alone since it might be due to the LH contamination.

In contrast there is considerable evidence that

L.H. can specifically influence steroidogenesis (Savard et al 1965; Channing and Short 1966). It has the effect of increasing both the rate of conversion of cholesterol into progesterone and the de-novo production of the latter.

In early pregnancy, it may be a specific action of chorionic gonadotrophin on steroidogenesis that maintains production of progesterone and oestrogen by the corpus luteum. The L.H. peak in the menstrual cycle (see Fig.10) is of short duration and occurs near the time of ovulation and not in the luteal phase of the cycle (Midgley and Jaffe 1966). It appears to be associated with ovulation and possibly with corpus luteum formation but does not appear to maintain steroid secretion during the luteal phase. There does not seem to be a sharp decline in L.H. associated with the decline of the luteal steroidogenesis and it is difficult to explain how rising levels of chorionic gonadotrophin continue the life span and steroid secretion of the corpus luteum when conception occurs. The presence of a uterine luteolytic factor, for which there is evidence in several animal species (Short 1966)

does not seem to obtain in the human and removal of the uterus appears to prolong the life of the corpus luteum by only a few days (Andreoli 1965).

The effect of gonadotrophins on ovarian cellular metabolism in vivo is almost completely unknown although the resultant steroid excretion is well documented, e.g. Loraine and Bell (1968), at any rate as far as oestrogen and pregnanediol are concerned. Where other steroids and particularly pregnanetriol are concerned evidence is much more scanty.

The other steroid of particular interest is pregnanetriol (5β-pregnane-3α,17α,20α-triol) and its origin and excretion in the menstrual cycle and in pregnancy will now be discussed.

Pregnanetriol (5β-pregnane-3α,17α,20α-triol).

In the biosynthesis of the steroid hormones 17α hydroxyprogesterone is an important intermediate (Samuels 1960). The principal urinary metabolite of 17α hydroxyprogesterone is pregnanetriol (Axelrod and Goldzieher 1960; Fukushima et al 1961).

There are three possible precursors of pregnanetriol namely, 11-deoxycortisol, 17α hydroxypregnenolone, and 17α hydroxyprogesterone. Fotherby and Love (1960) studied the conversion of these precursors to pregnanetriol and found that by far the greatest conversion (35%) occurred when 17α hydroxyprogesterone was injected. These workers concluded that pregnanetriol was therefore the main metabolite of 17α hydroxyprogesterone.

The ovarian production of a pregnanetriol precursor

It has been suggested (Brown 1956) that ovulation occurs at about the time that the excretion of oestrone and oestradiol reached a maximum towards the end of the follicular phase of the menstrual cycle. The data of Fotherby (1962) showed that there was a significant rise in pregnanetriol excretion on the day that the excretion of oestrone and oestradiol reached a peak. The magnitude of the rise suggests that the precursor responsible in the second half of the menstrual cycle was 17α hydroxyprogesterone since oestrogen levels are lower at this time. 17α Hydroxyprogesterone is known to be secreted by the adrenal cortex and the majority

of pregnanetriol in the urine may arise from this source. From the day of ovulation this adrenal pregnanetriol is supplemented by some arising from precursors secreted by the ovary.

Although the evidence (Fotherby 1962) suggests that the increase in pregnanetriol excretion in the menstrual cycle (Fig. 11) is due to an increased secretion of precursor from the ovary it could possibly be due to increased adrenal production at this time. However, that the ovary is responsible, is supported by the fact that the biosynthesis of oestrogens from cholesterol involves 17α hydroxyprogesterone as an intermediate (see Fig. 9 ) (Ryan and Smith 1965) and this steroid has been identified in extracts of human ovaries (Zander 1958) and in human follicular fluid (Short and London 1961). Further support for the ovarian secretion of a pregnanetriol precursor is provided by Fotherby (1962) who administered norethisterone to normally menstruating women and found that the rise of pregnanetriol excretion in the second half of the cycle did not occur. Since the second oestrogen peak and the pregnanediol peak did not

occur in these women, Fotherby (1962) concluded that the decrease in the excretion of pregnanetriol during the administration of the synthetic steroids was due to a suppression of precursors from the ovary which are metabolised to pregnanetriol viz 17α hydroxyprogesterone in the corpus luteum.

It would appear, therefore, that the excretion of pregnanetriol in the urine might give some estimate of the corpus luteum activity of the ovary and that this might be a valuable steroid to measure in the urine in early pregnancy.

Pregnanetriol excretion in human pregnancy

The most reliable estimates of urinary pregnanetriol output during normal pregnancy are probably those of Harkness and Love (1966). Previous methods i.e. Fotherby and Love (1960) were probably not specific for the determination of pregnanetriol in the urine of pregnant women (Fotherby et al 1965). These previous methods (Bongiovanni and Clayton 1954; Stern 1957) used a sulphuric acid colour reaction for estimating the steroid and there are compounds present in the urine of women during pregnancy that interfere with this colour

reaction. These interfering sulphuric acid chromogens in pregnancy urine increased with duration of the pregnancy and Fotherby et al (1965) found that at least some of the interfering compounds in pregnancy urine are polar metabolites of progesterone. In non-pregnant women and in early pregnancy the method of Fotherby and Love (1960) is suitable since the amount of interfering substances is minimal.

In their paper Harkness and Love (1966) measured urinary pregnanetriol excretion in the urine after the 22nd week of pregnancy and Fig. 12 is taken from their results. This shows an increase in the last trimester reaching a maximum at about 36 - 37th weeks of pregnancy. There were large variations between individual patients. The increase found by these workers was not so great as that described by Herrmann and Silverman (1953) and Ronan et al (1960). Harkness and Love (1966) also estimated pregnanetriol excretion during pregnancy in an adrenalectomised woman and found that the level also increased. They suggested that the foeto-placental unit is probably the source of the increased amounts of urinary pregnanetriol in the adrenalatomised pregnant

woman and presumably it is also the origin of some of the increase observed in normal pregnant women in the second and third trimesters. The occurrance of the rise in the last trimester of pregnancy suggests that the foetal component of the foeto-placental unit may be the more important source of the increase in urinary pregnanetriol. This suggestion is consistant with the scheme proposed by Diczfalusy (1964) for the steroid metabolism of the foeto-placental unit at mid pregnancy.

All the workers already referred to have measured the urinary excretion of pregnanetriol from mid pregnancy and there is no information as to what happens in very early pregnancy.

It is well known how difficult it is to obtain information at very early stages of human pregnancy but the advent of gonadotrophin therapy for stimulation of ovulation in amenorrhoeic women has meant that a number of women are under observation at the time of conception and during the very early stages of pregnancy. It is therefore possible to measure urinary steroids in these patients in the cycle in which pregnancy occurs and

observe the changes which take place in steroid excretion at this very early stage of pregnancy, when, in fact implantation and the growth of the blastocyst are taking place. It is at this time that the corpus luteum of pregnancy, in the human, is of some importance since after 35 days, it has been shown by Tulsky and Koff (1957) that human pregnancy can continue even if the corpus luteum has been removed.

Clinical Histories of Patients

Case No. 1. P.R. Age 34, Secondary amenorrhoea for 8 years.

The menarche occurred at the age of 14 years and the menstrual cycle was regular at first. At the age of 18 years it became less regular and finally stopped when the patient was 26 years old.

She was first seen at the age of 34 years and tested for suitability for gonadotrophin therapy. The details of this investigation are not pertinant to the aspect of the subject being discussed and will not be given here. She was judged to be suitable for the induction of ovulation with human menopausal gonadotrophin.

This patient became pregnant after treatment and abortion occurred at about 2-3 months of pregnancy. In a second pregnancy twins were conceived. This pregnancy proceeded to term when she was successfully delivered by Caesarean Section.

The results of steroid excretion assays during the aborted and twin pregnancy will be discussed.

Case No. 2. B. di F. Age 27 yrs. Secondary amenorrhoea for 4 years following the birth of her only child.

The menarche occurred at age 13 years and her menstrual cycle was regular until her first pregnancy occurred at age 23 years. The pregnancy was uneventful and she had a normal delivery at term with no complications. Menstruation did not return and after 4 years she was tested for suitability for gonadotrophin therapy and found to be suitable.

She was treated with human menopausal gonadotrophin in a number of cycles before a pregnancy occurred. In the first half of this pregnancy she had intermittent vaginal bleeding and rested in hospital for prolonged periods at this time. She remained well during the

middle trimester of her pregnancy and then had further bleeding and was admitted to hospital again for rest and supervision at 34 weeks. She was delivered successfully by Caesarean Section at 39 weeks of pregnancy. The results of steroid excretion in a cycle and during the early part of pregnancy will be discussed.

Case No. 3. S.F. Age 32 years. Secondary amenorrhoea for 7 years.

The menarche occurred at the age of 13 years and the menstrual cycle continued to be fairly regular until the age of 23 when the amount of bleeding became scanty and menstruation finally ceased altogether.

This patient was found to be suitable for gonadotrophin therapy and several cycles of treatment have been given.

The results of steroid excretion during these cycles will be discussed.

Case No. 4. C.H. Age 35 years. Nullipara.

This patient suffered from severe endometriosis and total hysterectomy and bilateral salpingo-

oopherectomy were performed. She kindly agreed to cooperate in an experiment to determine the effect of administered F.S.H. and L.H. to an oopherectomised woman. She was given 750 IU. F.S.H. plus 1000 IU. H.C.G. and her urinary steroids were measured for 14 days thereafter. The object of this experiment was to find out whether pregnanetriol excretion rose after gonadotrophin stimulation in the absence of the ovary i.e. whether some of the increase was due to the adrenal.

Assay methods: Urinary pregnanediol was measured by the method of Klopper et al (1955), and urinary oestriol by the method of Brown (1956) as modified by Brown et al (1957). Pregnanetriol was measured by the method of Fotherby and Love (1960) which is suitable for measuring this substance in the non-pregnant state and in early pregnancy where there is little interference with the sulphuric acid colour reaction.

Results:

Fig. 13 shows the pregnanediol excretion and the oestriol excretion in 48 hour urine specimens in Case

No. 1 in the pregnancy in which abortion occurred. The levels of both hormones rose to above non-pregnancy levels until bleeding occurred (B) after which time there was a gradual fall in both steroids, the 'Gravindex' pregnancy test became negative and abortion finally took place.

Fig. 14 shows the first 20 weeks of a twin pregnancy which resulted in a successful conclusion. In this pregnancy the excretion of oestriol and pregnanediol rose in the usual manner associated with early pregnancy and the hormone pattern in the urine remained normal till term.

Fig. 15 shows the excretion of pregnanediol, pregnanetriol and oestriol in a gonadotrophin stimulated cycle in Case No. 2. Only 1 oestrogen peak is present in this cycle and there are peaks of pregnanediol and pregnanetriol after ovulation thereafter the levels falling toward the end of the cycle.

Fig. 16 shows the excretion of these three steroids in the urine of Case No. 2, in the cycle in which pregnancy occurred and the excretion thereafter till 30 weeks gestation. There is a fall in oestriol

after 5-6 weeks of pregnancy but the level then begins to rise again to normal pregnancy levels. Pregnanediol excretion follows the same pattern with a fall to relatively low levels at about 12 weeks - it was during this time that bleeding occurred and there may have been deficient steroid biosynthesis in the trophoblastic tissue around this time accounting for the fall in the urinary excretion of steroid metabolites.

The excretion of pregnanetriol in this case also rose pare passu with pregnanediol up to 6 weeks of pregnancy and then fell to the levels usually reported for human pregnancy.

Figs. 17, 18 and 19 show the excretion of pregnanediol, pregnanetriol and oestriol in 3 consecutive cycles stimulated by gonadotrophin therapy in Case No. 3. In the 1st. and 3rd. cycles the response is more marked than in the 2nd. cycle and this confirms the variability of response by the same woman to the same dose of gonadotrophin in different cycles that has been commented upon by Crooke et al (1966) and others.

Fig. 20 shows the urinary excretion of the three hormones in the urine in Case No. 4, where the ovaries

were absent. There is a little rise in oestriol excretion but little change in the excretion of pregnanediol and pregnanetriol.

## Discussion

In Fig. 13 where abortion occurred the initial levels of oestriol and pregnanediol rose as would be expected in normal pregnancy. At about 60 days gestation there was some bleeding and thereafter the levels of hormone excretion fell. It seems probable that when bleeding occurred there was some disruption of the trophoblast which resulted in death and subsequent falling of the levels of urinary metabolites of the hormones manufactured by the trophoblastic tissue.

This case confirms the results discussed in Chapter 3, and shows that measurement of these two metabolites, viz, pregnanediol and oestriol, in the urine during pregnancy, is not likely to give an indication of impending abortion. This result would indicate that, at this stage of pregnancy when abortion occurred the ovarian contribution to the level of these

steroids in the maternal urine must be minimal. Diczfalusy and Borell (1961) measured the output of oestrogens and pregnanediol before and after removal of the ovary seventy eight days after the date of the last menstrual period. In the removed ovary these workers found a fully mature corpus luteum of pregnancy. Since the level of both the oestrogens and pregnanediol remained the same after oophorectomy - indeed that of pregnanediol actually rose after the operation - it was concluded that during the third month of pregnancy a histologically mature corpus luteum of pregnancy did not seem to be a significant source of urinary oestrogen and pregnanediol.

It may be true, and seems likely, that at even earlier stages of pregnancy the ovarian contribution may be significant but this would appear to be at very early stages only.

The pattern of excretion in the twin pregnancy is shown in Fig. 14. There is a steady rise in hormone level. The levels in this case are rather higher than those reported for twin pregnancy. Gemzell and Roos (1966) found the average excretion of pregnanediol to

be almost the same in those women who conceived a single infant or twins but was significantly higher only in those women conceiving triplets or more foetuses. On the other hand the average excretion of oestrogen has been found by these workers to be somewhat lower in those women who gave birth to a single infant than in those who gave birth to twins, triplets or more foetuses. The findings in the present patient would support this view. Unfortunately the levels of pregnanetriol were not available in this woman.

Pregnanetriol excretion in the gonadotrophin stimulated cycle

The origins and precursors of urinary pregnanetriol have already been discussed. Very little information is available in the literature about the excretion of pregnanetriol in the urine of women being treated with gonadotrophins although much has been written about oestrogens and pregnanediol (Loraine and Bell 1968). Loraine et al (1966) have reported a few cycles of pregnanetriol excretion in women who have been given clomiphene for induction of ovulation, and the pattern

in these cycles has been of the same order as that found in the normal cycle where stimulation has been by endogenous gonadotrophin.

Four gonadotrophin stimulated cycles are shown where the levels of pregnanetriol have been measured in the urine. Figs. 15, 17, 18 & 19. Three of these (17, 18 & 19) were successive cycles in the same woman and the fourth (15) was in a patient who became pregnant in a subsequent cycle.

The results show that in the gonadotrophin stimulated cycle the urinary excretion of pregnanetriol rises during follicle growth and is greatest just after ovulation i.e. between day 14 and 16 when the corpus luteum is most active.

As has been mentioned before the principle precursor of pregnanetriol is 17α-hydroxyprogesterone (Fotherby 1962). Strott and Lipsett (1968) have shown that there is a significant difference in plasma 17α-hydroxy-progesterone levels between women in the follicular and luteal phases of the menstrual cycle. These workers indicate that the higher 17α-hydroxyprogesterone levels

during the lutcal phase of the cycle suggest that the corpus luteum is the source of the 17α-hydroxy-progesterone. There is no doubt that this steroid is secreted by the ovary and Mikhail et al (1963) showed that the levels of 17α-hydroxyprogesterone in ovarian venous blood were 60 times those found in peripheral blood thereby proving ovarian secretion of 17α-hydroxyprogesterone. It is notable that in Case 4, where the ovaries are absent, there is no rise in the level of pregnanetriol following administration of F.S.H. and L.H. This gives further support to the ovarian origin of the increased amounts of this substance found in urine during the menstrual cycle.

The suggestion therefore is that the corpus luteum does in fact secrete moderate amounts of 17α-hydroxyprogesterone and this accords with the findings reported in Chapter 6, where this compound was detected in the theca lutein cyst fluid from a woman with hydatidiform mole. That the secretion is from the corpus luteum is further supported by the fact that in men, where the levels of 17α-hydroxyprogesterone are higher than those in the follicular phase of the

cycle in women, there is a substantial increase in urinary pregnanetriol following the administration of Human Chorionic Gonadotrophin to normal men (Jayle 1965; Landau and Lanes 1959). There appears to be a gradual decline in steroid synthesis in the corpus luteum during the luteal phase of the cycle since the level of both pregnanediol and pregnanetriol fall together. Other evidence that this is so is based on the progesterone content of the tissue, and the progesterone content of ovarian and peripheral venous blood (Zander et al, 1958; Mikhail et al, 1963). On the other hand recent work by Le Maire et al (1968) on measurement of progesterone synthesis in the corpus luteum failed to reflect the expected gradual decline through later phases of the menstrual cycle. These workers found that only the abrupt complete failure of corpora lutea, 14 days and over, to incorporate 14C acetate, accorded with previous observations of cessation of function of the corpus luteum at the end of the cycle. The reasons for this latter finding are not clear but an obvious shortcoming of <u>in vitro</u> studies of the type performed by Le Maire et al (1968) is the great variability in the synthetic capacities

of individual specimens from different patients, a factor which is absent from sequential studies conducted in the same patient. This of course is impossible to do in the human subject.

Pregnanetriol excretion in early gonadotrophin stimulated pregnancy

In the patients who became pregnant the levels of oestriol, pregnanetriol and pregnanediol rose together following ovarian stimulation by F.S.H. and H.C.G. The interesting features are that, the pregnanetriol level rose to over 10mg/24 hr., very much higher than any result previously reported in pregnancy. The level of all three hormones then fell and at this time the patient was resting in hospital with threatened abortion. The level of oestriol and pregnanediol thereafter rose to the usual figures found in pregnancy and the level of pregnanetriol remained at that reported for pregnancy by previous authors and discussed earlier.

The suggestion is therefore made that this high production of pregnanetriol at this very early stage of

pregnancy is a function of the corpus luteum of pregnancy and that the amount found does, in fact, give a measurement of this corpus luteum activity. A second patient (Case 3) has recently become pregnant and has shown the same findings in very early pregnancy.

It has been difficult to assess directly the function of the corpus luteum of pregnancy. Ovarian and peripheral venous progesterone levels have been measured at intervals throughout pregnancy (Mikhail and Allen 1967) and this data suggested that the corpus luteum was the important source of progesterone during the first 12 weeks of pregnancy. More recently Yoshima et al (1969) have measured plasma 17α-hydroxy-progesterone in women who became pregnant after ovulation was induced with gonadotrophin. Since the placenta has no or only very limited capacity for 17αhydroxylation (Palmer et al 1966; Jungmann and Schweppe 1967) measurement of plasma 17α-hydroxy-progesterone or its main urinary metabolite pregnanetriol should reflect corpus luteum function. The findings in the present case fit exactly those of Yoshima et al (1969). These workers found that the peak of plasma 17α-hydroxyprogesterone was between

4 and 8 weeks of gestation and by 12 weeks this compound had stabilised to a level which was maintained for the remainder of the pregnancy (Fig. 21). Plasma progesterone, on the other hand rose to a peak about 3 - 4 weeks after H.C.G. The level then fell reaching a nadir at six to eight weeks. This was followed by increasing levels, almost certainly due to placental production of progesterone.

Note on the functional life of the corpus luteum of pregnancy

The functional life of the corpus luteum of pregnancy in man has given rise to controversy over the years. Scattered reports indicate that its function may not be important to the continuation of pregnancy after 6 weeks, (Deansly 1966), but as has been suggested earlier in this chapter the work of Tulsky and Koff (1957) and Froewis (1963) indicate that abortions tend to occur after removal of the corpus luteum during the fifth to sixth week after conception whereas after the sixth week there were no spontaneous abortions. These data are consistent with an early important role for the corpus luteum.

It does not, however, help with respect to continuing corpus luteum function in pregnancy. Histological studies of the corpus luteum of pregnancy showed the persistence of granulosa lutein cells throughout pregnancy (Gillman et al 1941). These seemed to reach their greatest development between the second and third month and to disappear shortly thereafter, Nelson and Greene (1958) after extensive study concluded that the corpus luteum of pregnancy actively flourished and was functional during the first six weeks of pregnancy, deteriorated markedly from the 8th to the 16th week and was passively maintained from then on until the termination of the pregnancy. These workers concluded that the ovary during the larger portion of pregnancy appeared from a histological point of view, to act only as an end organ influenced by extrinsic substances probably of placental origin.

Elevated plasma 17α-hydroxyprogesterone levels were found in the luteal phase of the cycle (Yoshima et al 1969) and these workers found that the plasma 17α-hydroxyprogesterone level was a good index of

follicular development and luteal function. They suggested that they could examine one aspect of steroid production by the corpus luteum in the presence of a functional placenta. They estimated, from the divergence of curves of plasma progesterone and 17α-hydroxyprogesterone (Fig. 22), that the life of the corpus luteum of pregnancy was about 10 weeks.

If, therefore, the plasma 17α-hydroxyprogesterone does monitor the early corpus luteum, and the evidence that it does seems good, then the main metabolite pregnanetriol could also be used in this way. The evidence of the present two cases suggests that it does and therefore it is put forward that the measurement of pregnanetriol in the urine in very early pregnancy may be helpful in monitoring abnormalities of early pregnancy due to deficiency of corpus luteum function such as early abortion.

During the first six weeks of gestation the corpus luteum is the predominant source of progesterone and after the nadir of plasma progesterone is reached the placenta assumes the important role in progesterone production. The occurrence of the nadir in the plasma

progesterone curve suggests that this should be a critical period in pregnancy since either an unusually rapid fall in progesterone production by the corpus luteum or a too slow increase in placental progesterone production could result in plasma progesterone levels incompatible with the continuation of pregnancy. The value of urinary pregnanediol measurements in early pregnancy have been shown earlier in this work to be unreliable in forecasting abortion and giving information about the corpus luteum, and it is now suggested that urinary pregnanetriol estimations should be used instead unless suitable methods of assay of plasma levels of hormones can be developed for this, but this involves more complex methodology and repeated veni puncture.

One point which must be noted, however, is that urinary pregnanediol excretion is well known to be greater after induced ovulation than after normal ovulation (Gemzell 1965). The levels of 17-hydroxy-progesterone and progesterone noted one week after ovulation induced by gonadotrophins were higher than those seen at a comparable time of the normal luteal

phase and the levels of pregnanetriol seen in the urine in the present cases are also higher than those previously reported.

These higher levels may be due to multiple corpora lutea, or perhaps to a larger corpus luteum and are most probably attributable to the dose of gonadotrophin used to induce ovulation.

It is impossible, therefore, to consider gonadotrophin induced ovulation as normal and the high excretion of pregnanediol and oestrogens noted by many investigators attests to this. There is however, no reason to suppose that the time relationships of corpus luteum growth and persistence are altered by the previous injection of gonadotrophins. The increased excretion of pregnanetriol in gonadotrophin stimulated cycles where the level is higher than that normally reported are similar to those seen in cases of hydatidiform mole where there is also excessive stimulation as described in Chapter 5.

# CHAPTER 8.

## Summary and Conclusions.

### 1. Summary

Chapter 1. Introduction to the Thesis.

The introduction discusses the thoughts leading to the studies and shows the relationship of the different chapters to each other.

Chapter 2. Epidemiological studies on abortion and subsequent reproductive performance.

1. Women who start childbearing with 1 or 2 consecutive abortions are compared with those who start with 1 or 2 normal pregnancies in respect of age, height, husband's social class, obstetric performance in subsequent continuing pregnancy and later reproductive performance.

2. Women starting childbearing life with 2 abortions are older, shorter and of lower socio-economic status than the other 3 groups. Their problem is not 'fertility' but the difficulty of 'holding on' to the pregnancy.

3. In the first continuing pregnancy these women have a higher incidence of threatened abortion and premature labour. The perinatal mortality is increased mainly due to "prematurity" and "foetal deformity". There is also an increased rate of operative delivery, of forceps after 1 previous abortion and of Caesarean Section after 2 previous abortions.

4. In the group with 2 previous abortions there is an increased tendency to poor foetal growth and the women with poor foetal growth also have premature labour. Meticulous antenatal care is indicated in these women.

5. Eight percent of women who abort in their first pregnancy aborted in all their subsequent pregnancies and 10% of this group had no subsequent successful pregnancy.

6. The recurrent abortion risk increases with consecutive abortions from 25% after 1 previous abortion to 58% after 3 previous abortions.

7. One third of abortions take place before 2 months

gestation and 50% have occurred by 3 months.

8. The results suggest that in abortion studies the type of patient who should be studied is the 'primary recurrent aborter' with two or more previous consecutive abortions and no pregnancies which have progressed past 28 weeks.

9. Initial observations in abortion studies should be made, at the latest, by 8 weeks of gestation. If a previous abortion has ended at an earlier stage than 8 weeks the initial observations must be made before this time.

Chapter 3. Hormone assays in normal early pregnancy, in abortion and in hydatidiform mole.

1. The metabolic pathways from precursors to pregnanediol and oestriol are discussed.

2. Urinary pregnanediol and oestriol assays are made in 3 groups of women (1) Normal pregnancy, (2) Women with at least 2 previous abortions and no successful pregnancies and (3) Women who aborted in the pregnancy studied.

3. There is no significant difference in oestriol and pregnanediol excretion between the women who aborted and those in which the pregnancy continued successfully until 16 weeks gestation. Thereafter, when the foetal component of oestriol production takes effect low oestriol excretion is significant. There is no difference in the pregnanediol/oestriol ratio between successful and unsuccessful cases.

4. Urinary oestriol and pregnanediol assays are of no value in forecasting abortion until after 16 weeks of pregnancy.

5. These two assays are performed in 5 cases of hydatidiform mole where the trophoblast is abnormal and the foetus is absent. Urinary pregnanediol levels are higher than normal in 2 cases and lower in 3. The level of oestriol was normal until 16 weeks in 1 case and then flattened out. In the other 4 cases it was low.

6. In hydatidiform mole urinary pregnanediol and oestriol levels may be normal but are more likely to be low. There may be some change in steroid metabolism in this type of case.

## Chapter 4. Conversion of progesterone to pregnanediol.

1. Pregnanediol is the characteristic urinary metabolite of progesterone but other more polar metabolites are also excreted in the urine especially 6 and 16 hydroxylated compounds.

2. After I.V. injection of 100μc. tritiated progesterone the percentage radioactivity excreted as pregnanediol was measured in 4 groups of women (1) Non-pregnant (2) early pregnancy (3) late pregnancy (4) abnormal pregnancy.

3. There was no significant difference between the non-pregnant, early pregnancy and late pregnancy subjects, but there was a significant difference between abnormal pregnancy and the other 3 groups. In particular the conversion in cases of hydatidiform mole was low.

4. There was no difference in conversion in the same subject - pregnant and non-pregnant.

## Chapter 5. Progesterone metabolism in the human previable foetus.

1. The levels of progesterone in the umbilical vessels indicate that the human foetus metabolises this hormone.

2. Perfusion of previable foetuses with 4-$C^{14}$ progesterone for 14 and 45 minutes followed by extraction of the tissues and identification of metabolites shows that 40% of the radioactivity is present in the liver after 14 minutes mainly as 20α dihydroprogesterone. The adrenals at 14 minutes contain 3.4% of the radioactivity mainly in the form of polar compounds. After 45 minutes the main compound in the liver was pregnanediol and in the adrenals polar compounds - probably corticosteroids.

3. The foetal liver is the chief site of metabolism of progesterone and this organ produces mainly reduced metabolites. The adrenal also metabolises progesterone to polar compounds, such as corticosteroids.

4. Progesterone from the placenta is used by the foetus to produce corticosteroids for its own homeostasis.

Chapter 6. Steroid studies in a case of hydatidiform mole.

1. The increase in urinary pregnanetriol excretion in cases of hydatidiform mole suggests an alternative metabolic pathway in these cases. The pregnanediol:pregnanetriol ratio in molar pregnancy is 2:1 compared with that of 20:1 in normal pregnancy.

2. Mole tissue was incubated with [4-$^{14}$C] pregnenolone as precursor, and 17α hydroxypregnenolone, progesterone, 16α hydroxyprogesterone and 16β hydroxyprogesterone were isolated.

3. Cholesterol, pregnenolone, 17α hydroxypregnenolone, pregnanediol, pregnanetriol and androstenedione were isolated from extracts of mole tissue and theca lutein cyst fluid. 17α Hydroxyprogesterone was isolated from cyst fluid but not from mole tissue.

4. Mole tissue is capable of steroidogenesis but there was less synthesis of progesterone than normal.

5. The increase in 17α hydroxylation indicated by the elevation of the urinary excretion of pregnanetriol, is ovarian in origin, perhaps due to stimulation by the large amounts of H.C.G. circulating in these cases.

Chapter 7. Urinary steroid excretion after gonadotrophin therapy.

1. Ovarian steroid biogenesis is controlled by F.S.H. and L.H. It is not clear that F.S.H. has any specific effect on steroidogenesis but L.H. can increase the rate of conversion of cholesterol into progesterone and the de-novo production of the latter.

2. The major precursor of pregnanetriol is 17α-hydroxyprogesterone and there is a significant increase in the excretion of this metabolite in the luteal phase of the menstrual cycle. This is due to ovarian production of 17α hydroxy-

progesterone.

3. Urinary pregnanetriol excretion also rises in the luteal phase of gonadotrophin stimulated cycles and this may be a valuable measure of corpus luteum function.

4. The excretion of this substance also rises in early pregnancy during the growth of the corpus luteum of pregnancy and falls to normal pregnancy levels after 6 - 8 weeks of gestation when the main function of the corpus luteum of pregnancy appears to be past.

5. The pattern of urinary pregnanetriol levels in very early pregnancy agrees with that of the plasma levels of precursor 17α hydroxyprogesterone.

6. Urinary pregnanetriol excretion up to 6 - 8 weeks of pregnancy may be valuable in detecting the hyperstimulation syndrome after gonadotrophin therapy, and also in monitoring the corpus luteum of pregnancy. It may indicate when the latter is deficient with the resultant possibility of early abortion.

## 2. Conclusions

1. Women who begin childbearing with one or two abortions have a relatively poor subsequent reproductive performance.

2. Urinary oestriol and pregnanediol assays do not help in forecasting abortion up to 16 weeks of gestation and may not be helpful in diagnosing hydatidiform mole.

3. The conversion of progesterone to pregnanediol is low in abnormal pregnancy.

4. The foetus uses progesterone to manufacture adrenal steroids for its own homeostasis.

5. The increased 17-hydroxylation which takes place in hydatidiform mole occurs in the ovaries probably due to stimulation by H.C.G.

6. Urinary pregnanetriol excretion is a measure of corpus luteum function in the menstrual cycle and of the corpus luteum of early pregnancy.

Further work arising from this thesis.

1. Investigation into the control of steroid metabolism in the human foetus and foeto-placental unit.

2. Measurement of other steroid metabolites e.g. 6 and 16 hydroxylated metabolites of progesterone in the urine in pregnancy, normal and abnormal.

3. Further investigation into the use of pregnanetriol and also of plasma 17α hydroxyprogesterone in early pregnancy to assess corpus luteum function and its possible use in forecasting abortion.

4. The use of these assays in the prevention of ovarian hyperstimulation with subsequent multiple ovulation and multiple pregnancy in therapy with human gonadotrophins.

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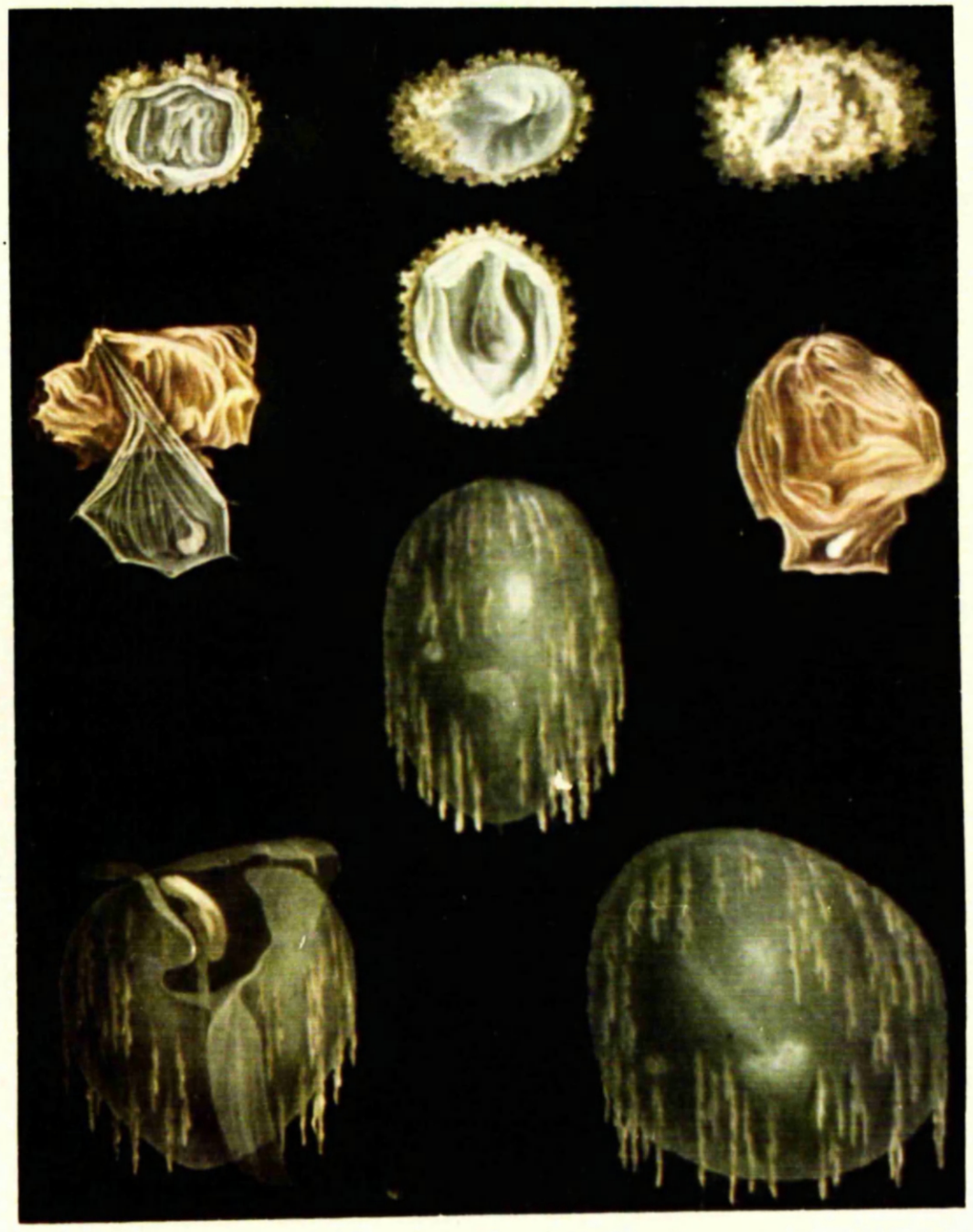
# EPIDEMIOLOGICAL AND HORMONE STUDIES IN EARLY HUMAN PREGNANCY - NORMAL AND ABNORMAL

## APPENDIX

1. Plates.
2. Diagrams
3. Tables.
4. References.
5. Publications.

PLATE I.



SPECIMENS OF VERY EARLY MISCARRIAGES $3\frac{1}{2}$ WEEKS TO 8 WEEKS AFTER MENSTRUATION (from Granville 1833).

# PLATE II.


SPECIMENS OF MISCARRIAGE 9 AND 10 WEEKS AFTER MENSTRUATION

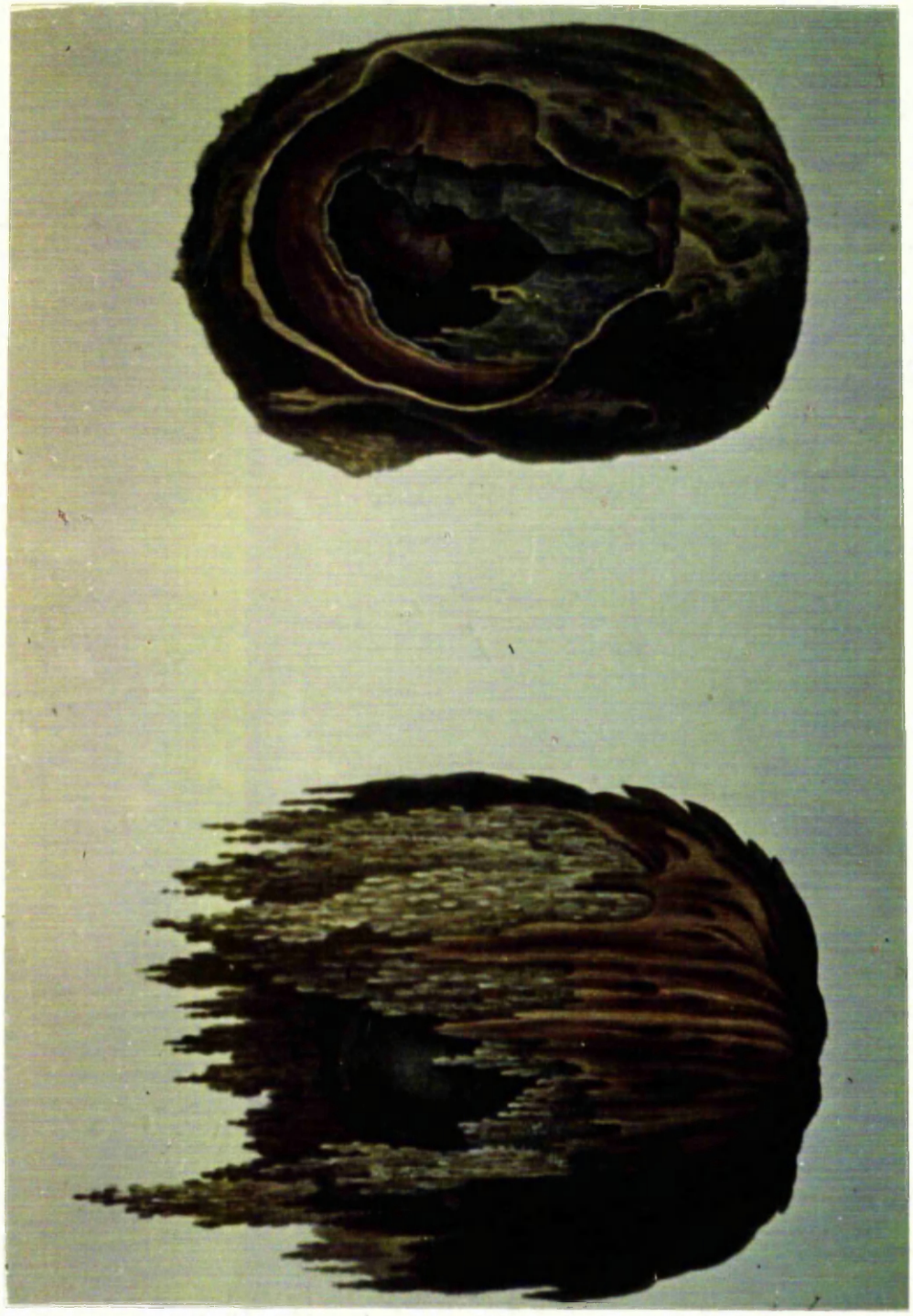
(from Granville 1833).

# PLATE III.

SPECIMENS OF MISCARRIAGE 10 - 11 WEEKS AFTER MENSTRUATION

(from Granville 1833).

PLATE IV.



SPECIMENS OF MISCARRIAGE BETWEEN 12 AND 16 WEEKS AFTER MENSTRUATION

(from Granville 1833).

PLATE V.

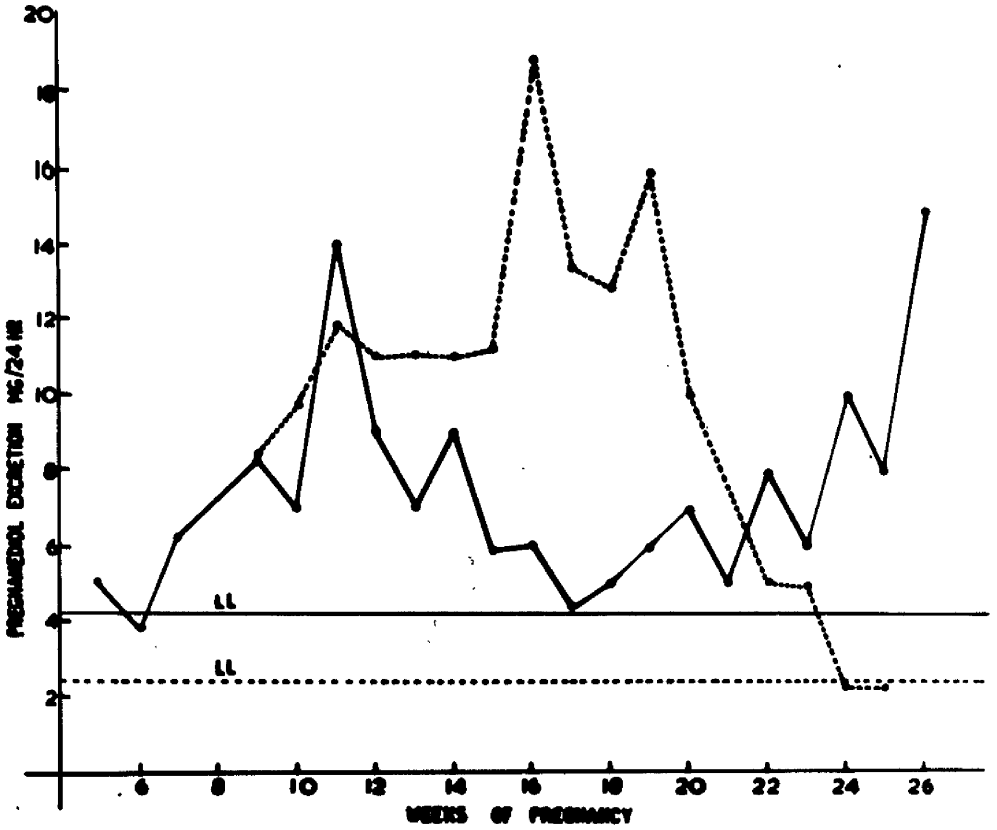
SPECIMENS OF MISCARRIAGE BETWEEN 12 AND 16 WEEKS AFTER MENSTRUATION (from Granville 1833).

PLATE VI.

SPECIMEN OF A MISCARRIAGE AT FIVE MONTHS

(from Granville 1833)

FIG. 1.

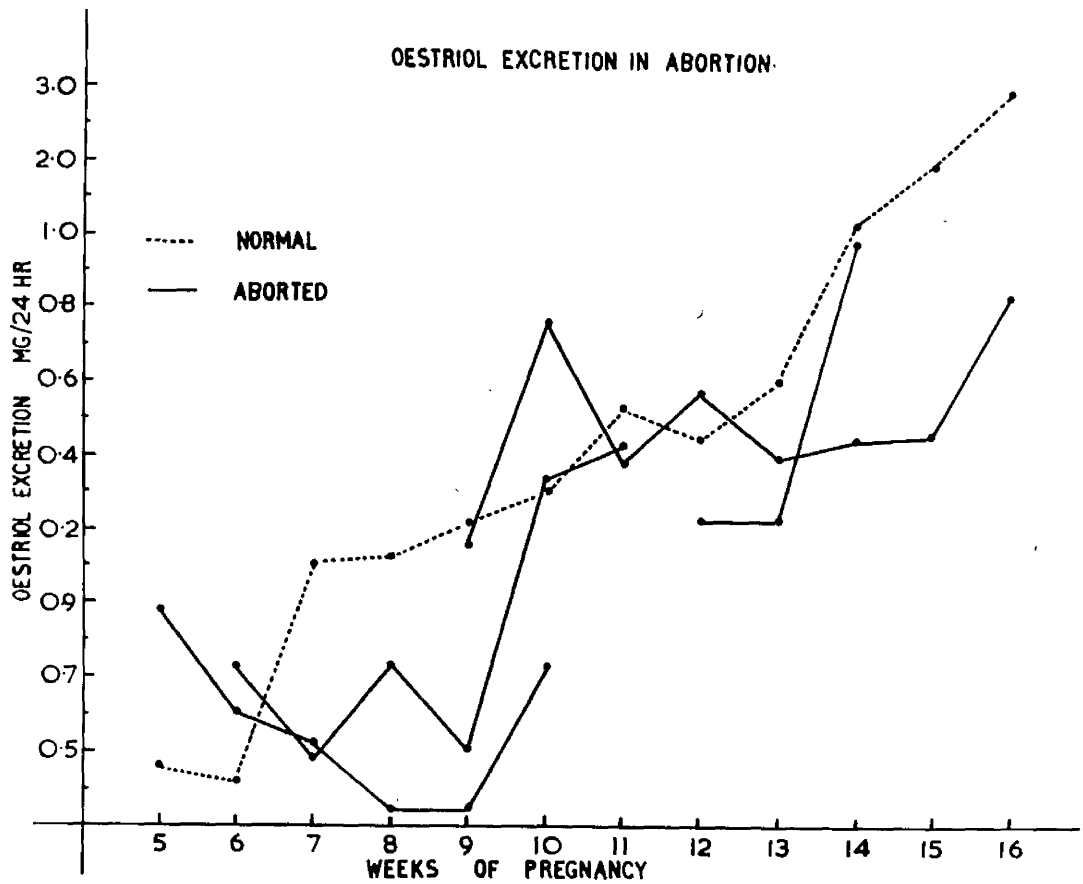



EARLY PREGNANCY AND LUTEAL LEVELS OF 2 WOMEN.

IN CASE 1 ●——● THE PREGNANCY CONTINUED.

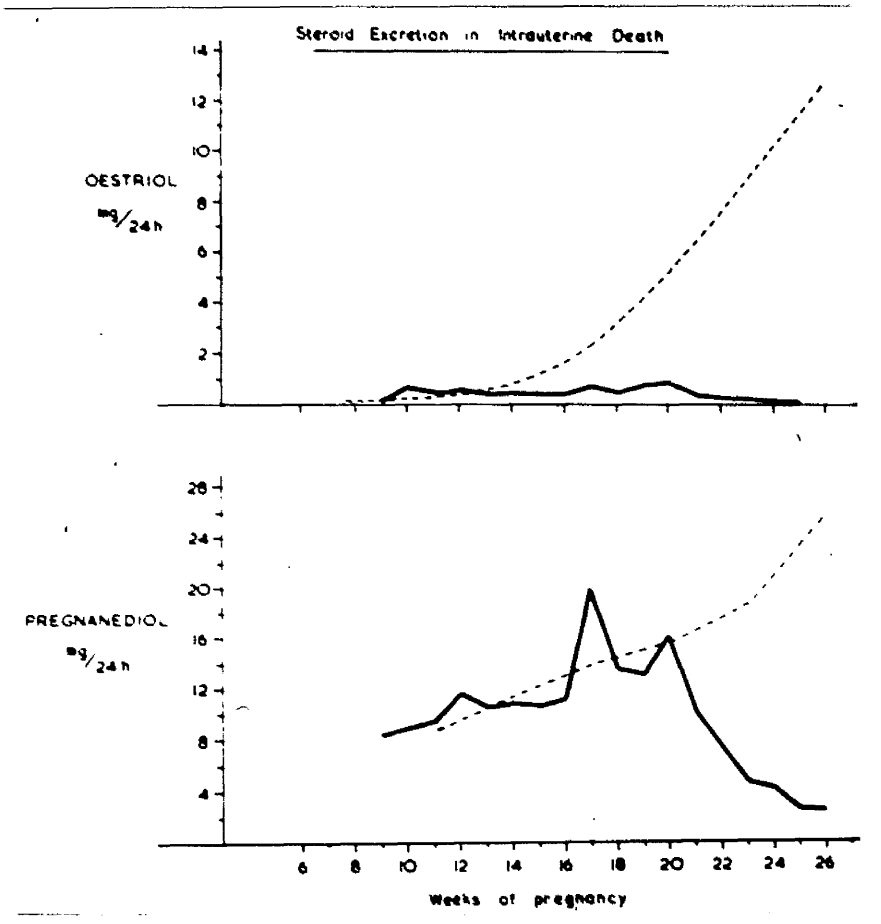
IN CASE 2 ●------● ABORTION OCCURRED.

FIG. 2.



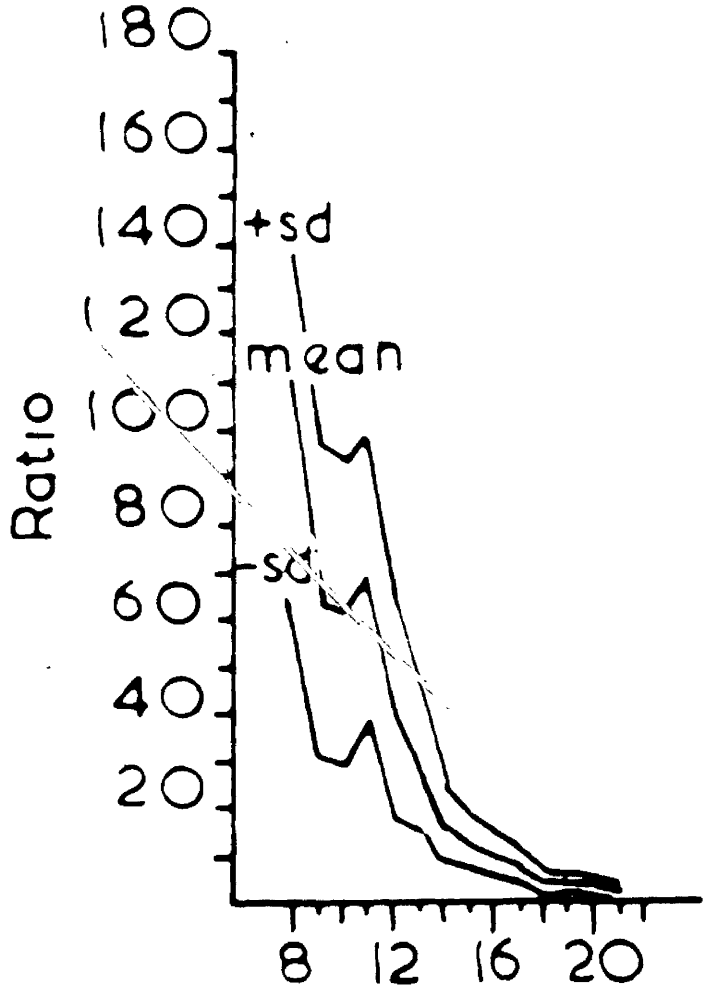

URINARY OESTRIOL LEVELS IN WOMEN WHO ABORTED.

FIG. 3.



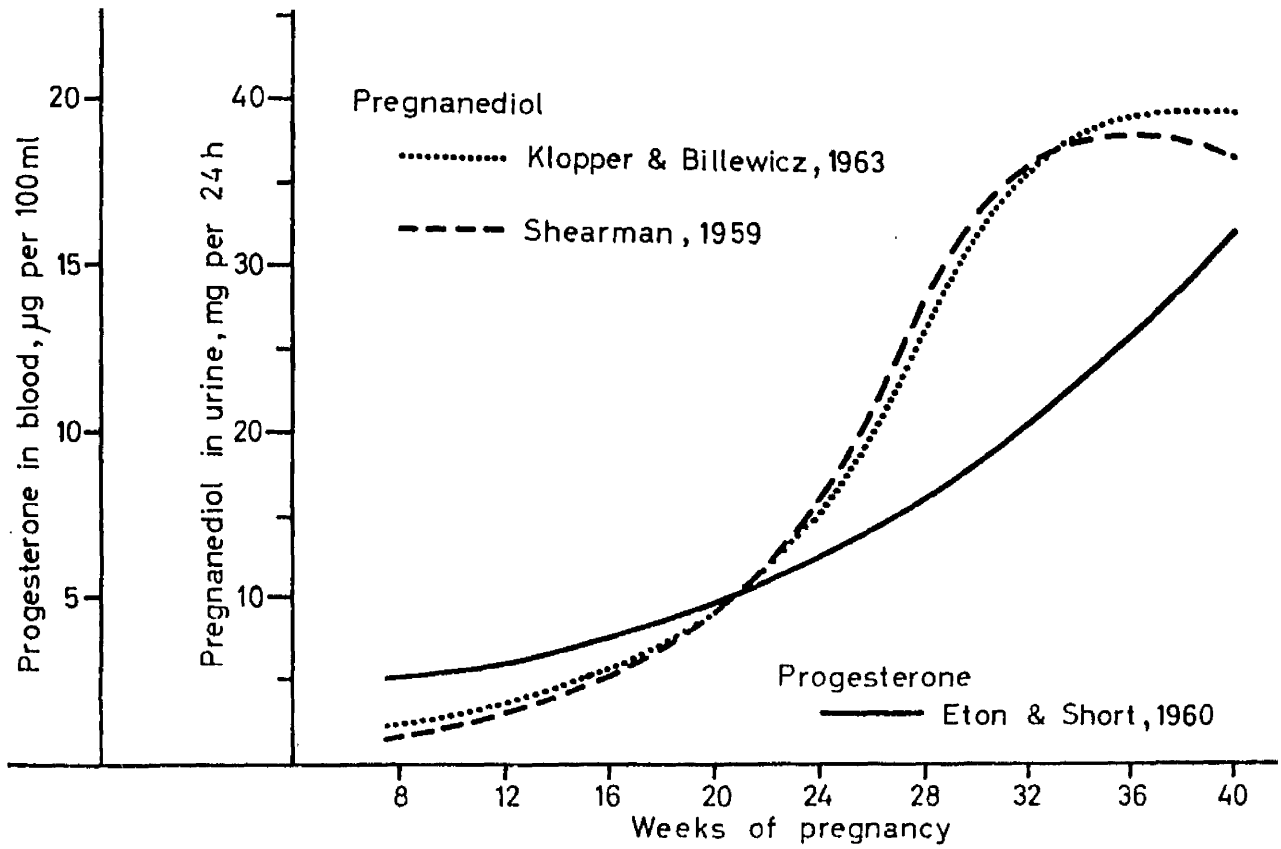

OESTRIOL AND PREGNANEDIOL EXCRETION IN A WOMAN WHO ABORTED AT 26 WEEKS

FIG. 4.



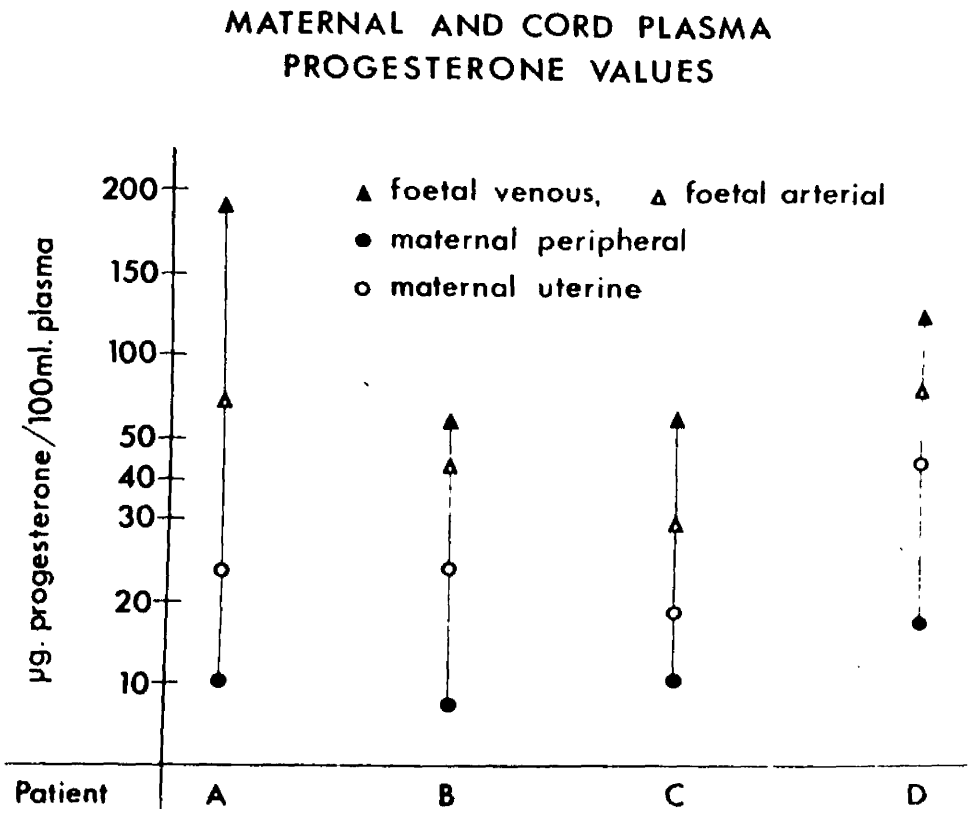

PREGNANEDIOL/OESTRIOL RATIO IN NORMAL PREGNANCY.
ABSCISSA AXIS - WEEKS OF PREGNANCY.
ORDINATE AXIS - PREGNANEDIOL/OESTRIOL RATIO.
(From Klopper and Billewicz 1963)

FIG. 5.




RELATIONSHIP OF BLOOD PROGESTERONE AND URINARY PREGNANEDIOL LEVELS IN PREGNANCY.

FIG. 6.

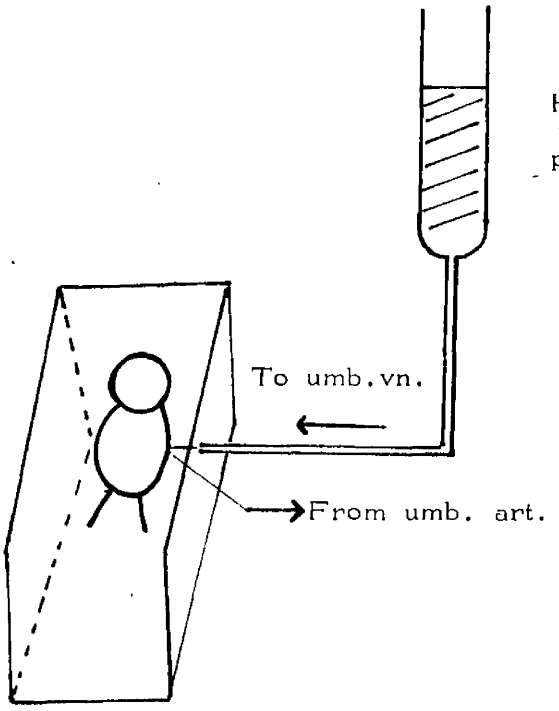



MATERNAL AND CORD PLASMA PROGESTERONE VALUES

(from Greig et al 1962).

**FIG. 7.**

FOETAL PERFUSION APPARATUS

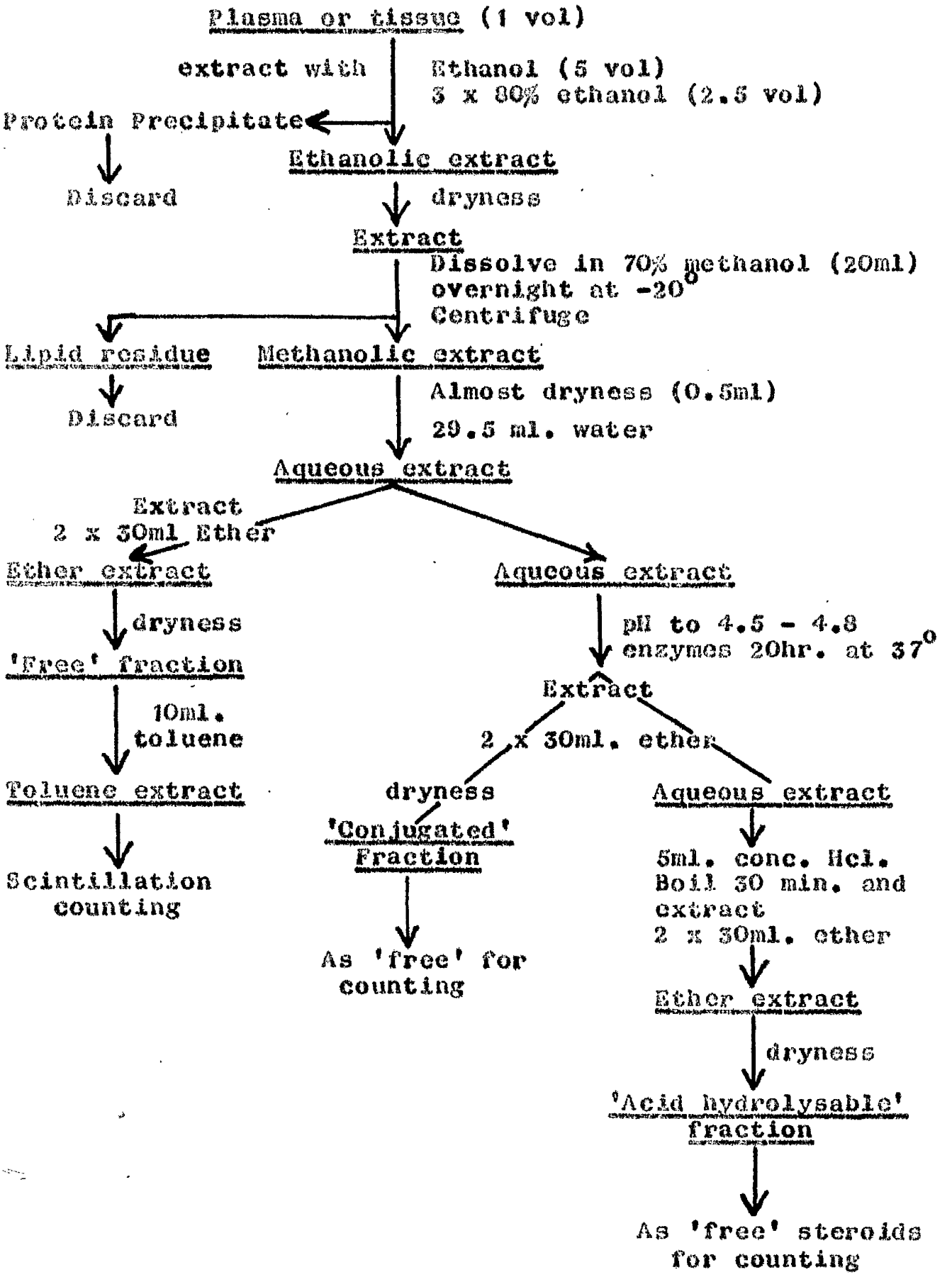



Perfusion Chamber with foetus
in 50% Hartmann's solution.

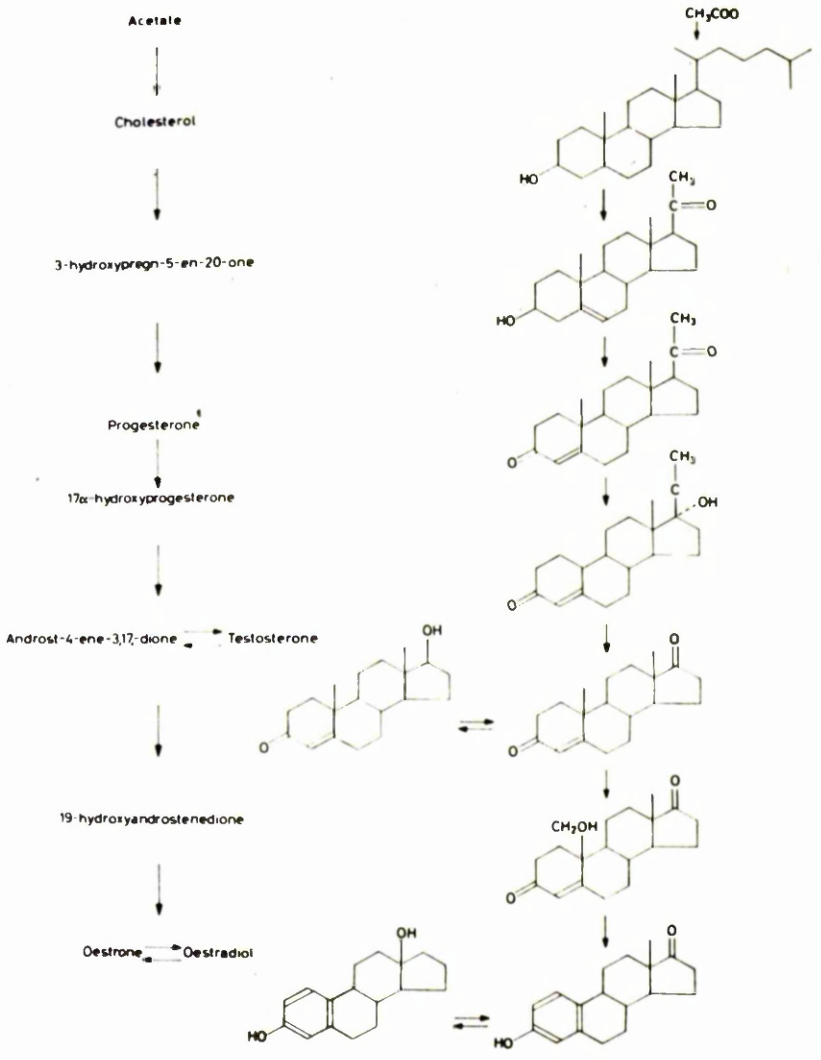
**FOETAL PERFUSION APPARATUS.**

FIG. 8.

FLOW SHEET - TISSUE AND PLASMA EXTRACTION

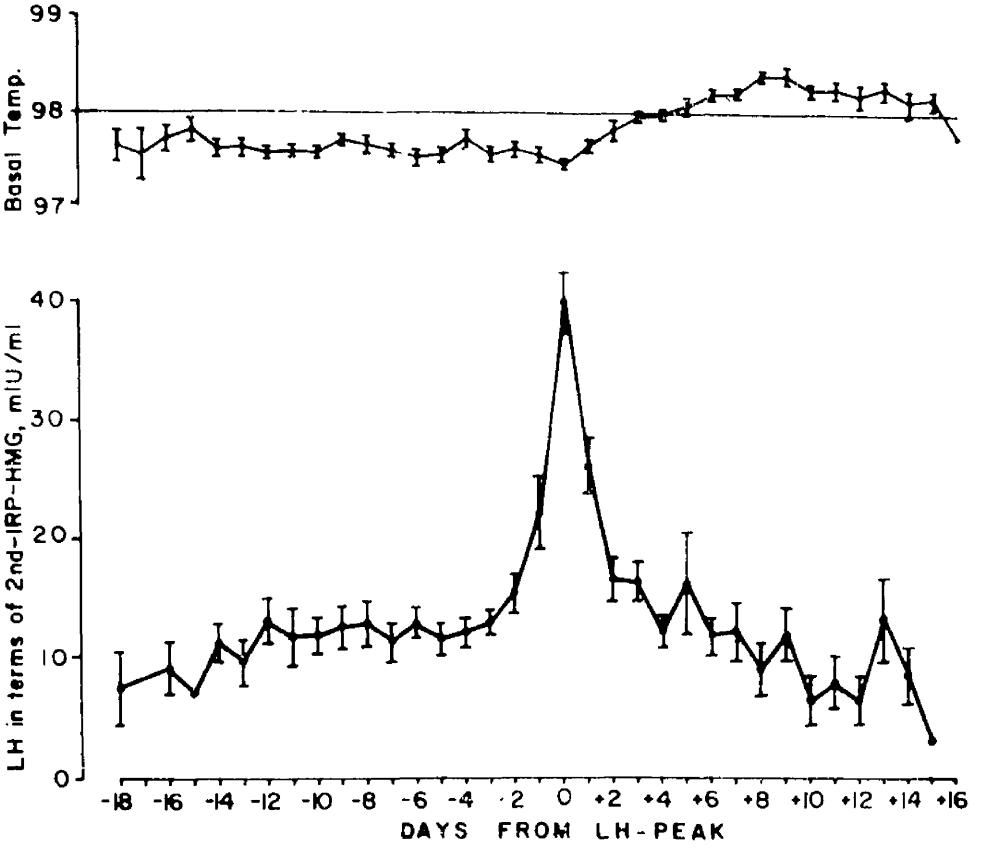


# FIG. 9.




**BIOSYNTHETIC PATHWAY OF STEROIDS FROM CHOLESTEROL TO OESTRONE AND OESTRADIOL.**

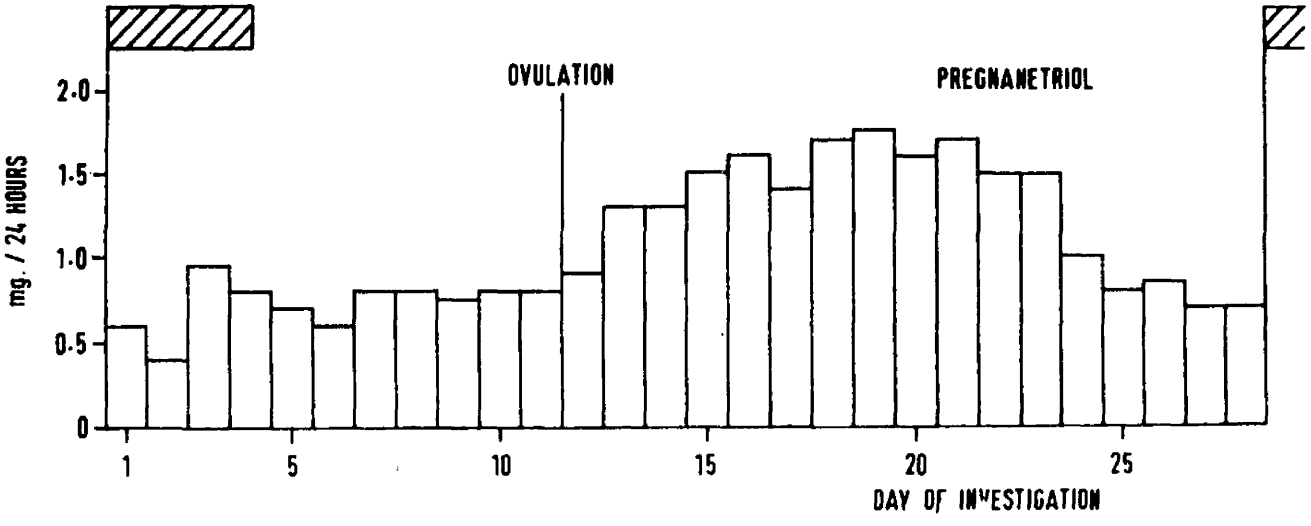
FIG. 10.




MEAN SERUM L.H. LEVELS ± S.E. IN 16 NORMAL MENSTRUAL CYCLES.

(from Midgley and Jaffe 1966).

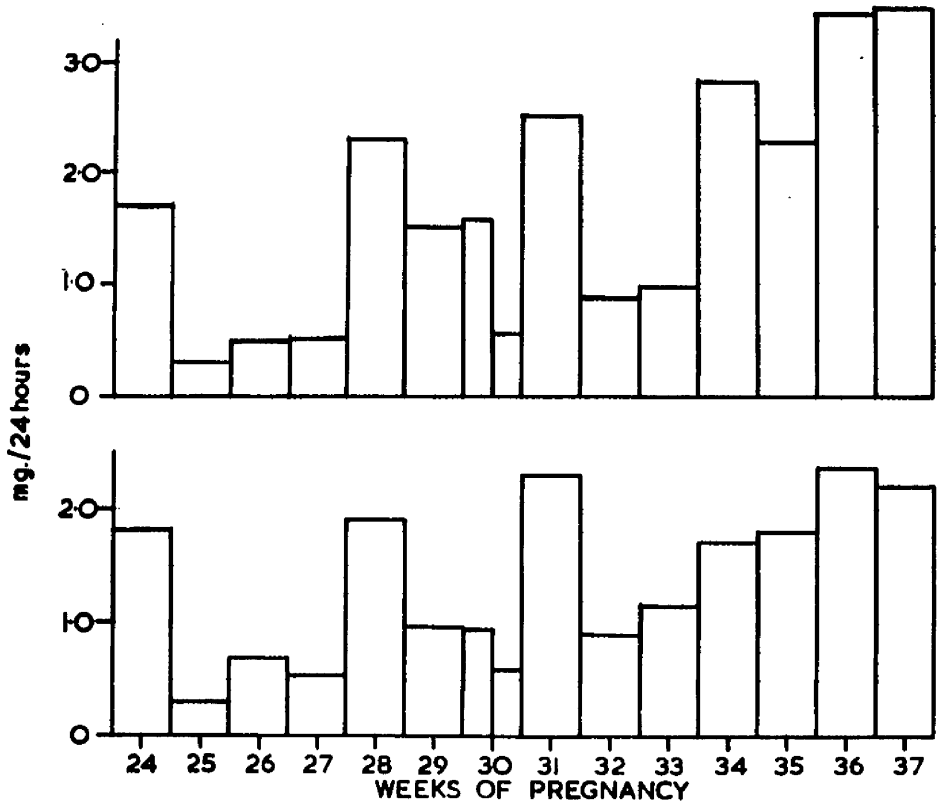
FIG. 11.




PREGNANETRIOL EXCRETION IN THE MENSTRUAL CYCLE.

(from Bell et al 1962).

FIG. 12.



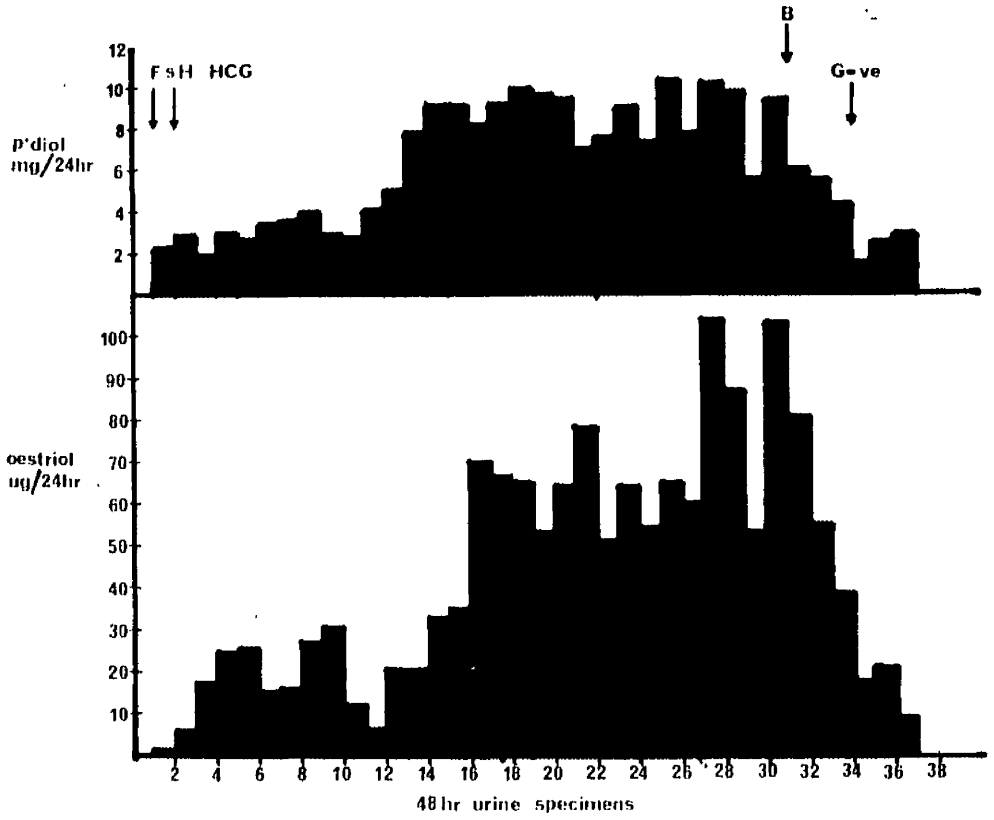

URINARY PREGNANETRIOL EXCRETION DURING PREGNANCY.

UPPER DIAGRAM - USING ZIMMERMANN REACTION.

LOWER DIAGRAM - USING SULPHURIC ACID COLOUR REACTION.

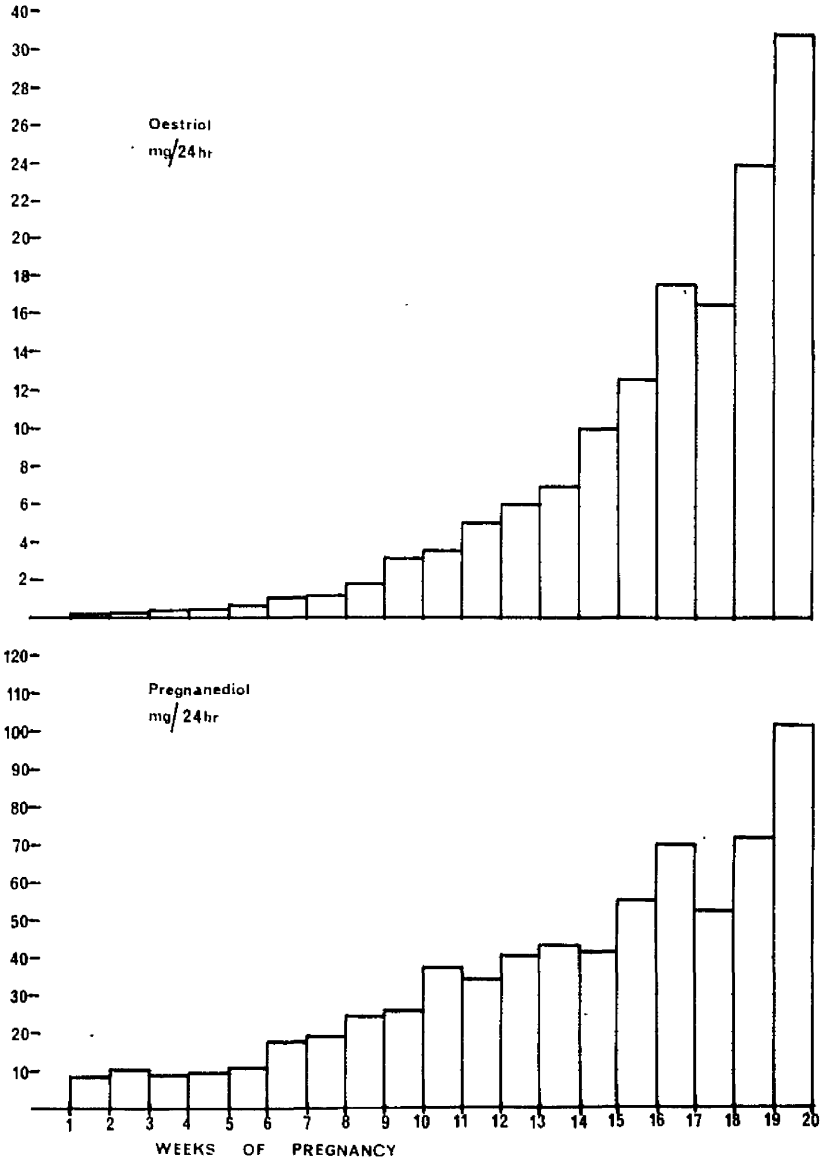
(from Harkness and Love 1966).

FIG. 13.



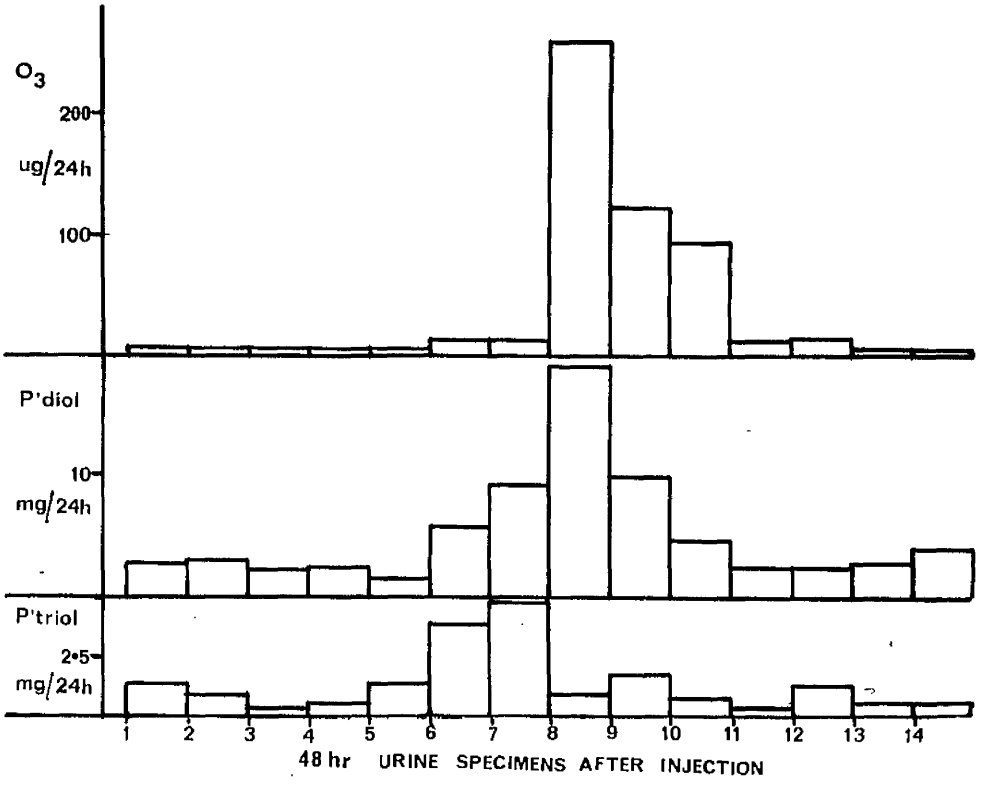

URINARY PREGNANEDIOL AND OESTRIOL EXCRETION IN CASE No. 1 WHERE ABORTION OCCURRED.

# FIG. 14.



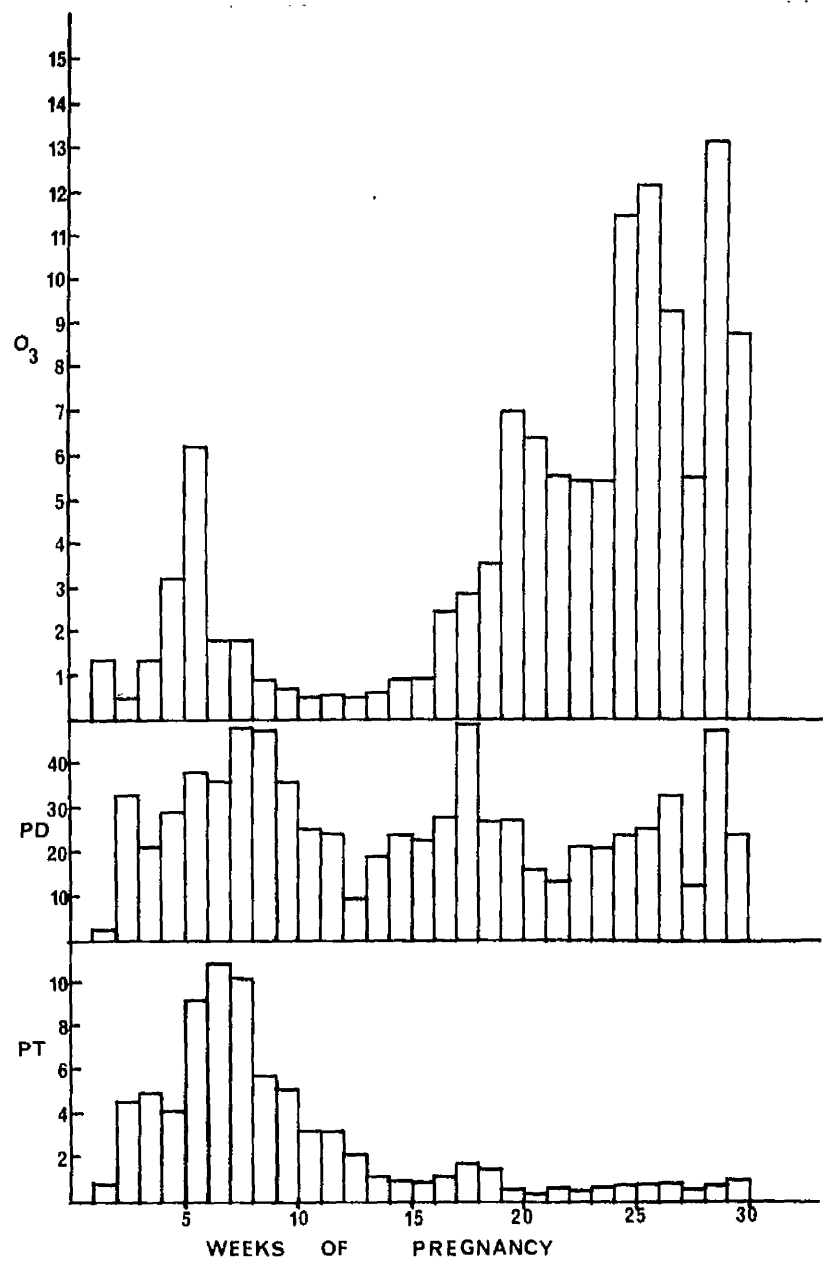

URINARY PREGNANEDIOL EXCRETION DURING TWIN PREGNANCY IN CASE No. 1.

FIG. 15.



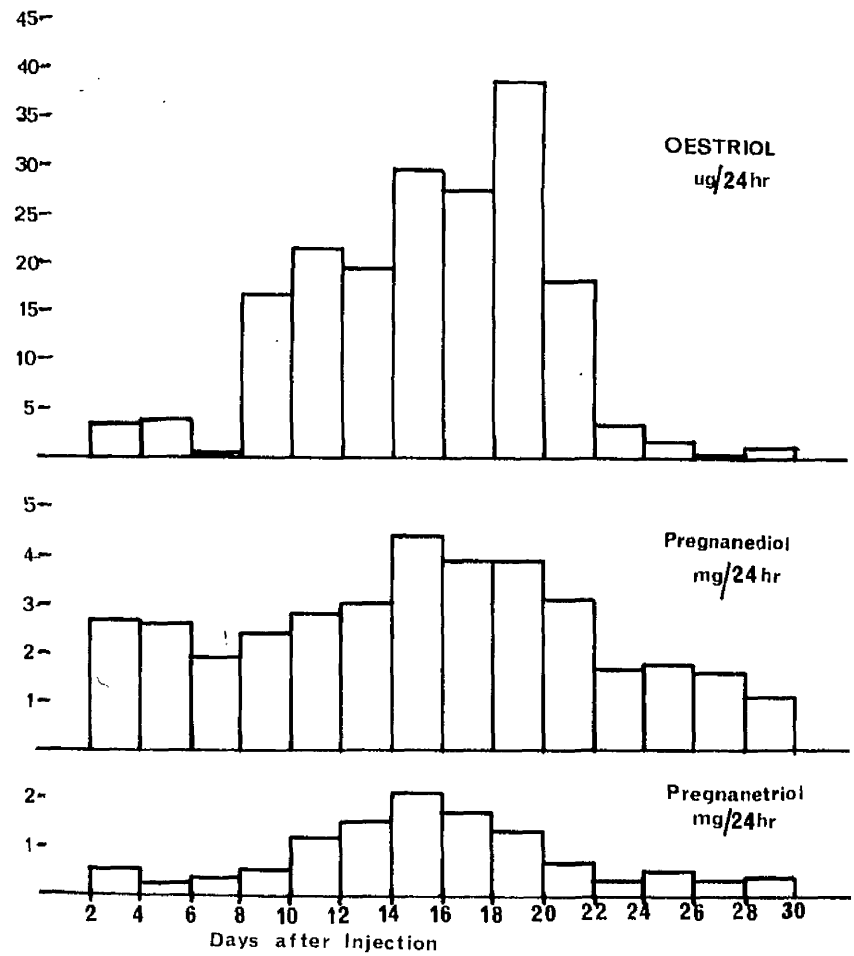

URINARY OESTRIOL, PREGNANEDIOL AND PREGNANETRIOL EXCRETION IN THE CYCLE IN CASE No. 2 BEFORE THE CONCEPTION CYCLE

FIG. 16.



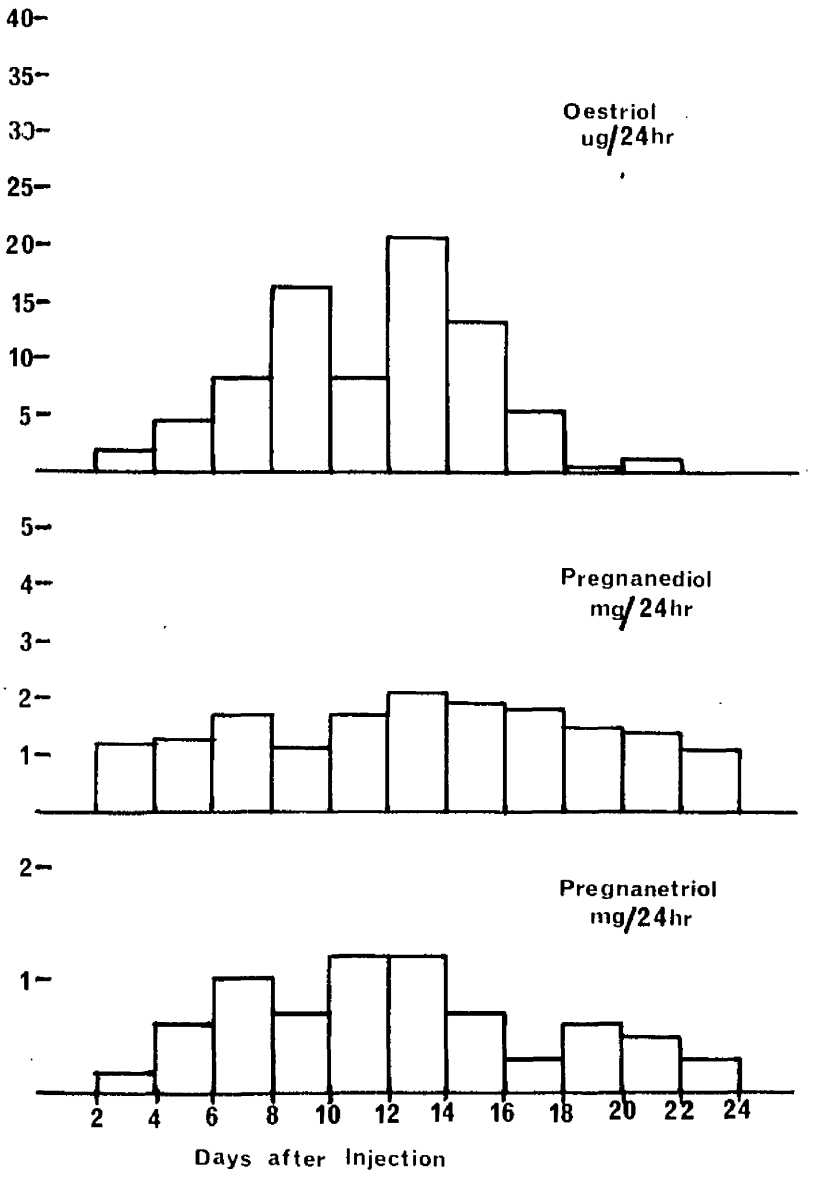

URINARY PREGNANEDIOL, PREGNANETRIOL, AND OESTRIOL EXCRETION IN CASE No. 2, IN PREGNANCY UNTIL 30 WEEKS OF GESTATION

FIG. 17.



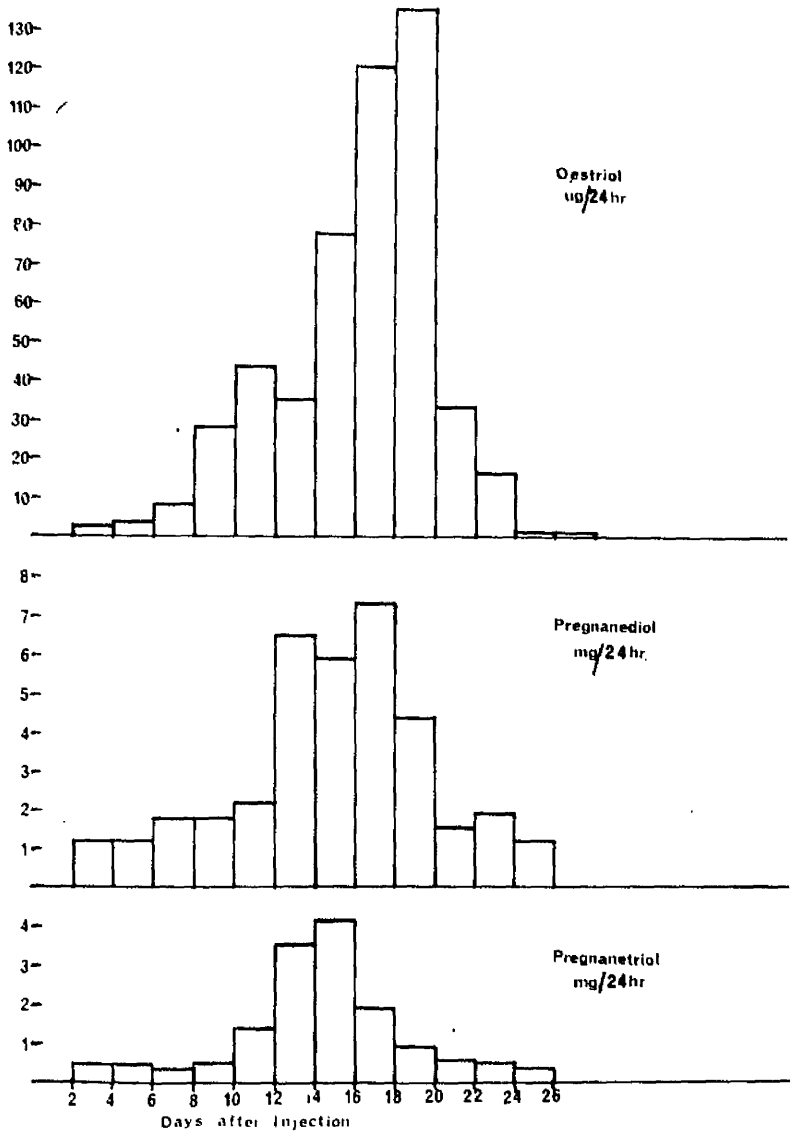

URINARY PREGNANEDIOL, PREGNANETRIOL AND OESTRIOL EXCRETION IN FIRST GONADOTROPHIN STIMULATED CYCLE IN CASE No. 3.

FIG. 18.



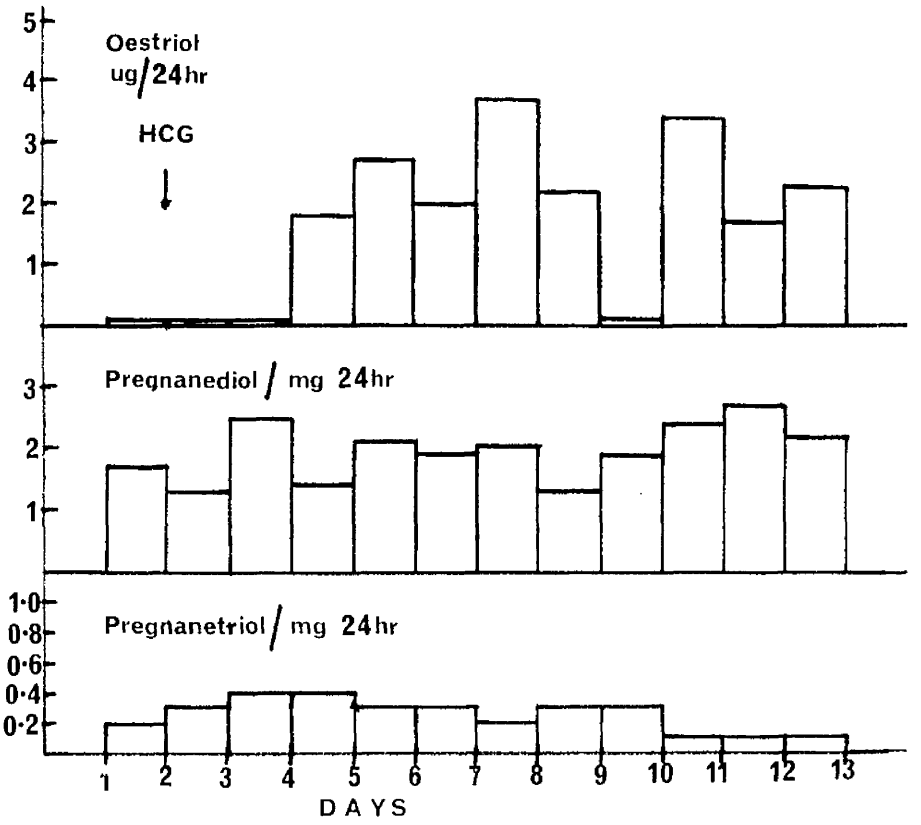

URINARY PREGNANEDIOL, PREGNANETRIOL AND OESTRIOL EXCRETION IN SECOND GONADOTROPHIN STIMULATED CYCLE IN CASE No. 3.

# FIG. 19.




URINARY PREGNANEDIOL, PREGNANETRIOL AND OESTRIOL EXCRETION IN THIRD GONADOTROPHIN STIMULATED CYCLE IN CASE No. 3.

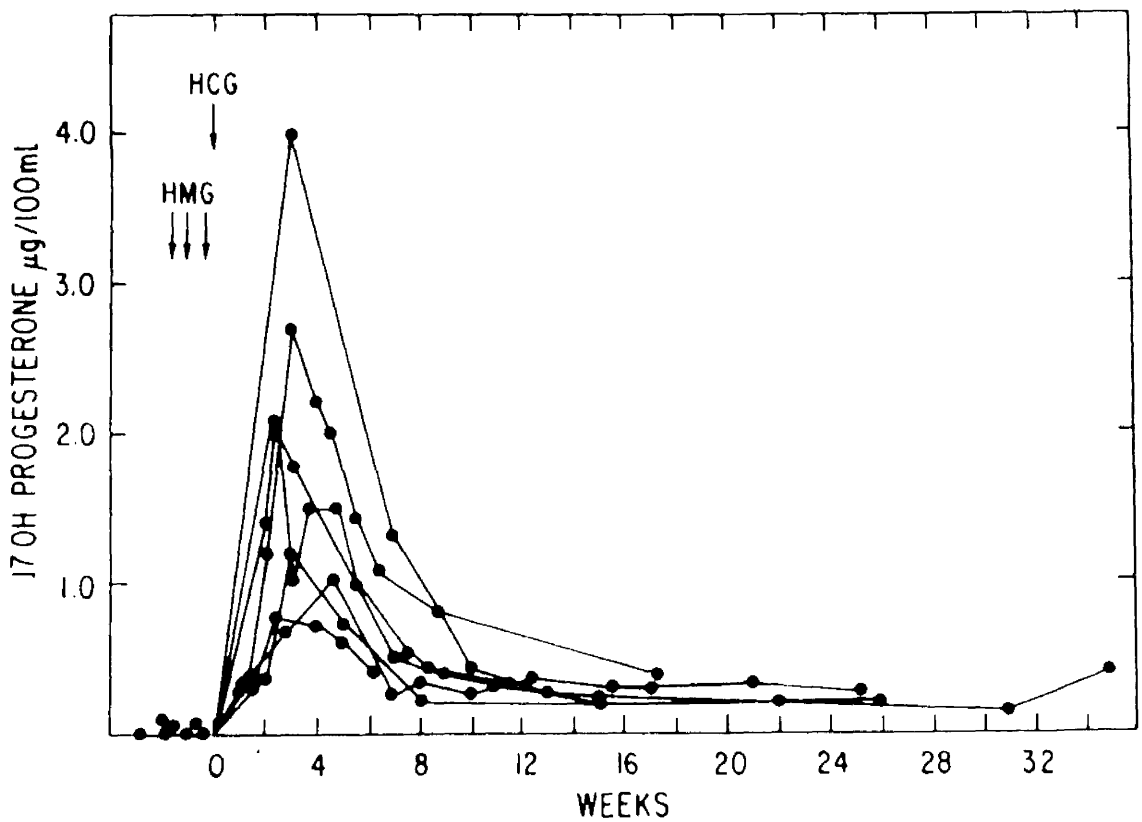
FIG. 20.




URINARY EXCRETION OF OESTRIOL, PREGNANEDIOL AND PREGNANETRIOL IN AN OOPHORECTOMISED, HYSTERECTOMISED WOMAN AFTER STIMULATION WITH F.S.H. AND H.C.G.

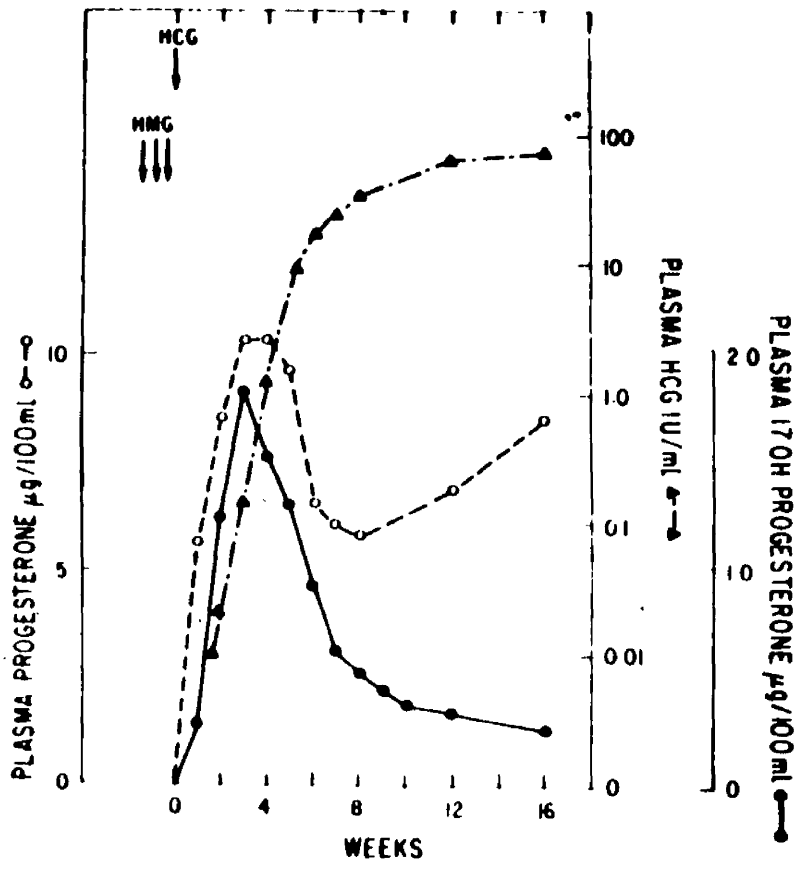
CASE No. 4.

FIG. 21.




PLASMA 17α HYDROXYPROGESTERONE LEVELS DURING GONADOTROPHIN INDUCED PREGNANCY.
(from Yoshimi et al 1969).

FIG. 22.




PLASMA 17α HYDROXYPROGESTERONE AND PLASMA PROGESTERONE LEVELS DURING GONADOTROPHIN INDUCED PREGNANCY SHOWING DIVERGENCE OF THE CURVES AT 6 - 8 WEEKS GESTATION

(from Yoshimi et al 1969)

TABLE 1.

INCIDENCE OF ABORTION.

| AUTHOR | Date | Incidence |
|---|---|---|
| TIETZE, GUTTMACHER AND RUBIN | 1950 | 7.0% |
| HUDSON AND RUCKER | 1945 | 10.0% |
| REPORT OF BIOL. & MED. COMMIS. | 1950 | 10.0% |
| DAVIS | 1950 | 10.0% |
| BAIRD | 1957 | 10.0% |
| STALLWORTHY | 1955 | 10.0% |
| HERTIG AND LIVINGSTONE | 1944 | 10.6% |
| STEVENSON, DUDGEON AND McCLURE | 1959 | 11.8% |
| INTERDEPARTMENTAL COMM. ON ABORTION | 1939 | 16 - 20% |
| TIETZE AND MARTIN | 1957 | 17.0% |
| WHITEHOUSE | 1929 | 17.2% |
| MALPAS | 1938 | 18.0% |

TABLE 2.

COMPARISON OF 4 GROUPS BY AGE. (%)

| AGE | 15-19 | 20-24 | 25-29 | 30+ | |
|---|---|---|---|---|---|
| 2nd Preg. 1 Abortion | 6.5 | 38.8 | 34.9 | 20.8 | 100.0 |
| 3rd Preg. 2 Abortions | 3.1 | 34.5 | 32.6 | 30.2 | 100.4 |
| 1st Preg. Normal | 11.9 | 51.8 | 26.3 | 10.0 | 100.0 |
| 2nd Preg. No Abortions | 2.4 | 34.7 | 37.8 | 25.1 | 100.0 |

TABLE 3.

INCIDENCE OF 1st ABORTION FOR MARRIAGE DURATION

| Marriage Duration | Incidence of 1st Abortion under 25 years, % | Incidence of 1st Abortion over 25 years. % |
|---|---|---|
| 0-2 years | 21.3 | 27.9 |
| 2-4 years | 6.3 | 9.2 |
| 5+ years | 2.1 | 5.0 |

TABLE 4.

COMPARISON OF 4 GROUPS BY HEIGHT (%).

| HEIGHT | Under 5'1" | 5'1"-5'4" | 5'4"+ | |
|---|---|---|---|---|
| 2nd Preg. 1 Abortion | 20.7 | 51.7 | 27.6 | 100.0 |
| 3rd Preg. 2 Abortions | 33.3 | 47.5 | 19.2 | 100.0 |
| 1st Preg. | 23.0 | 49.6 | 27.4 | 100.0 |
| 2nd Preg. No Abortions | 24.0 | 50.0 | 26.0 | 100.0 |

## TABLE 5.

COMPARISON OF 4 GROUPS BY HUSBANDS SOCIAL CLASS (%).

| SOCIAL CLASS | 1 & 2 | 3 | 4 & 5 | |
|---|---|---|---|---|
| 2nd Preg. 1 Abortion | 12.4 | 64.1 | 23.5 | 100.0 |
| 3rd Preg. 2 Abortions | 14.7 | 55.0 | 30.3 | 100.0 |
| 1st Preg. | 11.1 | 64.9 | 24.0 | 100.0 |
| 2nd Preg. No Abortions | 9.6 | 62.5 | 27.8 | 99.9 |

## TABLE 6.

### DISTRIBUTION PERCENT OF MOTHERS OF DIFFERENT HEIGHT GROUPS

### WITHIN SOCIO-ECONOMIC GROUPS

| Socio-economic Group | Tall (65" & over) % | Medium (64"-62") % | Short (Under 62") % | Not Stated % | All Heights % | No. of Births (Control week) |
|---|---|---|---|---|---|---|
| Professional | 40 | 44 | 13 | 3 | 100 | 2162 |
| Non-manual | 32 | 44 | 20 | 4 | 100 | 1733 |
| Skilled manual | 27 | 47 | 22 | 4 | 100 | 6811 |
| Semi-skilled manual | 26 | 47 | 22 | 5 | 100 | 2763 |
| Unskilled manual | 22 | 45 | 28 | 5 | 100 | 1552 |
| Remainder | 30 | 46 | 18 | 6 | 100 | 1264 |
| ALL GROUPS | 29 | 46 | 21 | 4 | 100 | 16285 |

TABLE 7.

EFFECT OF HEIGHT AND SOCIO-ECONOMIC GROUP ON PERINATAL MORTALITY -

NUMBER OF DEATHS AND RATES/1000

| Socio-Economic Group | Tall (65" & over) | Medium 64"-62" | Short (under 62") | Not Stated | TOTAL |
|---|---|---|---|---|---|
| Professional | 201 (19.4) | 270 (23.5) | 95 (27.8) | 64 (95.2) | 630 (24.3) |
| Non-manual | 154 (23.0) | 233 (25.5) | 121 (28.6) | 53 (70.1) | 561 (27.0) |
| Skilled manual | 583 (26.7) | 1116 (29.0) | 617 (33.6) | 264 (84.9) | 2580 (31.6) |
| Semi-skilled manual | 230 (26.7) | 489 (31.4) | 309 (41.9) | 147 (92.1) | 1175 (35.4) |
| Unskilled manual | 132 (31.5) | 323 (38.5) | 254 (48.9) | 66 (77.5) | 775 (41.6) |
| Remainder | 122 (26.4) | 209 (30.2) | 114 (41.7) | 86 (96.8) | 531 (35.0) |
| TOTAL | 1422 (25.3) | 2640 (29.4) | 1510 (36.6) | 680 (86.4) | 6252 (32.0) |

## TABLE 8.

### 1st ABORTIONS AND LEGITIMATE FIRST BIRTHS (R.G. SCOTLAND 1966) BY DURATION OF MARRIAGE

| Duration of Marriage (Yrs) | Legitimate First Births | | | 1st. Abortions | | |
|---|---|---|---|---|---|---|
| | Total | % | Cumulative Total % | Total | % | Cumulative Total % |
| Under 1 | 12,856 | 41.9 | | 54 | 48.6 | |
| 1 | 8,613 | 28.1 | 70.0 | 24 | 21.6 | 70.2 |
| 2 | 3,498 | 11.4 | 81.4 | 16 | 14.4 | 84.6 |
| 3 | 1,941 | 6.3 | 87.7 | 7 | 6.3 | 90.9 |
| 4 | 1,171 | 3.8 | 91.5 | 3 | 2.7 | 93.6 |
| 5 | 780 | 2.5 | 94.0 | 3 | 2.7 | 96.3 |
| 6 | 505 | 1.6 | 95.6 | 0 | 0 | 96.3 |
| 7 | 313 | 1.0 | 96.6 | 2 | 1.8 | 98.1 |
| 8 | 254 | 0.8 | 97.4 | - | - | - |
| 9 | 195 | 0.6 | 98.0 | - | - | - |
| 10-14 | 471 | 1.5 | 99.5 | 1 | 0.9 | 99.0 |
| 15-19 | 92 | 0.3 | 99.8 | 1 | 0.9 | 99.9 |
| TOTAL | 30,689 | 99.8 | | 111 | 99.9 | |

## TABLE 9.

### INCIDENCES OF OBSTETRIC PERFORMANCE

| | Threatened Abortion % | Antepartum Haemorrhage % | Prematurity % | Perinatal Deaths / 1000 |
|---|---|---|---|---|
| 2nd Preg. 1 Abortion | 5.7 | 2.6 | 8.3 | 43.2 |
| 3rd Preg. 2 Abortions | 20.1 | 3.1 | 11.6 | 38.8 |
| 1st Preg. | 2.6 | 2.6 | 7.5 | 30.7 |
| 2nd Preg. No Abortions | 1.2 | 1.6 | 4.8 | 20.9 |

## TABLE 10.

### AVERAGE BIRTH WEIGHTS (lbs) AT VARIOUS GESTATION PERIODS

| Gestation Weeks | Normal Prim. | Normal 2nd Preg. 1st Normal | 2nd Preg. 1 Abortion | 3rd Preg. 2 Abortions |
|---|---|---|---|---|
| 35 and Under | 4.38 | 5.26 | 3.79 | 2.25 |
| 36 - 37 | 6.05 | 6.30 | 6.00 | 5.5 |
| 38 - 39 | 6.75 | 7.05 | 6.81 | 6.7 |
| 40 - 41 | 7.26 | 7.54 | 7.29 | 7.5 |
| 42 and Over | 7.46 | 7.71 | 7.46 | 6.5 |

## TABLE 11.

### PROPORTION OF DELIVERIES BEFORE 36 WEEKS

| | |
|---|---|
| PRIMIGRAVIDAE | 4.6% |
| NORMAL SECOND PREGNANCY | 3.5% |
| 2nd PREG. 1 PREV. ABORTION | 5.3% |
| 3rd PREG. 2 PREV. ABORTIONS | 9.1% |

TABLE 12.

PERINATAL MORTALITY BY CAUSE %

(Numbers of cases in brackets)

| | P.U. | M.U. | A.P.H. | TOX | TRAUMA | DEF. | OTHER | TOTAL | RATE/1000 |
|---|---|---|---|---|---|---|---|---|---|
| 2nd Preg. 1 Abortion | 11.4 (7) | 4.9 (3) | 1.6 (1) | 6.5 (4) | - | 11.4 (7) | 6.5 (4) | 26 | 42.2 |
| 3rd Preg. 2 Abortions | (1) | - | - | (1) | - | (3) | - | 5 | 38.8 |
| 1st Preg. | 7.7 (62) | 4.3 (35) | 3.1 (25) | 4.2 (34) | 4.4 (36) | 4.3 (35) | 2.8 (23) | 250 | 30.9 |
| 2nd Normal Preg. | 5.8 (44) | 3.7 (24) | 3.5 (23) | 1.2 (8) | 2.6 (17) | 4.2 (27) | 1.7 (11) | 154 | 23.7 |

TABLE 13.

INCIDENCE OF TYPE OF DELIVERY

| | Caesarean Section % | Forceps Delivery % | Assisted Delivery % | Spontaneous % |
|---|---|---|---|---|
| 1st. Preg. | 4.0 | 16.1 | 1.7 | 78.2 |
| 2nd. Preg. 1st. Normal | 2.6 | 2.0 | 0.9 | 94.6 |
| 2nd. Preg. 1 Abortion | 5.9 | 19.6 | 2.9 | 71.6 |
| 3rd. Preg. 2 Abortions | 11.7 | 15.1 | 1.5 | 71.8 |

## TABLE 14.

## ANALYSIS OF CAESAREAN SECTIONS IN WOMEN HAVING 3rd. PREGNANCY WITH TWO PREVIOUS ABORTIONS

| Age | Height | Duration of Preg. (Wks) | Wt. of Baby (lbs & oz) lbs. | oz. | Reason for C.S. |
|---|---|---|---|---|---|
| 25 | 5' 1" | 37 | 5 | 13 | Placenta Praevia |
| 27 | 5' 4" | 39 | 6 | 1 | B.O.H. - Elective |
| 34 | ? | 39 | 5 | 9 | B.O.H. - Elective |
| 32 | 5' 1½" | 42 | 6 | 6 | B.O.H. - Elective |
| 31 | 5' 0½" | 40 | 7 | 15 | Breech |
| 35 | 5' 1" | 41 | 6 | 8 | B.O.H. - Elective |
| 33 | 5' 3" | 40 | 8 | 7 | B.O.H. - Elective |
| 26 | 5' 1½" | 38 | 3 | 8 | Foetal Distress |
| 26 | 5' 2½" | 38 | 6 | 13 | Foetal Distress |
| 25 | 4' 10" | 39 | 9 | 0 | Disproportion |
| 24 | 4' 11" | ? | 5 | 11 | Foetal Distress |
| 33 | 5' 0" | 41 | 7 | 11 | Incoordinate Uterine Action |
| 30 | 5' 2" | 38 | 6 | 13 | Breech - Elective |
| 31 | 5' 5" | 41 | 7 | 13 | B.O.H. - Elective |

## TABLE 15.

### SUBSEQUENT FERTILITY FOLLOWING 1st ABORTION and 1st NORMAL PREGNANCY

| | 1st Normal Pregnancy - (83 patients) | | | 1st Abortions - (104 patients) | | |
|---|---|---|---|---|---|---|
| No. of children | No. | % | % Cumulative | No. | % | Cum % |
| 1 | 32 | 38.5 | | 28 | 26.9 | |
| 2 | 21 | 25.3 | 63.8 | 41 | 39.4 | 66.3 |
| 3 | 6 | 7.2 | 71.0 | 14 | 13.4 | 79.7 |
| 4 | 6 | 7.2 | 78.2 | 4 | 3.8 | 83.5 |
| 5 | 6 | 7.2 | 85.4 | 0 | 0 | 83.5 |
| Nil | 12 | 14.4 | | 7 | 6.7 | |
| Abortions only | 0 | 0 | | 8 | 7.7 | |
| Pregnancies over 28 weeks but none alive | 0 | 0 | | 2 | 1.9 | |
| TOTALS | 83 | 99.8 | | 104 | 99.8 | |

TABLE 16.

TOTAL NUMBER OF ABORTIONS IN TWO GROUPS.

| 1 Previous Abortion Group (104 patients) | | | 1st. Normal Pregnancy Group (83 patients) | |
|---|---|---|---|---|
| Total No. of Abortions | No. of Patients | % | No. of Patients | % |
| 1 | 20 | 19.3 | 10 | 12.1 |
| 2 | 8 | 7.7 | 5 | 6.0 |
| 3 | 6 | 6.6 | 1 | 1.2 |
| 4 | 2 | 1.9 | 1 | 1.2 |
| 5 | 0 | | - | |
| 6 | 0 | | - | |
| 7 | 1 | 0.9 | - | |

## TABLE 17.

### NUMBERS WHO ABORTED IN SUBSEQUENT PREGNANCIES FOLLOWING 1st. ABORTION (TOTAL 83)

| | | |
|---|---|---|
| No. of women who aborted in 2nd Pregnancy | - | 26 (25%) |
| No. of women who aborted in 3rd Pregnancy after 2 previous abortions | - | 12 (11.5%) |
| % of those who aborted in 2nd Pregnancy | - | 46% |
| No. of women who aborted in 4th Pregnancy after 3 previous abortions | - | 7 (6.7%) |
| % of those who aborted in 3rd Pregnancy | - | 58.3% |

TABLE 18.

STUDIES OF RECURRENT ABORTION (Modified from Goldzieher & Benigno, 1958)

| Author | % of pregnancies aborted after 2 previous pregs. | 3 previous pregs. | 4 previous pregs. |
|---|---|---|---|
| ALDER (1953) | 30 | - | - |
| BEVIS (1951) | - | 20 | 20 |
| COLVIN ET AL (1950) | 23 | - | - |
| GUTERMAN (1953) | 6 | 25 | 67 |
| JONES & DELFS (1951) | 26 | 39 | 50 |
| ROBSON & GORNALL (1955) | - | 20 | - |
| RUCKER (1952) | - | 17 | - |
| SCHOENECK (1953) | 44 | 0 | - |
| SPEERT (1954) | - | 19 | 33 |
| PRESENT STUDY | 25 | 46 | 58 |
| RANGE OF ABORTION FREQUENCY | 6 - 44 | 0 - 46 | 20 - 67 |
| AVERAGE | 25.6% | 23.2% | 45.4% |

## TABLE 19.

### GESTATION PERIOD AT WHICH ABORTION OCCURS

1st. Pregnancy ending in abortion

| Time of Abortion | Number | % |
|---|---|---|
| Up to 2 months | 285 | 32.8 |
| 2 months - 3 months | 131 | 15.1 |
| 3 months - 4 months | 296 | 34.1 |
| Over 4 months | 157 | 18.1 |
| TOTAL | 869 | 100.1 |

## TABLE 20.

## GESTATION PERIOD AT WHICH ABORTION OCCURS

2nd. Pregnancy ending in abortion

| Time of Abortion | Number | % |
|---|---|---|
| Up to 2 months | 37 | 30.3 |
| 2 months - 3 months | 24 | 19.7 |
| 3 months - 4 months | 39 | 32.0 |
| Over 4 months | 22 | 18.0 |
| TOTAL | 122 | 100.0 |

## TABLE 21.

## GESTATION PERIOD AT WHICH ABORTION OCCURS

Third Pregnancy ending in abortion

| Time of Abortion | Number | % |
|---|---|---|
| Up to 2 months | 8 | 33.3 |
| 2 months - 3 months | 2 | 8.3 |
| 3 months - 4 months | 9 | 37.5 |
| Over 4 months | 5 | 20.8 |
| TOTAL | 24 | 99.9 |

TABLE 22.

COMPARISON OF GESTATION PERIODS OF FIRST AND SECOND ABORTIONS IN THE SAME SUBJECTS

| | | Second Abortion | | | | |
|---|---|---|---|---|---|---|
| | Gestation Period | 8 wks or less | 9-12 wks incl. | 13-16 wks incl. | 17 wks and over | TOTAL |
| 1st. Abortion | 8 weeks or less | 21 (56.8%) | 4 | 8 | 2 | 35 |
| | 9-12 weeks incl. | 1 | 16 (66.6%) | 3 | 4 | 24 |
| | 13-16 weeks incl. | 8 | 1 | 22 (56.4%) | 7 | 38 |
| | 17 weeks and over | 7 | 3 | 6 | 9 (41.0%) | 25 |
| | TOTAL | 37 | 24 | 39 | 22 | 122 |

TABLE 23.

INTERVAL BETWEEN 1st. & 2nd. PREGNANCY

(to nearest year)

| Interval in Years | Normal | | 1st. Abortion | |
|---|---|---|---|---|
| | No. | % | No. | % |
| 1 | 14 | 17.1 | 64 | 50.1 |
| 2 | 17 | 21.0 | 38 | 30.0 |
| 3 | 16 | 20.0 | 9 | 7.0 |
| 4 | 8 | 10.0 | 2 | 1.6 |
| 5 | 4 | 5.0 | 5 | 3.9 |
| 6 | 4 | 5.0 | 0 | - |
| 7 | 3 | 3.7 | 1 | 0.8 |
| 8 | 4 | 5.0 | 0 | - |
| 9 | 1 | 1.2 | 0 | - |
| 10 | 0 | - | 0 | - |
| Nil | 10 | 12.4 | 8 | 6.3 |
| TOTAL | 81 | 100.4 | 127 | 99.7 |

## TABLE 24.

## INTERVAL BETWEEN 2nd. & 3rd. PREGNANCY

| Interval in Years | 1st. Preg. Normal | | 1st. Preg. Abortion | |
|---|---|---|---|---|
| | No. | % | No. | % |
| 1 | 17 | 24.0 | 24 | 28.0 |
| 2 | 6 | 8.6 | 19 | 22.0 |
| 3 | 11 | 15.8 | 8 | 9.3 |
| 4 | 8 | 11.4 | 12 | 14.0 |
| 5 | 1 | 1.4 | 3 | 3.5 |
| 6 | 2 | 2.85 | 4 | 4.6 |
| 7 | 0 | - | 0 | - |
| 8 | 1 | 1.4 | 0 | - |
| 9 | 0 | - | 0 | - |
| 10 | 0 | - | 0 | - |
| Nil | 24 | 34.5 | 16 | 18.6 |
| TOTAL | 70 | 99.95% | 86 | 100.0% |

TABLE 25.

PREGNANEDIOL EXCRETION. NORMAL PREGNANCY

(from Klopper & Billewicz (1963)).

| Weeks of Pregnancy | Excretion in mg/24hr. (± SD). (Number of cases in brackets) | | |
|---|---|---|---|
| 5 | - | - | - |
| 6 | 6.18 | | (2) |
| 7 | 6.18 | | (2) |
| 8 | 7.67 | ± 1.169 | (5) |
| 9 | 7.91 | ± 2.544 | (6) |
| 10 | 8.43 | ± 1.825 | (7) |
| 11 | 10.06 | ± 2.751 | (7) |
| 12 | 10.58 | ± 2.239 | (8) |
| 13 | 11.94 | ± 3.003 | (8) |
| 14 | 11.77 | ± 3.019 | (5) |
| 15 | 12.91 | ± 3.560 | (5) |
| 16 | 12.38 | ± 5.166 | (5) |

## TABLE 26.

PREGNANEDIOL EXCRETION (± SD) IN PATIENTS WITH 2 OR MORE PREVIOUS ABORTIONS. PRESENT PREGNANCY SUCCESSFUL. (NUMBER OF CASES IN BRACKETS).

| Weeks of Pregnancy | Pregnanediol excretion mg/24hr. | | | |
|---|---|---|---|---|
| 5 | 8.6 | | | ( 3) |
| 6 | 5.6 | ± | 2.98 | ( 4) |
| 7 | 8.4 | ± | 1.09 | ( 9) |
| 8 | 8.8 | ± | 2.33 | ( 8) |
| 9 | 9.3 | ± | 2.49 | (11) |
| 10 | 10.2 | ± | 2.91 | (16) |
| 11 | 11.7 | ± | 3.26 | (14) |
| 12 | 12.7 | ± | 3.66 | (14) |
| 13 | 12.0 | ± | 4.27 | (14) |
| 14 | 14.1 | ± | 5.02 | (14) |
| 15 | 16.9 | ± | 5.83 | (10) |
| 16 | 15.0 | ± | 0.34 | (14) |

TABLE 27.

PREGNANEDIOL EXCRETION (± SD)

PATIENTS WHO ABORTED IN PRESENT PREGNANCY.

(NUMBER OF CASES IN BRACKETS).

| Weeks of Pregnancy | Pregnanediol excretion mg/24hr. | | | |
|---|---|---|---|---|
| 5 | 0.5 | | | ( 3) |
| 6 | 0.6 | ± | 2.47 | ( 5) |
| 7 | 5.7 | ± | 1.38 | ( 5) |
| 8 | 6.9 | ± | 3.47 | ( 8) |
| 9 | 7.9 | ± | 3.02 | (10) |
| 10 | 8.0 | ± | 3.16 | ( 9) |
| 11 | 7.7 | ± | 2.39 | ( 6) |
| 12 | 7.9 | ± | 2.73 | ( 6) |
| 13 | 7.8 | ± | 3.88 | ( 7) |
| 14 | 12.8 | ± | 1.59 | ( 4) |
| 15 | 10.6 | | | ( 3) |
| 16 | 13.5 | | | ( 2) |

TABLE 28.

OESTRIOL EXCRETION (± SD).

NORMAL EXCRETION.

(NUMBER OF CASES IN BRACKETS).

| Weeks of Pregnancy | Oestriol excretion mg/24hr. | | | |
|---|---|---|---|---|
| 5 | 0.045 | ± | 0.345 | (4) |
| 6 | 0.041 | ± | 0.169 | (5) |
| 7 | 0.101 | ± | 0.0433 | (7) |
| 8 | 0.119 | ± | 0.0545 | (8) |
| 9 | 0.254 | ± | 0.092 | (8) |
| 10 | 0.263 | ± | 0.222 | (7) |
| 11 | 0.52 | ± | 0.54 | (8) |
| 12 | 0.45 | ± | 0.267 | (8) |
| 13 | 0.56 | ± | 0.379 | (6) |
| 14 | 1.07 | ± | 0.734 | (7) |
| 15 | 1.78 | ± | 0.699 | (6) |
| 16 | 2.88 | ± | 0.749 | (5) |

TABLE 29.

OESTRIOL EXCRETION (± SD).

PATIENTS WITH 2 OR MORE PREVIOUS ABORTIONS.

PRESENT PREGNANCY SUCCESSFUL.

(NUMBER OF PATIENTS IN BRACKETS).

| Weeks of Pregnancy | Oestriol excretion mg/24hr. |
|---|---|
| 5 | 0.058 ± 0.012 ( 4) |
| 6 | 0.112 ± 0.049 ( 5) |
| 7 | 0.082 ± 0.031 ( 6) |
| 8 | 0.219 ± 0.126 ( 8) |
| 9 | 0.216 ± 0.134 ( 9) |
| 10 | 0.304 ± 0.167 (11) |
| 11 | 0.400 ± 0.364 (12) |
| 12 | 0.633 ± 0.258 (11) |
| 13 | 0.799 ± 0.502 (10) |
| 14 | 0.980 ± 0.340 (12) |
| 15 | 1.03 ± 0.598 (11) |
| 16 | 2.06 ± 0.626 ( 8) |

TABLE 30.

OESTRIOL EXCRETION (± SD).

PATIENTS WHO ABORTED IN PRESENT PREGNANCY.

(NUMBER OF PATIENTS IN BRACKETS).

| Weeks of Pregnancy | Oestriol excretion mg/24hr. | | | |
|---|---|---|---|---|
| 5 | 0.078 | | | (2) |
| 6 | 0.065 | | | (3) |
| 7 | 0.049 | ± | 0.0024 | (4) |
| 8 | 0.052 | ± | 0.0414 | (4) |
| 9 | 0.063 | ± | 0.0591 | (4) |
| 10 | 0.063 | | | (3) |
| 11 | 0.13 | ± | 0.198 | (4) |
| 12 | 0.39 | | | (2) |
| 13 | 0.3 | | | (2) |
| 14 | 0.93 | | | (3) |
| 15 | 0.09 | | | (2) |
| 16 | 1.765 | | | (2) |

TABLE 11.

RATIO OF EXCRETION OF PREGNANEDIOL & OESTRIOL (±SD).

NORMAL RATIO.

(NUMBER OF CASES IN BRACKETS).

| Weeks of Pregnancy | Ratio |
|---|---|
| 5 | 181.7 ± 93.8 (4) |
| 6 | 147.9 ± 64.2 (5) |
| 7 | 92.8 ± 32.1 (7) |
| 8 | 65.4 ± 20.6 (8) |
| 9 | 51.1 ± 33.4 (8) |
| 10 | 46.6 ± 17.2 (7) |
| 11 | 36.4 ± 29.2 (8) |
| 12 | 37.8 ± 33.5 (8) |
| 13 | 34.6 ± 41.1 (6) |
| 14 | 22.0 ± 24.1 (7) |
| 15 | 7.5 ± 6.71 (6) |
| 16 | 5.2 ± 3.16 (5) |

TABLE 32.

RATIO OF EXCRETION OF PREGNANEDIOL & OESTRIOL (± SD).

PATIENTS WITH 2 OR MORE PREVIOUS ABORTIONS.

PRESENT PREGNANCY SUCCESSFUL.

(NUMBER OF CASES IN BRACKETS).

| Weeks of Pregnancy | Ratio |
|---|---|
| 5 | 159.8 ± 57.9 ( 5) |
| 6 | 57.65 ± 46.8 ( 4) |
| 7 | 79.49 ± 41.5 ( 6) |
| 8 | 49.57 ± 26.9 ( 8) |
| 9 | 60.25 ± 40.12 ( 9) |
| 10 | 43.3 ± 24.4 (11) |
| 11 | 55.95 ± 24.8 (12) |
| 12 | 31.85 ± 20.9 (11) |
| 13 | 18.26 ± 10.4 (10) |
| 14 | 14.4 ± 7.46 (12) |
| 15 | 10.85 ± 6.5 (11) |
| 16 | 7.5 ± 6.5 ( 8) |

TABLE 33.

RATIO OF EXCRETION OF PREGNANEDIOL & OESTRIOL (± SD).

PATIENTS WHO ABORTED IN PRESENT PREGNANCY.

(NUMBER OF CASES IN BRACKETS).

| Weeks of Pregnancy | Ratio | | | |
|---|---|---|---|---|
| 5 | 74.6 | | | (2) |
| 6 | 93.8 | ± | 29.4 | (3) |
| 7 | 112.3 | ± | 31.7 | (4) |
| 8 | 112.6 | ± | 24.5 | (4) |
| 9 | 74.5 | ± | 35.6 | (4) |
| 10 | 72.7 | ± | 59.6 | (3) |
| 11 | 92.4 | ± | 57.8 | (4) |
| 12 | 29.3 | | | (2) |
| 13 | 51.6 | | | (2) |
| 14 | 15.1 | ± | 6.7 | (3) |
| 15 | 18.6 | | | (2) |
| 16 | - | | | |

## TABLE 34.

### URINARY EXCRETION OF OESTROGEN AND PREGNANEDIOL IN HYDATIDIFORM MOLE

| Case No. | Weeks of Pregnancy from L.M.P. | Oestrone µg/24hr. | Oestradiol µg/24hr. | Oestriol µg/24hr. | Ratio of Oestriol: Oestrone & Oestradiol µg/24hr. | Pregnanediol mg/24hr. |
|---|---|---|---|---|---|---|
| 1 | 10 | 40 | 26.5 | 155 | 2.3 | 5.92 |
| | 11 | 16.3 | 73.0 | 385 | 1.6 | 10.5 |
| | 12 | 290 | 125 | 565 | 1.3 | 22.0 |
| | 13 | 308 | 107 | 635 | 1.5 | 27.0 |
| | 14 | 388 | 176 | 742 | 1.4 | 31.0 |
| | 16 | 264 | 100 | 828 | 2.2 | 21.0 |
| | 17 | 504 | 108 | 840 | 1.3 | 31.5 |
| 2 | 11 | 25 | 17.5 | 122 | 2.8 | 6.5 |
| | 12 | 5.5 | 9.0 | 44 | 3.0 | 6.0 |
| | 14 | 4.0 | 2.5 | 31 | 4.7 | 2.43 |
| | 17 | 27.0 | 9.5 | 44.5 | 1.2 | 2.5 |
| | 19 | 11.7 | 2.7 | 14.8 | 1.0 | 0.8 |
| 3 | 15 | - | - | - | - | 23 |
| | 16 | - | - | - | - | 21 |
| | 17 | - | - | - | - | 23 |
| | 18 | - | - | - | - | 17 |
| | 19 | - | - | 1832 | - | 24.5 |
| 4 | 16 | - | - | 146 | - | 4.5 |
| | 17 | - | - | 104 | - | 7.5 |
| | 18 | - | - | - | - | 7.5 |
| | 19 | - | - | - | - | 8.3 |
| | 20 | - | - | 173 | - | 9.0 |
| 5 | 11 | - | - | 134 | - | 4.0 |
| | 12 | - | - | 56 | - | 3.0 |
| | 13 | - | - | 144 | - | 4.0 |
| | 14 | - | - | 154 | - | 5.5 |
| | 15 | - | - | 160 | - | 6.5 |
| | 16 | - | - | 84 | - | 6.5 |

## EXCRETION OF OESTROGEN, GONADOTROPHIN AND PREGNANEDIOL IN CASES OF HYDATIDIFORM MOLE

(from Frandsen & Stakeman, 1964)

| Case | Weeks of Amenorrhoea | $OE_1$ µg/24hr. | $OE_2$ µg/24hr. | $OE_3$ µg/24hr. | $OE_3/OE_2$ | G IU/24hr. | P mg/24hr. |
|---|---|---|---|---|---|---|---|
| K.K. | 19 | - | - | 85 | | - | |
| | 20 | - | - | 210 | | - | |
| I.N. | 25 | 21 | 12 | 104 | 3.1 | 132,000 | 3.2 |
| | 26 | 26 | 12 | 138 | 3.1 | 650,000 | 3.2 |
| A.O. | 15 | - | - | 269 | | 700,000 | |
| B.A. | 16 | 106 | 45 | 219 | 1.9 | 600,000 | 3.6 |
| | 17 | - | - | 280 | | | |
| G.B. | 15 | - | - | 525 | | - | |
| | 18 | - | - | 1100 | | - | |
| K.S. | 18 | - | - | 1100 | | 24,000 | |
| L.M. | 20 | - | - | 43 | | - | |
| L.B. | 12 | 117 | 29 | 125 | 0.9 | 854,000 | 7.6 |
| | 13 | - | - | 132 | | 2470,000 | |
| | 14 | 77 | 55 | 130 | 1.0 | 2500,000 | 7.7 |
| | 15 | 252 | 187 | 240 | 0.5 | 2540,000 | 5.6 |
| | 16 | - | - | 194 | | 5700,000 | |
| | 17 | - | - | 425 | | 8400,000 | |
| B.H. | 11 | - | - | 184 | | - | |
| | 15 | 62 | 19 | 102 | 1.3 | 240,000 | 1.9 |
| I.E. | 32 (?) | 94 | 58 | 335 | 2.2 | 46,000 | 3.4 |

$OE_1$ - Oestrone
$OE_2$ - Oestradiol
$OE_3$ - Oestriol
G - Gonadotrophin
P - Pregnanediol

## TABLE 36.

DAILY AND 5 DAY TOTAL PERCENTAGE RECOVERY OF I.V. INJECTED TRITIATED PROGESTERONE AS URINARY PREGNANEDIOL IN A GROUP OF NON-PREGNANT PATIENTS.

| | Daily % Recovery | | | | | |
|---|---|---|---|---|---|---|
| Case | 1 | 2 | 3 | 4 | 5 | Total % Recovery |
| 1 | 3.56 | 1.45 | 0.43 | 0.15 | 0.07 | 5.69 |
| 2 | 8.91 | 1.45 | 0.77 | 0.08 | 0.13 | 11.34 |
| 3 | 3.47 | 6.05 | 3.47 | 1.19 | 0.03 | 14.21 |
| 4 | 6.57 | 0.96 | 0.54 | 0.33 | 0.11 | 8.51 |
| 5 | 2.92 | 2.07 | 0.54 | 0.28 | 0.25 | 6.06 |

TABLE 37.

DAILY AND 5 DAY TOTAL PERCENTAGE RECOVERY OF I.V. INJECTED TRITIATED PROGESTERONE AS URINARY PREGNANEDIOL IN ELEVEN SUBJECTS 7 - 19 WEEKS PREGNANT

| Case | Week of Gestation | Daily % Recovery 1 | 2 | 3 | 4 | 5 | Total % Recovery |
|---|---|---|---|---|---|---|---|
| 6 | 7 | 5.02 | 1.60 | 0.77 | 0.79 | 0.46 | 8.64 |
| 7 | 8 | 10.60 | 3.32 | 1.18 | 0.14 | 0.43 | 15.67 |
| 8 | 10 | 2.31 | 1.50 | 0.30 | 0.29 | 0.26 | 4.66 |
| 9 | 12 | 7.10 | 1.70 | 1.10 | 0.50 | 0.15 | 10.55 |
| 10 | 13 | 11.36 | 2.10 | 1.30 | 0.56 | 0.09 | 15.14 |
| 11 | 15 | 8.80 | | 0.78 | 0.75 | 0.46 | 10.79 |
| 12 | 15 | 3.84 | 0.70 | 0.38 | 0.16 | 0.14 | 5.22 |
| 13 | 18 | 15.70 | 2.60 | 0.86 | 0.30 | 0.13 | 19.59 |
| 14 | 16 | 5.67 | 0.38 | 0.33 | 0.19 | 0.24 | 6.81 |
| 15 | 18 | 8.28 | 2.00 | 0.62 | 0.24 | 0.14 | 11.28 |
| 16 | 19 | 5.95 | 0.88 | 0.13 | 0.34 | 0.14 | 7.44 |

TABLE 36.

DAILY AND 5 DAY TOTAL PERCENTAGE RECOVERY OF I.V. INJECTED TRITIATED PROGESTERONE AS URINARY PREGNANEDIOL IN SUBJECTS 39 and 40 WEEKS PREGNANT

| Case | Week of Gestation | Daily % Recovery 1 | 2 | 3 | 4 | 5 | Total % Recovery |
|---|---|---|---|---|---|---|---|
| 17 | 39 | 4.68 | 0.34 | 0.45 | 0.22 | 0.11 | 5.80 |
| 18 | 40 | 13.60 | 2.24 | 0.28 | 0.16 | 0.07 | 16.35 |
| 19 | 40 | 6.23 | 1.97 | 0.53 | 0.18 | 0.07 | 8.98 |

TABLE 39.

DAILY AND 5 DAY TOTAL PERCENTAGE RECOVERY OF I.V. INJECTED TRITIATED PROGESTERONE AS URINARY PREGNANEDIOL IN ABNORMAL PREGNANCY

| Case | Week of Gestation | Abnormality | Daily % Recovery 1 | 2 | 3 | 4 | 5 | Total % Recovery |
|---|---|---|---|---|---|---|---|---|
| 20 | 10 | 'mole | 3.60 | 0.88 | 0.33 | 0.21 | 0.07 | 5.09 |
| 21 | 10 | Abortion | 2.02 | 1.29 | 0.84 | 0.08 | 0.25 | 4.28 |
| 22 | 11 | 'mole | 1.04 | 0.25 | 0.13 | - | - | 1.42 |
| 23 | 14 | 'mole | 1.96 | 0.75 | 0.26 | 0.05 | 0.15 | 3.17 |
| 24 | 18 | 'mole | 7.38 | 1.55 | 0.58 | 0.29 | 0.10 | 9.90 |
| 25 | 34 | Sev. P.E.T. | 3.85 | 1.22 | 0.28 | 0.23 | 0.12 | 5.70 |
| 26 | 34 | Sev. P.E.T. | 2.32 | 1.03 | 0.15 | 0.08 | 0.03 | 3.61 |
| 27 | 35 | Sev. P.E.T. | 5.92 | 0.82 | 0.25 | 0.15 | 0.08 | 7.22 |
| 28 | 37 | Anenceph. | 6.14 | 0.42 | 0.54 | 0.23 | 0.13 | 7.46 |

'Mole - Hydatidiform mole.

Anenceph - Anencephalic foetus.

Sev. P.E.T. - Severe pre-eclamptic toxaemia.

## TABLE 40.

COMPARISON OF THE AVERAGE TOTAL PERCENTAGE RECOVERED OF THE TRITIATED PROGESTERONE INJECTED AS URINARY PREGNANEDIOL IN THE FIRST FIVE DAYS POST INJECTION IN FOUR GROUPS OF WOMEN

| | No. of Cases | Average % Recovered | Standard Deviation |
|---|---|---|---|
| Non-pregnant | 5 | 9.16 | ± 3.62 |
| 7-10 week gestation | 11 | 10.53 | ± 4.69 |
| 39 & 40 week gestation | 3 | 10.38 | ± 5.41 |
| Abnormal pregnancy | 9 | 5.32 | ± 2.58 |

TABLE 41.

DAILY SPECIFIC ACTIVITIES OF

mg/24hr. urinary pregnanediol diacetate isolated

| Case | | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| N.P. | 1 | 5.73 | 7.89 | 6.60 | 5.23 | 5.57 |
| | 2 | 7.31 | 6.30 | 8.82 | 2.52 | 4.33 |
| | 3 | 1.40 | 17.20 | 7.06 | 3.65 | 2.70 |
| | 4 | 3.26 | 2.27 | 2.77 | 2.77 | 3.54 |
| | 5 | 5.65 | 4.03 | 3.86 | 4.37 | 2.86 |
| E.P. | 6 | 17.64 | 13.23 | 12.60 | 12.60 | 12.60 |
| | 7 | 15.75 | 11.97 | 9.14 | 10.10 | 15.75 |
| | 8 | 5.04 | 10.46 | 3.65 | 5.54 | 16.70 |
| | 9 | 6.30 | 5.88 | 7.80 | 8.19 | 5.04 |
| | 10 | 18.90 | 19.28 | 16.38 | 16.38 | 5.67 |
| | 11 | 10.71 | | 6.30 | 10.08 | 8.82 |
| | 12 | 10.40 | 7.88 | 8.32 | 3.65 | 15.12 |
| | 13 | 25.20 | 25.20 | 22.68 | 22.05 | 25.94 |
| | 14 | 12.60 | 10.08 | 8.82 | 10.08 | 8.19 |
| | 15 | 7.56 | 8.19 | 11.34 | 5.80 | 9.70 |
| | 16 | 17.64 | 20.79 | 15.75 | 14.49 | 13.23 |
| L.P. | 17 | 12.92 | 8.82 | 21.42 | 25.33 | 21.42 |
| | 18 | 74.34 | 99.54 | 79.38 | 85.68 | 90.72 |
| | 19 | 40.95 | 63.00 | 64.26 | 54.18 | 52.92 |
| A.P. | 20 | 9.77 | 13.23 | 10.71 | 16.38 | 13.23 |
| | 21 | 8.82 | 12.60 | 8.82 | 5.04 | 9.45 |
| | 22 | 6.68 | 10.58 | 6.30 | - | - |
| | 23 | 6.30 | 8.19 | 3.78 | 3.15 | 8.19 |
| | 24 | 9.45 | 13.80 | 8.32 | 11.21 | 10.52 |
| | 25 | 18.14 | 19.03 | 12.16 | 16.00 | 13.29 |
| | 26 | 18.90 | 18.77 | 19.66 | 12.73 | 9.51 |
| | 27 | 11.34 | 8.82 | 7.06 | 10.71 | 14.49 |
| | 28 | 18.90 | 8.19 | 22.68 | 22.68 | 22.68 |

PREGNANEDIOL DIACETATE ISOLATED

Daily Specific Activity in Muc/mg.

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| 621 | 183.8 | 65.2 | 34.4 | 12.6 |
| 1218.9 | 230.2 | 87.3 | 31.7 | 30.0 |
| 2478.6 | 351.7 | 491.5 | 326.0 | 11.1 |
| 2015.3 | 422.9 | 195.0 | 119.1 | 31.1 |
| 516.8 | 513.6 | 139.9 | 64.1 | 87.4 |
| 284.6 | 120.9 | 61.1 | 62.7 | 36.5 |
| 673.0 | 277.8 | 129.1 | 13.9 | 27.5 |
| 458.3 | 143.4 | 82.2 | 52.4 | 15.6 |
| 1127.0 | 289.1 | 141.0 | 61.1 | 29.8 |
| 601.1 | 108.9 | 62.9 | 34.2 | 15.9 |
| 821.7 | | 123.8 | 74.4 | 52.2 |
| 369.2 | 88.8 | 45.6 | 43.8 | 9.3 |
| 623.0 | 103.2 | 57.9 | 13.6 | 5.4 |
| 472.2 | 85.4 | 14.7 | 33.7 | 17.1 |
| 750.0 | 46.4 | 29.1 | 32.8 | 24.7 |
| 469.4 | 96.2 | 39.4 | 16.6 | 10.6 |
| 362.2 | 38.5 | 21.0 | 8.7 | 5.1 |
| 182.9 | 22.5 | 3.5 | 1.9 | 0.8 |
| 152.1 | 31.1 | 8.3 | 3.3 | 1.3 |
| 368.5 | 66.5 | 30.8 | 12.8 | 5.3 |
| 229.0 | 102.4 | 72.6 | 15.9 | 26.5 |
| 155.7 | 25.6 | 20.6 | - | - |
| 311.1 | 91.6 | 68.8 | 15.9 | 18.3 |
| 781.0 | 112.3 | 70.0 | 25.9 | 9.5 |
| 212.2 | 64.1 | 23.0 | 14.4 | 9.0 |
| 122.8 | 54.9 | 7.6 | 6.3 | 3.2 |
| 522.1 | 93.0 | 35.4 | 14.0 | 5.5 |
| 324.9 | 51.3 | 23.8 | 10.1 | 5.7 |

TABLE 42.

URINARY PREGNANEDIOL EXCRETION PERCENTAGE CONVERSION OF TRITIATED PROGESTERONE TO URINARY PREGNANEDIOL AND SPECIFIC ACTIVITY OF THE PREGNANEDIOL IN A WOMAN WITH NO UTERUS

| Day | Pregnanediol mg. | % Conversion to pregnanediol | Specific Activity of pregnanediol diacetate (mμc/mg). |
|---|---|---|---|
| 1 | 1.36 | 0.16 | 360.2 |
| 2 | 1.48 | 0.08 | 363.6 |
| 3 | 1.24 | 0.62 | 397.4 |
| 4 | 1.70 | 0.25 | 116.8 |
| 5 | 1.27 | 0.15 | 93.8 |

Total % converted - 7.86%

## TABLE 43.

## COMPARISON OF RESULTS IN TWO PATIENTS IN THE NON-PREGNANT AND PREGNANT STATES.

| Patient 1. | Case 5. Non-Pregnant | | Case 21 Pregnant | |
|---|---|---|---|---|
| Day | % Recovered | S.A. | % Recovered | S.A. |
| 1 | 2.92 | 516.8 | 2.02 | 329.0 |
| 2 | 2.07 | 515.6 | 1.29 | 102.4 |
| 3 | 0.54 | 139.9 | 0.64 | 72.6 |
| 4 | 0.25 | 64.8 | 0.08 | 15.9 |
| 5 | 0.26 | 87.4 | 0.25 | 26.5 |
| Total | 6.06 | | 4.28 | |

| Patient 2. | Case 4. Non-Pregnant | | Case 8 Pregnant | |
|---|---|---|---|---|
| Day | % Recovered | S.A. | % Recovered | S.A. |
| 1 | 6.57 | 2015.3 | 2.31 | 458.3 |
| 2 | 0.96 | 422.9 | 1.50 | 143.4 |
| 3 | 0.54 | 195.0 | 0.30 | 82.2 |
| 4 | 0.33 | 119.1 | 0.29 | 52.4 |
| 5 | 0.11 | 31.1 | 0.26 | 15.6 |
| Total | 8.51 | | 4.66 | |

(S.A. = Specific Activity)

TABLE 44.

URINARY PREGNANEDIOL EXCRETION, PERCENTAGE CONVERSION OF TRITIATED PROGESTERONE TO URINARY PREGNANEDIOL, AND SPECIFIC ACTIVITY OF THE PREGNANEDIOL DIACETATE ISOLATED IN CASE 30 - 38 WEEKS PREGNANT, LOW PREGNANEDIOL EXCRETION AND DELIVERY OF LIVE CHILD

| Day | Pregnanediol mg. | % Converted to Pregnanediol | Specific Activity of pregnanediol diacetate (mμc/mg) |
|---|---|---|---|
| 1 | 3.25 | 0.39 | 95.1 |
| 2 | 2.5 | 0.06 | 19.1 |
| 3 | 2.0 | 0.02 | 7.9 |
| 4 | 2.85 | 0.01 | 2.8 |
| 5 | 2.15 | 0.005 | 1.9 |

Total % converted - 0.49%

TABLE 45.

DISTRIBUTION OF RADIOACTIVE COMPOUNDS IN THE 'FREE' FRACTION OF LIVER AND ADRENALS 14 MIN. AFTER INJECTION OF [4-$^{14}$C] PROGESTERONE INTO A HUMAN FOETUS

| Metabolite | % of Total Radioactivity present in | |
|---|---|---|
| | Liver | Adrenals |
| Polar Material | 16.6 | 47.0 |
| Pregnanediol | 19.2 | 13.3 |
| 20α-dihydroprogesterone | 36.5 | 17.8 |
| 20β-dihydroprogesterone | 11.0 | 6.7 |
| Pregnanolone | 3.2 | 0 |
| Progesterone | 13.4 | 15.6 |

TABLE 46.

DISTRIBUTION OF RADIOACTIVE COMPOUNDS IN THE 'CONJUGATED' FRACTIONS OF LIVER AND ADRENALS 14 MIN. AFTER INJECTION OF [4-$^{14}$C] PROGESTERONE INTO A HUMAN FOETUS

| Metabolite | % of Total Radioactivity present in the two conjugated fractions | | | |
|---|---|---|---|---|
| | Liver | | Adrenals | |
| | 1 | 2 | 1 | 2 |
| Polar Material | 20.5 | 29.8 | 45.8 | 100 |
| Pregnanediol | 31.9 | 54.6 | 39.9 | - |
| 20α-dihydroprogesterone | 19.4 | - | 0.0 | - |
| 20β-dihydroprogesterone | 9.9 | - | 0.0 | - |
| Pregnanolone | 7.5 | 9.4 | 0.0 | - |
| Progesterone | 11.1 | 6.2 | 14.3 | - |

TABLE 47.

COMPARISON BETWEEN 'FREE' AND 'CONJUGATED' STEROIDS PRESENT IN LIVER AND ADRENALS SHOWING THE PERCENTAGE PRESENT AS 'FREE' STEROID 14 MIN. AFTER INJECTION OF [4-$^{14}$C] PROGESTERONE INTO A HUMAN FOETUS

| Metabolite | LIVER | | | ADRENALS | | |
|---|---|---|---|---|---|---|
| | Amount of Radio-activity present (mμc) | | % of metabolite in free form | Amount of Radio-activity present (mμc) | | % of metabolite in free form |
| | Free | Conj | | Free | Conj | |
| Polar Material | 122.0 | 15.9 | 88 | 26.3 | 7.0 | 79 |
| Pregnanediol | 141.0 | 25.4 | 85 | 7.5 | 3.5 | 68 |
| 20α-dihydroprogesterone | 272.0 | 12.2 | 96 | 10.0 | - | 100 |
| 20β-dihydroprogesterone | 81.3 | 6.2 | 93 | 3.7 | - | 100 |
| Pregnanolone | 23.7 | 5.6 | 81 | - | - | - |
| Progesterone | 98.7 | 7.6 | 93 | 8.7 | 1.2 | 88 |

TABLE 48.

FREE COMPOUNDS PRESENT IN FOETAL TISSUES AND PLASMA AFTER PERFUSION WITH [4-$^{14}$C] PROGESTERONE

| Tissue | % of Steroid in Tissue | | | | | |
|---|---|---|---|---|---|---|
| | Polar Material | Pregnane-diol | 20αdihydro-progesterone | Pregnan-olone | Prog-esterone | Pregnane-dione |
| Gonads | 100 | - | - | - | - | - |
| Spleen | - | - | - | - | - | - |
| Brain | - | 34.5 | - | - | 65.5 | - |
| Kidneys | 52.8 | - | - | - | 47.2 | - |
| Adrenals | 60.0 | - | - | - | 40.0 | - |
| Lungs | 19.4 | 16.9 | 10.2 | 12.7 | 35.2 | 5.5 |
| Heart | - | - | 8.8 | 5.5 | 76.3 | 10.8 |
| Intestine | 8.8 | 31.4 | 4.8 | 23.0 | 19.9 | 12.1 |
| Liver | 17.1 | 69.0 | - | - | 13.1 | - |
| Foetus (residual tissues) | 28.2 | - | 18.6 | 27.3 | 12.1 | 12.7 |
| Plasma | | | | | | |
| 0 - 15 min. | 15.8 | - | 16.1 | - | 66.5 | 1.4 |
| 15 - 30 min. | 9.3 | - | 27.7 | - | 63.0 | - |
| 30 - 45 min. | 7.0 | - | 36.0 | - | 57.0 | 6.3 |

TABLE 49.

METABOLITES ISOLATED FROM THE INCUBATION OF MOLE TISSUE WITH [4-$^{14}$C] PREGNENOLONE

| Material Investigated | Chemical Reaction | Chromatographic mobility identical with |
|---|---|---|
| Pregnenolone | -- | Pregnenolone |
| | Acetylation | Pregnenolone acetate |
| | Reduction | Pregn-5-ene-3β,20β-diol |
| Progesterone | -- | Progesterone |
| | Acetylation | Progesterone |
| | Oxime formation | Progesterone dioxime (2 isomers) |
| | Reduction | 20β-hydroxypregn-4-ene-3-one |
| 17α-Hydroxy-pregnenolone | -- | 17α-hydroxypregnenolone |
| | Acetylation | 17α-hydroxypregnenolone acetate |
| 16α-Hydroxy-progesterone | -- | 16α-hydroxyprogesterone |
| | Acetylation | 16α-hydroxyprogesterone acetate |
| | Oxidation | 16-oxo progesterone |
| 16β-Hydroxy-progesterone | -- | 16β-hydroxyprogesterone |
| | Acetylation | 16α-hydroxyprogesterone acetate |

## TABLE 50.

### CONCENTRATIONS OF STEROIDS AND STEROID PRECURSORS FOUND IN THE EXTRACTS OF MOLE TISSUE AND CYST FLUID

| Substance | Mole Tissue (µg/100g. wet weight) | Cyst Fluid (µg/100ml) |
|---|---|---|
| Cholesterol | 30,000 | 12,500 |
| Pregnenolone | 1,200 | 850 |
| 17α-Hydroxypregnenolone | 120 | 1,000 |
| Progesterone | Trace (not characterised) | 1,100 |
| 17α-Hydroxyprogesterone | - | 1,000 |
| Pregnanediol | 370 | 1,600 |
| Pregnanetriol | 230 | 1,350 |
| Androstenedione | 300 | 400 |

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## Publications

1. Some observations on the exeretion of pregnanediol in the urine of women with a bad obstetric history. (1960). Advance Abstract, 1st. International Congress of Endocrinology, Copenhagen.

2. Pregnancy following abortion. (1961). J. Obstet. Gynaec. Br. Commonw. 68, 789.

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