THE PRODUCTION AND METABOLISM OF CORTICOSTEROIDS IN PREGNANCY

by

Stella Sybulski, B.Sc., M.Sc.

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Department of Biochemistry,
McGill University,
Montreal.

April 1959.

ACKNOWLEDGEMENTS

The work presented in this thesis was carried out in the Department of Investigative Medicine at McGill University. I wish to thank Dr. J.S.L. Browne for the laboratory facilities provided and for financial assistance. I am grateful to the Banting Research Foundation for financial support during the greater part of this study.

The criticism and guidance of Dr. E.H. Venning is greatly appreciated.

I wish to thank Dr. I. Dyrenfurth for her interest and suggestions. A fellow student, Mr. O.J. Lucis, kindly gave assistance upon many occassions for which I am most grateful.

Dr. C.J.P. Giroud and Dr. D. Das Gupta are thanked for carrying out the difficult bioassay of isolated aldosterone, Mr. W. Zaharia for the determination of an infrared spectrum on isolated progesterone, and Dr. V. Pollak for providing urine collections from 2 of his patients.

Mr. Frank Dylak, Dr. I. Silvaszy, Mr. W. Otterstatter and Mr. J. Druyan are also thanked for their cooperation and Mrs. R. Ross and Miss J. McKenzie for the typing of this thesis.

TABLE OF CONTENTS

INDEX OF TABLES

INTRODUCTION

The numerous observations of increased levels of blood and urinary 17-hydroxycorticosteroids in human pregnancy associated with a rising output of 17-ketosteroids and aldosterone have suggested to many investigators that adrenal cortical function is increased in this condition. In some pregnant women with adrenal insufficiency increased urinary corticosteroids and 17-ketosteroids have also been reported and the possibility that the placenta may be elaborating corticosteroids has frequently been suggested.

The following study was undertaken to determine whether it was possible to demonstrate placental elaboration of corticosteroids and to investigate the possibility of increased adrenal secretion of these steroids during pregnancy. It was also considered that changes in the rate of corticosteroid metabolism might occur during gestation which could also account for the estimation of increased amounts of these steroids in the blood and urine.

The first experiments carried out were the incubations of human placentae obtained at caesarian section under a variety of 'in vitro' conditions; the placental tissue was minced and incubated alone and also with certain trophic hormones and precursors of adrenal cortical steroids. It subsequently became possible to study the urinary excretion of aldosterone, 17-hydroxycorticosteroids and certain metabolites of progesterone

in 2 totally adrenalectomized women in the last trimester of gestation. The findings in these patients were compared with those obtained in normal pregnant and non-pregnant subjects and permitted an evaluation of the role of the placenta and maternal and foetal adrenal glands with respect to production of 21 carbon steroids.

In addition certain studies were carried out on the rat to determine if pregnancy affected the rate of corticosteroid production and metabolism. The rate of corticosteroid secretion by adrenal glands of pregnant and non-pregnant female rats was compared in both 'in vitro' and 'in vivo' studies which included incubations of adrenal glands and collection of adrenal venous blood. The possibility that alterations in the rate of corticosteroid metabolism in pregnancy could be demonstrated directly at the level of liver function was investigated in incubations of liver tissue from pregnant and non-pregnant female rats with steroids under 'in vitro' conditions. Rat placental tissue was also incubated 'in vitro' to determine if an elaboration of adrenal-like hormones could be observed.

THE PRODUCTION AND METABOLISM OF CORTICOSTEROIDS IN PREGNANCY

A REVIEW OF THE LITERATURE

1. ADRENAL FUNCTION IN HUMANS.

A. PRODUCTION OF ADRENAL CORTICAL HORMONES

The human adrenal cortex secretes several hormones of steroidal nature which are necessary for the maintenance of life.

These corticosteroids are essentially of two main types. One group, the so-called glucocorticoid, plays an important role in the regulation of carbohydrate metabolism and includes hydrocortisone, cortisone, corticosterone and 11-dehydrocorticosterone. The other group, the mineralocorticoid, is active in the regulation of salt and water metabolism. It includes aldosterone (which is the most potent sodium-retaining hormone known), desoxycorticosterone and 17-hydroxy-11-desoxycorticosterone.

Experimental work on rat and beef adrenals by Giroud et al (1)(2) and subsequently on human adrenals by Ayres et al (13) has shown that the glucocorticoids are secreted mainly by the zona fasciculata and reticularis while aldosterone is produced predominantly by cells of the zona glomerulosa.

The secretion of glucorcorticoids by the adrenal glands was shown to be regulated by the secretion of adrenocorticotrophin of the anterior pituitary (15)(16)(17). While ACTH stimulated aldosterone secretion in vivo (3)(4)(5)(6)(7) and under

in vitro conditions (8) it does not appear to play the major role in the regulation of the secretion of this hormone. Other factors including salt intake (9), body fluid volume (10)(11) (12) and lesions of certain hypothalamic areas were shown to influence aldosterone secretion as well (14).

B. OBSERVATIONS ON ADRENAL FUNCTION IN PREGNANCY

1. Pregnancy in Normal Women

A number of determinations of plasma and urinary levels of adrenal cortical hormones in human pregnancy have given indication of an increased adrenal function.

Venning (20) found elevated glucocorticoid levels, especially during the last trimester when they increased from about 40 to 200 glycogenic units per day. Tobian (21) reported double the non-pregnancy levels of formaldehydogenic steroids in urine of pregnant women. Gemzell (22) observed a four-fold increase of plasma 17-hydroxycorticosteroids (17-OHCS) above non-pregnancy values. Garst and Assali (23) and Christy et al (24) reported similar increases.

A still more pronounced rise in the urinary excretion of aldosterone has been observed during pregnancy. Venning and Dyrenfurth (25) and Venning et al (26) reported a progressive increase in the excretion of this Na-retaining hormone. Genest et al (27) found values of more than 100 ug per 24 hours. Martin and Mills (28), Rinsler and Rigby (29) and Laidlaw et al (30) have all made similar observations.

2. Pregnancy in Women with Rheumatoid Arthritis

Hench (31) first reported that some patients with rheumatoid arthritis became considerably improved in health during pregnancy. A similar observation following cortisone administration to non-pregnant patients with this disease was made by Hench et al (32). The authors concluded that the beneficial effect of pregnancy in patients with rheumatoid arthritis was related to an increased plasma level of 17-hydroxycorticosteroids as later observed by Robinson et al (33).

3. Pregnancy in Patients with Adrenal Insufficiency

The study of adrenal function in cases of Addison's disease complicated by pregnancy has been of special interest to a number of investigators.

Prior to the advent of corticosteroid replacement therapy pregnancy was considered to be fatal in these patients. In 1908 French (34) reported on two Addisonian women who died during pregnancy and Fitzpatrick (35) in a 1922 review of 11 cases stated that all died during pregnancy or shortly after delivery.

Since substitution therapy has become available treated patients usually survive pregnancy and deliver normally; however, they generally require careful supervision throughout gestation. Perkins (36), Sheldon (37), Samuels et al (38) and Cohen (39) have all reported the successful outcome of pregnancy in patients with Addison's disease on a constant corticosteroid therapy.

In some cases a rise in the excretion of corticosteroids has been observed during pregnancy which decreased immediately following delivery. In 1950 Jailer and Knowlton (40) described a pregnancy in an Addisonian woman which was associated with an

increased excretion of urinary neutral reducing lipids and 17-ketosteroids. Similar observations were made by Hunt and McConakey (41) and by Hills et al (42) during pregnancy in adrenal deficient women. The latter investigators found as well that the urinary glucocorticoid levels during the latter half of pregnancy were 3 to 4 times those observed in the non-pregnant state of these patients; administration of adrenocorticotrophin did not cause a further rise of urinary corticosteroids either during pregnancy or later in the post-partum period. Administration of ACTH similarly did not effect a further rise in the levels of urinary and plasma 17-hydroxycorticosteroids which were increased in one pregnant Addisonian studied by Christy et al (24).

On the other hand, only very low urinary corticosteroid levels were observed in one Addisonian woman during the last trimester of pregnancy in an investigation by Halberg and Kaiser (44). Knowlton et al (45) could detect no corticosteroids in the urine of a similar patient.

Investigators generally have reported that pregnancy had a beneficial effect on the well-being of women with Addison's disease (57). These patients nevertheless usually required substitution therapy throughout gestation. Halberg and Kaiser (44) described one case who could tolerate withdrawal of cortisone therapy for about 6 days but thereafter showed signs of incipient adrenal insufficiency. Pregnant adrenal deficient women studied by Hills et al (42) could not tolerate a decrease in corticosteroid replacement therapy and, furthermore, were afforded no protection against the development of acute adrenal cortical

insufficiency during stress because of their pregnancy.

II INVESTIGATION OF SOURCE(S) OF ADRENAL CORTICAL AND OTHER HORMONES APPEARING IN BLOOD AND URINE OF PREGNANT WOMEN

A. ROLE OF MATERNAL ADRENAL GLANDS

1. Predominant Source of Corticosteroids in Pregnant Women

The more recent studies on normal pregnant women and, especially, on totally adrenalectomized ones during pregnancy suggest strongly that the maternal adrenal glands must be the predominant, if not the only source of both the glucocorticoid and mineralocorticoid hormones measured in the maternal blood and urine during gestation.

a. Production of 17-hydroxycorticosteroids

Migeon et al (46) measured the 17-hydroxycorticosteroid levels in the maternal circulation and cord blood immediately after delivery. The values of these steroids in cord blood were always one-fifth to one-half of those in the corresponding maternal blood samples. They remained within this range when either hydrocortisone or ACTH were administered to the mother prior to delivery and appeared, therefore, to be merely a reflection of maternal plasma 17-hydroxycorticosteroid concentrations.

Investigations on Addisonian and bilaterally adrenalectomized women also showed by indirect way that the hydrocortisone-like adrenal steroids appearing in the blood and urine in normal women are almost completely of maternal adrenal origin. Beaulieu et al (47) reported on a pregnant Addisonian woman whose urinary 17-hydroxycorticosteroid excretion became negligible upon withdrawal of cortisone thereapy. In the 31st week of pregnancy two days after cortisone withdrawal no free cortisone or hydrocortisone could be detected in the urine and only 50 ug. per day of

the reduced metabolites of these hormones, compounds THF and THE. All these values were well below those observed in normal non-pregnancy and pregnancy urine. No hydrocortisone could be found in the plasma of this patient (48) whereas the normal pregnancy values ranged from 8 to 32 ug. per cent. Of interest is the study of Little et al (49) on the adrenal function in a pregnant woman who was hypophysectomized 3 months before term and who at post-mortem examination was shown to have atrophied adrenals. In the last trimester of her pregnancy the urinary 17-hydroxycorticosteroid excretion was very low and upon withdrawal of exogenous cortisone decreased to zero. After 2 days without cortisone administration signs of adrenal insufficiency became apparent. Cohen et al (50) in 1958 reported a similar lack of excretion of compounds F, E, THF and THE in the urine of bilaterally adrenalectomized patients during pregnancy when no cortisone was given.

b) Production of Aldosterone

Aldosterone appears also to be derived predominantly from maternal adrenals according to the most recent studies. Beaulieu et al (48) could not find any aldosterone in the urine of a pregnant Addisonian in the 18, 34 and 36th weeks of pregnancy while she was being maintained on 9-α-fluorohydrocortisone alone. Laidlaw et al (30) reported an excretion of approximately 1 ug. of aldosterone per day in one totally adrenalectomized pregnant woman while another excreted 4.4. ug. per day. These patients were maintained on both hydrocortisone and 9-α-fluorohydrocortisone therapy. With their technique the authors obtained in normal pregnancy urine values which ranged from 21 to

125 ug. per day, the normal non-pregnancy values were less than 10 ug. per 24 hours (30).

2. Rate of Corticosteroid Production

a. During gestation

Some investigators have suggested that the adrenal glands are hyperactive during pregnancy while the studies of others have shown no significant change in the rate of corticosteroid production. An increase of 40% in the urinary excretion of 17-hydroxycorticosteroids in 26 normal pregnant women investigated by Appleby and Norymberski was considered to be insignificant by the authors (51). Cohen et al (50) also do not consider the maternal adrenals to be hyperactive for although in their studies plasma free 17-hydroxycorticosteroids were increased in pregnant women the urinary levels of total 17-hydroxycorticosteroids were the same as in normal non-pregnant women. Furthermore, although administration of ACTH to normal pregnant women resulted in a greater increase in the excretion of free hydrocortisone and cortisone than in non-pregnant women similarly treated, the increase in the total urinary excretion of compounds, F, E, THF and THE was the same in both groups of women.

On the other hand, Christy et al (52) considered that the increased plasma hydrocortisone in pregnancy is partly related to a greater production of this steroid. They reported a hyperactive response with respect to plasma 17-hydroxycorticosteroid increase upon ACTH administration to women in the last trimester of pregnancy. A similar response was not observed in the first 2 trimesters. Furthermore, the urinary corticoid excretion was greater than normal in 12 out of 15 pregnant women before term, in their investigation.

b) Production of corticosteroids at the time of labor and parturition.

Labor appears to be a stress as borne out by observations of Migeon et al (46) that plasma 17-hydroxycorticosteroid values rose to a peak during labor and increased still further during parturition. Women who were delivered by caesarian section did not show comparable increases in the plasma 17-hydroxycorticosteroids. Gemzell (53) also considers the presence of anxiety and stress to be an important influence on maternal adrenal function as his studies showed that women pregnant for the first time had significantly higher plasma 17-hydroxycorticosteroid levels at parturition than women who were multiparae. McKay et al (54) made similar observations.

3. Histological Study of Adrenal Glands from Pregnant Women

In a review of the early histological studies on adrenals of pregnant women Andersen and Kennedy (55) could find no conclusive evidence for hypertrophy of these glands. More recently Whitely and Stoner (56) studied the histology of adrenals obtained from women who died suddenly during pregnancy or immediately after delivery. The average weight of the adrenals of the pregnant women was slightly greater than that observed for non-pregnant subjects but the difference was not statistically significant. There was no significant alteration in the histology of the pregnancy adrenals that would suggest an increased activity of these glands during gestation.

B. INFLUENCE OF THE METABOLISM OF CORTICOSTEROIDS IN PREGNANCY

The levels of corticosteroids in plasma and urine are dependent not only on rate of production but also on the rate of metabolism (58)(59). In pregnancy, therefore, the increased

amounts of adrenal cortical hormones in the circulation and urine may be due either to an actual increase in production or to a decreased rate of metabolism or, perhaps, to a combination of these phenomena.

1. Normal Pathways of Metabolism

a) Reduction of the Δ4-3-ketonegrouping of ring A

One of the major metabolic pathways of inactivation of corticosteroids involves reduction of the Δ4-3ketone structure in ring A to the dihydro or tetrahydro derivative. This has been shown to occur mainly in the liver. These reduced metabolites of hydrocortisone or cortisone have been isolated from human urine following administration of compounds F or E by Burstein et al (60)(61), Gray and Lunnon (43) and Burton et al (62). Similar observations have been made following administration of compounds B or A by Engel et al (64)(65), Mont-Gomez (71) and Mason (63). A tetrahydro derivative of compound S (68)(67)(69) and of aldosterone (70) has also been isolated from human urine.

Human liver tissue incubated in vitro had the capacity to reduce the Δ4-3-ketone structure in ring A of compounds B, DOC, F and of aldosterone in experiments of Reaver (72). Gordon (73) has observed similar inactivation of aldosterone. In work on particulates of liver tissue (74) the conversion of the 3-ketone to the 3-hydroxyl grouping occurred in the presence of a reduced pyridine nucleotide, hydrogen ion and was catalysed by a 3-hydroxysteroid dehydrogenase as follows: $C=O + DPNH + H^+ \rightleftarrows CHOH + DPN$.

Tomkins and Isselbacher (75) and Tomkins (76)(77) have observed the conversion of cortisone to the dihydro pregnane

metabolite by a fraction of a rat liver homogenate which enzymatically generated TPNH as the reductant. The reaction was apparently TPNH specific, irreversible and had a high degree of substrate specificity.

b) Conjugation of Metabolites with glucuronic acid

The ring A reduced metabolites that have been isolated from human urine were chloroform extractable in greatest part only after beta-glucuronidase or acid hydrolysis. This is because reduction of corticosteroids is followed by conjugation of the 3 carbon hydroxyl with glucuronic acid in order to render the steroid molecule more water soluble for urinary excretion. This conjugation occurs in the liver and was shown to involve reaction of the 3 carbon hydroxyl with uridine diphosphate glucuronic acid (UDPGA) under the influence of a glucuronyl transferase which has been isolated from the microsomes of rat liver (78). Tetrahydrocortisone glucuronide has been isolated from human urine by Schneider et al (79).

c) Conjugation of metabolites with sulphate

Certain steroids may be excreted as sulphates which are labile to strong acid hydrolyses. Conjugation of the 3 carbon hydroxyl with sulphate was shown to occur in the last of a 3 step reaction where 3- phosphoadenosine-5-phosphate sulphate (PAPS) reacted with the hydroxyl group under the influence of a transferase or sulfokinase (80). This reaction was found to occur in the presence of a soluble rabbit liver extract (81).

d) Metabolism of the side chain at carbon 17

Alterations of the 17 carbon side chain also occur in the metabolism of corticosteroids. This metabolism may involve only

the reduction of the 20-ketone to a hydroxyl group and would render the steroid biologically inactive. A 20-hydroxy derivative of corticosterone has been isolated from human urine by Engel et al (64)(65) after its administration. This 20-ketone reduction has also been observed in vitro with rat liver homogenate by Hubener et al (85) and by De Courcy and Schneider (86).

More extensive degradation has been observed with certain 17-hydroxycorticosteroids which were in part converted to 17-ketosteroids according to 'in vivo' studies of Sandberg et al (87), Ungar et al (83), Savard and Goldfaden (82) and Rosselet et al (88). Glenn and Recknagel (84) also observed degradation of the 17 carbon side chain by a system found only in the liver.

2. Metabolism of Corticosteroids in Pregnancy

a) Alterations of pattern of free and conjugated corticosteroids in blood and urine.

The clearance of 4-C-14 hydrocortisone was shown by Migeon et al (89) to be decreased in human pregnancy. In the plasma the half-life of free radioactive steroid was double that observed in the plasma of non-pregnant subjects. The amount of radioactivity liberated by beta-glucuronidase hydrolysis was, however, smaller than that observed in normal plasma. The total amount of radioactivity recovered in the urine of pregnant women was decreased and was associated with an increase in the free fraction and a decrease in the glucuronide fraction. Mills (90), Christy et al (52) and Cohen et al (50) similarly observed a decreased rate of metabolism of hydrocortisone during pregnancy in normal or adrenalectomized women following administration of the hormone.

6. Hydrocortisone metabolism in newborn infants

A prolonged turnover rate of hydrocortisone in 1 day old infants has been observed by Bongiovanni et al (91). The metabolism was more rapid by the 3rd to the 9th day, but was still slower than in older children. Compound THE, which is reduced at ring A, was, however, metabolized at the same rate as that observed in older children and adults.

c) Effect of oestrogens on corticosteroid metabolism

It is of interest that Taliafero et al (93) reported increased plasma 17-hydroxycorticosteroids in a patient with cancer of the prostate when oestrogens were administered, but this effect was not observed in an Addisonian patient whose corticosteroid production was negligible. Wallace et al (94) found that post-menopausal women who showed a normal ACTH response had apparently greater responses when oestrogens were given with the ACTH. They also observed that oestrogens interfered with the plasma clearance of exogenously administered hydrocortisone. The latter observation has also been reported by Mills (90) and by Cohen et al (50).

There is now evidence that high oestrogen levels in the blood increase the protein binding of certain hydrocortisone-like corticosteroids thus rendering them less available to hepatic catabolism (100).

It has been well established that oestrogen production is greatly increased in human pregnancy. The high levels of oestrogen in the circulation may be responsible for the slower rate of metabolism of 17-hydroxycorticosteroids in pregnant women.

The prolonged turnover rate of hydrocortisone in newborn infants (91) may also be associated with the finding of Diczfalusy et al (92) that oestrogen excretion in newborn infants was high and diminished rapidly to negligible levels by the fifth day of life. The effect of oestrogens on the rate of aldosterone metabolism is not known.

C. ROLE OF FOETAL ADRENAL GLANDS

1. Histological Studies

During the second month of foetal life, a distinct adrenal cortex is recognizable (66). According to Uotila (95) the foetal adrenal consists of the permanent cortex which slowly increases in size throughout intrauterine life though in the foetus at term it forms scarcely more than 1/4 to 1/3 of the cortex. There is also a distinct foetal cortex which starts to degenerate during the last 10 weeks of intrauterine life. The zona glomerulosa was seen shortly before birth or at the end of the first post-natal month while the zona-reticularis and fasciculata appeared about 2 months later in studies of Keene and Hewer (97). The permanent cortex and, to a lesser extent, the foetal cortex was shown to contain fats during foetal life but at term only the permanent cortex contained lipids.

2. Possibility of Corticosteroid Production

a) Corticosteroids in extracts of foetal adrenals

Molybdenum reducing corticoids have been measured in extracts of foetal adrenals obtained at different periods of gestation by Staemmler (98). There was a gradual increase of the 21 carbon steroids after the 5th lunar month which was about

twentifold by the 10th lunar month. One to 2 days post-partum the adrenal content of these steroids was one-half of that measured at the time of delivery. From the fifth day onwards the corticoid concentration decreased to one-fifth of the early values.

Bloch et al (66) found hydrocortisone in extracts from the 16th to 21st weeks of gestation and a Na-retaining hormone was obtained from extracts as early as the 9th week of pregnancy.

The presence of corticoids in foetal adrenals is, however, not necessarily due to foetal production of these steroids. Migeon et al (46) have shown that hydrocortisone administered to the mother reaches the foetal circulation by passage through the placenta.

b) Incubation of adrenal glands of newborn infants.

A regular production of measurable amounts of corticosteroids could not be observed in incubations of adrenals of newborn infants by Lanman and Silverman (103). Progesterone added to these incubations was, however, converted to compounds F,B and, perhaps, to compound S. This means that the adrenal tissue of newborn infants was capable of carrying out certain hydroxylations 'in vitro'. Davis and Plotz (104) showed that upon C-14-acetate administration to the mother there was radioactive cholesterol in the human foetal adrenal glands. The authors concluded that the glands had the capacity to carry out preliminary steps of corticosteroidogenesis.

c) Significance of foetal adrenal glands in production of corticosteroids during gestation

Some investigators consider that the foetus can produce corticosteroids (53). Klein et al (116) have isolated a Na-excre-

ting substance from the urine of newborn infants given ACTH which could not be obtained from the urine of older children and adults. From 4.4. to 7.7. ug. per cent of hydrocortisone and 5 other unidentified compounds have been found by Ulstrom et al (101) in pooled cord plasma. One day old infants had about 2 ug. per cent of plasma hydrocortisone.

On the other hand, a number of workers discount foetal adrenal production of 17-hydroxycorticosteroids in normal human pregnancy (46)(102). Similar conclusions have been reached indirectly by studies of 17-hydroxycorticosteroid excretion in adrenal deficient women during pregnancy (49)(50). Aldosterone was absent from (48) or present in only small quantity (30) in the urine of similar patients and, therefore, was also not derived to any significant extent from foetal adrenal production.

3. Possibility of 17-ketosteroid Production

a) 17-ketosteroids in pregnancy urine

The 17-ketosteroid levels in pregnancy urine were the same or slightly greater than those in urines of non-pregnant women in studies of Venning (105), Davis and Plotz (106) and Birke et al (107). There have been reports of increased 17-ketosteroid excretion in the latter half of pregnancy in adrenal deficient women (38)(40)(41) but Kaiser (108) could not find any change in a similar patient. The increased values of 17-ketosteroids may, however, have been due to the presence of pregnanolones in the extracts. The latter compounds are excreted in increased amounts in pregnancy urine; they have a 20 ketone group and are measurable by the Zimmerman reaction which was the one used to measure the 17-ketosteroids (100).

b) 17-ketosteroids in urine of newborn infants

Traces of 17-ketosteroids were found to be excreted in the urine of newborn infants in studies of Ulstrom et al (101) and of Jailer and Knowlton (40).

c) 17-ketosteroids in amniotic fluid

Kinnunen (109) reported an average of 3 mgm. of 17-ketosteroids per litre of amniotic fluid of both male and female fetuses. No detectable amounts of these steroids could be found however, by Migeon et al (110).

d) Presence of androgens in extracts of foetal adrenal glands

Androgenic property could not be found in steroid extracts of normal human foetuses and newborn infants in earlier studies of Gersh and Grollman (111) and Carnes (112). Later Wilkins et al (113) observed androgenic property in adrenals of a single case of adrenal hyperplasia. Benirschke et al (114) have extracted two 19 carbon steroids with weak androgenic property and Block et al (66) (115) have reported the isolation of three 17-ketosteroids from extracts of adrenals of both male and female foetuses from the 9th to the 21st week of gestation.

e) Comparison of levels of 17-ketosteroids in cord blood and maternal plasma

The finding of greater 17-ketosteroid concentrations in cord plasma than in the corresponding maternal peripheral circulation by Gardner and Walton (117) suggests that the foetus was producing these steroids. Their content in cord plasma was greater in younger than in older foetuses. Migeon et al (110) reported the concentration of DHA to be consistently higher in cord plasma than in the maternal plasma, but that of androstenedione was not sig-

nificantly different. Further evidence of indirect nature for foetal production of 17-ketosteroids is afforded by Nichols et al (102). They attempted to measure 17-ketosteroid levels in cord plasma of anencephalic infants which have characteristically atrophied adrenals and found no or subnormal amounts of these steroids.

f) A postulated trophic factor for foetal adrenal glands

Lanman (121) and Lanman and Dinerstein (124) have put forth the theory that the foetal zone of the foetal adrenal may be under the influence of chronic gonadotrophin at certain periods of gestation. In this connection, Borell (122) has observed increased neutral 17-ketosteroid excretion in the urine of females given large doses of this hormone.

D. ROLE OF THE PLACENTA

1. Histological Studies

The extensive studies on human and monkey placentae by Dempsey and Wislocki (18)(118)(19) have shown that the trophoblast, the parenchyma of the placenta, consists essentially of two kinds of tissue. One kind is called the cytotrophoblast which in the regions of the secondary or definitive villi is also called Lanhgans or epithelial cells. It functions by perpetuating itself for a certain period and by giving rise to the second main type of tissue in the placenta, the syncytium. The syncytial tissue is present in greatest amount in the placenta in the regions of the secondary villi and smaller quantities are associated with cytotrophoblastic tissue in other parts of the placenta.

Histochemical studies by the same authors (19)(120) have presented evidence for the presence of fat droplets presumed to be the

sites of steroid production in the syncytium of the placenta. This region gave the type of fluorescence characteristic of oestrogens, the birefringence given by cholesterol and its derivatives and the Lieberman-Burchardt reaction for the unsaturated steroid molecule.

Sudanophilic droplets in decidual cells of the placenta, the maternal side, were neither birefringent nor fluorescent.

2. Production of Nitrogen-Containing Hormones

a) Chorionic gonadotrophin (HCG)

The cytotrophoblast gave no positive histochemical reactions for the steroid hormones (18)(19) but in tissue culture studies of Gey et al (123) and Stewart et al (125) was shown to be the site of production of chorionic gonadotrophin.

(i) Content of HCG in placenta throughout gestation

The concentration of chorionic gonadotrophin in human placenta reached a maximum between the second and third lunar months of pregnancy in studies of Diczfalusy (126). After the fifth lunar month, the concentration appeared to be approximately constant and usually below 20 units per gm. of tissue. These results agree with the pattern of urinary excretion of gonadotrophin in normal pregnancy reported by Venning (105).

(ii) Possible functions of HCG

Several theories have been put forth as to the function of chorionic gonadotrophin (HCG). One view is that it stimulates corpora luteal activity. Another is that HCG may stimulate secretion of steroid hormones by the syncytial cells of the placenta. The latter theory has been tested by Pincus and his associates (127). They have carried out perfusions of human placentae with

both HCG and a pituitary gonadotrophin preparation and obtained increased production of progesterone-like steroids but not in all experiments.

The effect of HCG on adrenal function has also been investigated. No direct influence could be observed either in 'in vivo' experiments of Clayton and Hammant (128) or in 'in vitro' perfusion studies on calf adrenal glands by Rosenfeld and Bascon (129).

Recently Troen and Gordon (130) reported that the addition of HCG stimulated utilization of citrate by perfused human placentae but this effect was greater when oestrogens were added as well.

b) Other trophic hormone(s) isolated from the placenta

Besides chorionic gonadotrophin other nitrogen containing hormones may be produced by the placenta. A material with ACTH-like activity has been found by Jailer and Knowlton (40), Badinand et al (131), Assali and Hamermesz (132) and by Sulman and Bergman (133).

3. Production of Steroid Hormones

a) Role of the placenta in the production of progesterone

(i) Early studies on progesterone

In 1903 Frankel (134), and a few years later, Loeb (135), (136) showed that corpora lutea contained a principle that was active in the preparation of the uterus for the implantation of the fertilized ovum. In 1929 Corner and Allen (137) isolated this principle; its structure was subsequently elucidated and the substance was synthesized in crystalline form as progesterone (138), (139), (140).

An inactive steroid was isolated from human pregnancy urine by

Marrian (141) which was later shown to be related to progesterone and called pregnanediol by Butenandt (142)(143). Venning and Browne (144)(145) reported the excretion of pregnanediol in the urine conjugated with glucuronic acid and then showed the 'in vivo' conversion of exogenous progesterone to pregnanediol glucuronide. Pregnanediol has been well established as the major urinary metabolite of progesterone.

(ii) Pregnanediol in urine of non-pregnant humans

Several investigators including Klopper et al (146) (147)(100) have reported the excretion of approximately 1 to 2 mgm. of pregnanediol per day in the urine of healthy men and post-menopausal women. Surgical stress and ACTH administration increased the excretion of this substance. Similar findings were not observed in adrenalectomized persons which means that the pregnanediol measured was presumably a metabolite of a secretion of the adrenal cortex, most probably progesterone.

Pregnanediol is also excreted in very small amounts by children according to Bergstrand and Gemzell (148).

(iii) Pregnanediol in urine of normal pregnant women

In investigations of Venning (105) the excretion of pregnanediol remained at a relatively constant level during the early days of pregnancy with usually a slight increase when HCG first appeared in the urine. After the peak in HCG excretion pregnanediol glucuronide values rose progressively to term and were within a range from 40 to 150 mgm. per day in the latter part of pregnancy.

(iv) Pregnanediol in urine of cases of abnormal pregnancy

Adrenal deficient women studied by Jailer and Knowlton

(140), Beaulieu et al (47) and Knowlton et al (45) all showed during pregnancy urinary pregnanediol values within the range observed for normal pregnant women or slightly less.

Venning (262) investigated 3 patients in whom the ovary containing the corpus luteum of pregnancy was removed on the 40th, 54th and 96th day of gestation. The increase in urinary pregnanediol continued but to a lesser extent than that observed in normal pregnancy.

Pregnanediol excretion remained normal in 5 cases of intrauterine death studied by Appleby and Norymberski (51).

All of these findings in pregnant women strongly suggest that the placenta is after the fourth or fifth month of pregnancy a major source of progesterone.

(v) Presence of pregnanolones in pregnancy urine

Steadily increasing amounts of pregnanolones have been measured in human pregnancy urine by Venning (149) and Venning and Ripstein (263) and others (150) have shown them to be metabolites of progesterone.

(vi) Assay of progesterone in maternal plasma and placental blood

In 1949 Hooker and Forbes (151) devised a very sensitive biossay for progestationally active steroids. Later Forbes (152) reported that levels of free plasma progesterone were approximately 2 ug. per ml. or less in pregnancy. With the same assay, however, Fujii et al (153) found a gradual increase during pregnancy from 6 to 26 ug. per ml. of maternal plasma.

With chemical techniques for Δ4-3-ketonic steroids Pearlman and Thomas (156) and Sommerville (154) estimated maternal

peripheral plasma to contain about 0.1 ug. progesterone per ml. Chemically determined progesterone values in human placental blood were higher, approximately 0.4 ug. per ml. (156)(157).

Sommerville and Deshpande (155) found that plasma pregnanediol increased from 0.05 to 0.1 ug/ml in the first trimester of pregnancy to levels of 0.3 to 0.6 ug/ml. in the third one. They failed to detect this steroid in the plasma 24 hours after delivery.

(vii) Progesterone and derivatives in extracts of placental tissue

Amounts of progesterone ranging from 0 to 1 mgm. per kilogram of extracted placental tissue were found by Pearlman and Cerceo (158). Haskins (157) reported that placentae obtained at caesarian section had appreciably greater concentrations of progesterone in comparison with full term ones - average of 1.63 and 0.69 ug./gm., respectively.

These values are much less than what might be expected from the high urinary pregnanediol levels in pregnancy. On the basis of isotopic dilution studies with 16^3-H-progesterone Pearlman (159) calculated endogenous progesterone production to be of the order of 250 mgm. per day in women in late pregnancy.

Salhanick et al (160), Short (161) and Zander et al (162) have all isolated Δ4-pregnene-20α-α-3-one in extracts of human placentae. The latter authors found the 20β-hydroxy epimer as well. Both compounds had progestational activity in bioassays and were together present in amounts approximately one-sixth of those of progesterone. All these steroids were obtained in greatest concentration in extracts of 2 and 3 month placentae.

(viii) 'In vitro' production of progesterone by placental tissue

Incubated human placental tissue converted Δ5-pregnen-3β-ol-20-one to progesterone in experiments of Nissim and Robson (164) and others (163)(165). However, beef adrenal glands, corpora lutea and testes (163) and even certain bacteria (166) and molds (167) could also carry out this oxidation.

Solomon et al (168) reported that cholesterol was converted to progesterone via this pregnenolone intermediate in perfused human placentae.

Perfusions of placentae were also carried out by Hagopian et al (172). The amount of steroid in the progesterone fraction was increased when ACTH was added to the perfusion medium. This fraction was, however, inactive in tests for progestational activity and found later to consist of 6-ketoprogesterone and an unknown steroid. Progesterone added to the perfusion medium was converted to both these compounds.

Pincus and his co-workers (127) similarly reported that placentae obtained at caesarian section when perfused over a 4 hour period regularly maintained a production of steroids in the progesterone fraction. Full term placentae, however, yielded no steroids in similar perfusion studies.

(ix) Hydroxylation of progesterone by placental tissue

Minced placentae obtained at caesarian section were found to have the capacity to convert 4-C-14-progesterone to a 6β- hydroxy derivative by Berliner and Salhanick (169).

The finding of pregnane-3α, 17α, 20α-triol in the urine of totally adrenalectomized pregnant women was considered by Herrmann and Silverman (170) and Herrmann (171) to be indirect

evidence that human placentae can hydroxylate the 17 carbon atom of the progesterone molecule.

b. Possibility of corticosteroid production by placenta

(i) Presence of corticosteroids in extracts of placenta

Cortisone, traces of hydrocortisone and 3 other Δ 4-3-ketonic steroids were found in ethyl acetate extracts of minced placentae by DeCourcy et al (173). Other investigators have also isolated small amounts of biologically active corticosteroids from placentae (174) (176). Berliner et al (177) obtained compounds E,F,A, THE and aldosterone from extracts of full term placentae. These compounds are considered by Salhanick et al (160) to be probably derived from extra-placental source (s). They have reported a concentration by placental tissue of radioactive cortisone injected into the maternal circulation prior to delivery.

(ii) Corticosteroids obtained from perfused placentae.

In 4 hour perfusions of placentae obtained at caesarian section Pincus (127) reported that Δ4-3-ketonic steroids were detected only in the desoxycorticosterone and progesterone fractions of the extracts. Most of the alpha-ketolic material was present in the more polar, hydrocortisone and 'tetrahydro' fractions. Often all fractions decreased to zero by the 2nd hour of perfusion. When ^{C}-14-progesterone was added to the perfusing medium radioactivity appeared only in the least polar fractions of steroids. Addition of ACTH did not result in an increase in the total steroid content but rather a decrease in the amount of less polar compounds. Full term placentae were non-functional in similar perfusions with whole beef blood.

A considerable production of Porter-Silber (PS) reactive material by full term placentae during 6 to 12 hour perfusions with Tyrodes solution has been described in an abstract by Troen (184). The author concluded that the human placenta can synthesize and conjugate corticosteroids.

C. Role of placenta in production of oestrogens

(i) Oestrogens in urine and blood during pregnancy

In studies of Venning (149) the excretion of urinary oestrogens increased progressively from about the 80th day of pregnancy to values of 40 to 50 mgm. per day in late pregnancy at which time oestriol was the predominant oestrogen excreted. Adrenal deficient women (40)(47) and women in which the corpus luteum was surgically removed (262) were found to have essentially normal urinary oestrogen levels throughout gestation. This strongly suggests that the placenta is the source of most of the oestrogens appearing in the urine in the latter half of pregnancy. Supporting this view is the report of Salhanick et al (178) that the ratio of oestrogen concentration in placental to peripheral blood is 100 to 1.

(ii) Presence of oestrogens in extracts of placentae

The three oestrogens, oestrone, oestradiol and oestriol were present in a ratio of 17 to 2 to 122 respectively in extracts of placentae prepared by Diczfalusy (126). Villee and Loring (179) obtained 230 ug. of oestradiol plus oestrone per kg. of fresh placental tissue.

(iii) In vitro interconversion of oestrogens by placental tissue

An interconversion of oestrone and oestradiol by perfused

human placentae was reported by Levitz et al (180). They could not detect any conversion of either of these steroids to oestriol, 'in vitro'.

(iv) Conversion of 17-ketosteroids to oestrogens by placental microsomes

Ryan (181) obtained oestrone production from androstenedione and testosterone by incubated human placental microsomes. Up to 50% of the added 17-ketosteroids were aromatized and this activity was noted as early as the 13th week of pregnancy.

4. Influences of Placental Hormones on Corticosteroid Metabolism and Function

a) Effect of oestrogens and progesterone on 17-hydroxycorticosteroid metabolism

It has been reported that oestrogens increase the protein binding of plasma 17-hydroxycorticosteroids (100). The high levels of oestrogens produced in pregnancy are responsible in part, at least, for the measurement of increased amounts of these steroids in pregnancy plasma. Oestrogens are also implicated in the alteration in the pattern of free and conjugated 17-hydroxycorticosteroids in the urine of pregnant women, (93)(94)(90)(50). This is described in a preceding section, Metabolism of Corticosteroids in Pregnancy.

Progesterone when administered intravenously to men did not alter the level of blood 17-hydroxycorticosteroids in the experiments of Brown and Migeon (182).

b) Inhibition of sodium retaining activity of aldosterone by progesterone.

Landau and Lugibihl (183) recently reported that 50 mgm. of administered progesterone led to pronounced sodium excretion in a woman with Addison's disease who was treated with aldosterone

and cortisone. This effect of progesterone was not observed following aldosterone discontinuation. The authors considered that during pregnancy progesterone and the Na-retaining aldosterone may compete in the control of sodium metabolism.

III OBSERVATIONS ON PREGNANCY IN ANIMALS

A. THE PRODUCTION OF CORTICOSTEROIDS BY RAT ADRENAL GLANDS

The production of corticosteroids by rat adrenal glands has been studied extensively with use of both 'in vitro' incubation and 'in vivo' techniques. Corticosterone and aldosterone were found to be the major glucocorticoid and mineralocorticoid secreted, respectively.

1. In Vivo Secretion of Steroids

Bush (185), Rosenman et al (188), Masson et al (190) and Reif and Longwell (189) have all obtained corticosterone from adrenal venous blood collected over various periods of time from rats. Singer and Stack-Dunne (186) and later Das Gupta and Giroud (187) reported aldosterone secretion as well.

2. In Vitro Secretion of Steroids

An in vitro production of corticosterone by rat adrenals incubated in an artificial medium, Krebs-Ringer solution, has been reported by Saffran and Bayliss (191), Saffran and Schally (192) and others (193)(194)(195). Giroud et al (1)(2) and Venning et al (8) have consistently found production of aldosterone as well as of corticosterone 'in vitro'. The former authors found that aldosterone was secreted mainly by the zona glomerulosa while corticosterone was produced chiefly by cells in the zona fasciculata and reticularis.

B. ADRENAL-LIKE FUNCTION DURING PREGNANCY IN THE RAT AND OTHER LABORATORY ANIMALS

1. Histology of Maternal Adrenal Glands

In the rat changes in the histology of maternal adrenal glands

could not be observed during pregnancy by Donaldson (199), Andersen and Kennedy (55), Blumenfeld (200) and, more recently, by Christianson and Jones (201).

On the other hand, adrenal hypertrophy during pregnancy has been reported in the guinea pig and rabbit by Guieysse (196) and Watrin (197), respectively. Histological changes in the mouse adrenal indicated increased activity during the first three-fourths of pregnancy followed by a decrease according to Tamura (198).

2. Studies on Adrenalectomized Pregnant Animals

Experiments on the effect of adrenalectomy in pregnant rats have shown conflicting results. In earlier studies Lewis (206) and others (207) reported normal reproduction following adrenalectomy but Wyman (208), Corey (204) and McKeown and Spurell (209) have all observed abortion in rats adrenalectomized during pregnancy. According to more recent work by Davis and Plotz (205) rats adrenalectomized from the 4th to 6th day of pregnancy aborted but when this was done from the 14th to 16th day of pregnancy there was normal delivery and survival time was increased in comparison with that of adrenalectomized non-pregnant rats. This latter effect was first observed in the dog by Rogoff and Stewart (202). Dogs adrenalectomized during pregnancy lived longer than non-pregnant ones similarly treated. As this influence was still seen in cases when the fetus was removed it was not due to fetal adrenal activity.

The increased life span in these animals following adrenalectomy during pregnancy was not, however, necessarily due to a placental secretion as it was also reported in rats when adrenal-

ectomized during pseudopregnancy by Emery and Schwabe (212). A similar effect was noted in the dog by Swingle et al (210) and in cats by Collings (211).

3. Role of the Steroid Secretions of the Corpus Luteum

The beneficial effects of pregnancy and pseudopregnancy in prolonging the life span of certain adrenalectomized animals appears, therefore, to be due to corpora luteal activity, presumably to progesterone secretion.

Wells and Greene (213), Schwabe and Emery (214) and others (215) have all reported that progesterone administration maintained non-pregnant adrenalectomized rats in good health and a similar effect was observed in the cat (216).

4. Role of the Foetal Adrenals of the Rat

Josimovich et al (237) made a histophysiological study of the developing adrenal cortex of the rat during foetal and early post-natal growth. Foetal glands grow steadily from the 16th day of gestation to birth i.e. to about 22nd day. A narrow zona glomerulosa and wider zones of fasciculata and reticularis were defined by the 18th day. Sudanophilic droplets were detectable at the 16th day and their number increased to term; between the 17th and 19th days tests for ketone and cholesterol were positive. According to these authors the foetal glands had secretory activity at least by the 19th day of gestation but this activity decreased immediately after birth. Cohen (238) observed a lipid discharge at the 19th and 20th days of foetal life.

The foetal and postnatal reticular zone were apparently active in the production of androgenic compounds in experiments of Blackman (239). Male sex hormones when administered to young rats

decreased the size of this zone.

Tobin (240) found that destruction of foetal adrenals at the 17th day of pregnancy did not influence the life span of the embryo. According to Christianson and Jones (201) the foetal adrenals of the rat do not secrete hormones which could influence water and salt metabolism such as aldosterone.

5. Role of the Rat Placenta

a) Histological study

Wislocki and Dempsey (241) have made a detailed study of the rat placenta with use of the electron microscope. In the 3rd week of pregnancy the cytoplasm of the trophoblastic cells contained numerous mitochondri and fat droplets which were characteristically present in the innermost cell layer. The presence of sudanophil lipoidal material in the placental syncytium of the rat has been observed (264) in the latter part of gestation; this fat surrounded the fetal blood channels and may have been a steroid secretion.

a) Possibility of production of trophic hormones

Aqueous extracts of rat placenta prepared by Astwood and Greep (242) contained a substance, perhaps a gonadotrophin, which stimulated the activity of corpora luteal glands. In experiments by Greer, however, no evidence could be found for adrenocorticotrophin activity in rats after they were hypophysectomized; the placenta was, presumably, not a source of this hormone (243).

c. Possibility of production of oestrogens and progesterone

Evidence for production of corpus luteum hormone by rat placenta was reported by Selye et al (244). A similar substance has

been isolated by McKeown and Zuckerman (246).

No evidence for oestrogen production by rat placenta could be found by Selye and McKeown (245).

d. Possibility of production of corticosteroids

In studies of Greep (247) the rat placenta did not produce a factor which could influence salt and water metabolism i.e. aldosterone-like material. Pregnant rats when hypophysectomized were not more capable in excreting a water load than non-pregnant ones similarly treated in experiments of Hofmann et al (248).

Administration of cortisone to pregnant rats resulted in decreased placental weight in experiments of Hisaw (249). This effect was also noted by Curry and Beaton (250). The latter authors found that administration of large amounts of cortisone to non-pregnant rats brought about marked biochemical changes. These changes were not observed in pregnant rats similarly treated. The presence of the placenta was found to be necessary for this resistance of pregnant rats to overdosage of cortisone. The authors concluded that either the placenta secreted an anti-cortisone material or, more likely, was able to concentrate or destroy the cortisone which was administered.

C. INFLUENCE OF OESTROGENIC SUBSTANCES ON THE ADRENAL FUNCTION OF THE RAT AND OTHER LABORATORY ANIMALS

1. Effect of Ovariectomy

Ovariectomy in the rat leads to a loss of adrenal weight according to Winter and Emery (217) and others (218). This was due to a narrowing of the zone of fasciculata and reticularis of the adrenal cortex in experiments of Blumenfeld (219). Smith (220)

reported evidence for increased adrenal activity after ovariectomy in the rat.

Spaying of guinea pigs of both sexes led to degenerative changes in the zona reticularis in experiments of Zalesky (221) but Deansley (222) was unable to demonstrate similar changes in spayed mice.

2. Effect of Administration of Oestrogenic Substances

a) On histology of adrenal glands

Adrenal hypertrophy following oestrogen administration in female rats has been observed by Andersen (223) and others (224) (225). Stilbestrol had similar effects according to Heskett and Hoffman (226) who detected also congestion and hemorrhage in the reticular zone of the cortex. Vogt (227) noted that implantation of hexestrol in rats led to adrenal hypertrophy with complete loss of cortical lipids in 10 days.

Estrone administration caused greater adrenal hypertrophy in castrated male rats than in normal ones (228). Upon prolonged administration of oestrogen to male rats atrophic changes were reported to follow the initial hypertrophy (229).

A 50 per cent increase in the cortical weight of adrenal glands was observed in guinea pigs given diethylstilbestrol by Allen and Bern (230).

b. On corticosteroid production

(i) 'in vivo' effect

Administration of hexoestrol to rats led to a decreased corticosterone secretion in adrenal venous blood of male rats in experiments of Vogt (231). She postulated that the oestrogen effect

was due to inhibition of cholesterol synthesis. Clayton and Hammant (128) reported that when oestrogens were given with ACTH to guinea pigs there was an inhibition of the normal response of increased urinary 17-ketogenic steroids seen when ACTH was given alone. Progesterone given with ACTH did not show this inhibition of steroid excretion.

(ii) 'in vitro' effect

McKerns (232) could find no inhibition by oestradiol of ACTH stimulated rat adrenal cortical tissue incubated 'in vitro' when the oestrogen was added directly to the medium. But it was later reported (233) that following a period of oestradiol administration to male rats the adrenal glands were removed and their rate of corticosteroid production 'in vitro' was below normal. Ethinyl estradiol and stilbestrol, however, had inhibitory effects in both types of experiments.

These results suggest that the action of the natural oestrogens on the adrenal cortex is indirect. According to Selye et al (234) and Carter (235) it is mediated by the anterior pituitary. Gompertz (236) found that in rats, oestrogens acted by influencing both hypophyseal secretion of ACTH and the sensitivity of the adrenal cortex to ACTH.

D. INVESTIGATIONS OF CORTICOSTEROID METABOLISM BY RAT LIVER

1. Metabolic Pathways Involved

The liver has been well established as the predominant site of corticosteroid metabolism. An 'in vivo' metabolism of cortisone added to the perfusion medium of rat liver has been reported by

Miller et al (253); a variety of alpha-ketolic compounds were derived from cortisone.

Rat liver slices or homogenates incubated 'in vitro' were observed to metabolize corticosteroids added to the incubation medium in a number of ways.

(1) A reduction of the Δ 4-3-ketone grouping at ring A of added corticosteroids to the corresponding 'dihydro' or 'tetrahydro' derivative was reported by Eisenstein (254)(255), Schneider and Horstmann (257)(258) and others (251)(256)(252)(261).

2) When cortisone was added to the incubations conversion of the 11 carbon ketone to a hydroxyl group was observed by Eisenstein (254)(255) and by Hubener et al (85). A similar conversion of compound THE to compound THF could not be detected by the latter authors.

(3) Destruction of the dihydroxyacetone grouping of certain 17-hydroxycorticosteroids by hydrogenation of the 20 carbon ketone has been reported (85)(86).

(4) More complete degradation of the side chain at carbon 17 has been demonstrated 'in vitro' by Glenn and Recknagel (84).

2. Factors Influencing Hepatic Activity

According to Yates et al (259) the 'in vitro' reduction of ring A of compounds E,F, and DOC and of aldosterone was greater by liver tissue obtained from female rats than from male liver tissue. The in vivo administration of oestrogens in both sexes increased the synthesis of the hepatic enzymes responsible for this inactivation; administration of androgens in both sexes had the opposite effect according to observations made in subsequent incubations of the excised liver tissue.

McGuire and Tomkins (260) reported that the rate of cortisone metabolism was increased in 'in vitro' incubations of homogenates of liver obtained from rats previously injected with thyroxin. There was observed a two to threefold increase in the activity of a particulate steroid reductase which catalysed the conversion of cortisone to allodihydrocortisone.

No data has been found on the influence of pregnancy on hepatic capacity to metabolize corticosteroids, however, an increased liver weight by the 15th day of gestation has been reported (250).

METHODS

INTRODUCTION:

The experiments carried out involved the use of both 'in vivo' and 'in vitro' techniques. The 'in vitro' method was used in the study of the activity of placental and other tissue during incubation in artificial medium while the 'in vivo' experiments included the collection of adrenal venous blood from rats and the collection of urine specimens from human subjects.

The analyses of the steroid content of the incubation media and body fluids obtained was carried out according to fundamental techniques developed in this field of research. Those used included (1) extraction of the steroids from the various fluids followed by (2), the separation and isolation of corticosteroids by paper chromatographic methods and (3), the subsequent estimation of the corticosteroids by chemical or biological assay. The techniques which were used repeatedly are described in detail only in the first section of methods (human placental incubations).

PART I: METHODS USED IN EXPERIMENTAL WORK ON HUMAN PREGNANCY

A. Incubations of Human Placentae

Human placentae obtained at caesarian section were incubated in Krebs-Ringer solution with the aim of demonstrating a production of corticosteroids and also of progesterone 'in vitro'. In some experiments different samples of tissue were incubated with various trophic hormones and precursors of adrenal cortical hormones in an effort to stimulate steroidogenesis. The possibility was also considered that placental tissue may produce these 21 carbon steroids

but not release them freely into the incubation medium due to protein binding or other phenomena. For this reason samples of placental tissue were hydrolysed with NaOH solution or homogenated to ensure a more complete recovery of steroids from the tissue following incubation.

1) Incubation Procedure

a) Preparation of tissue

Placentae removed by caesarian section about two weeks before term were placed immediately into ice-cold physiological saline for transportation to the laboratory. Cord blood was allowed to run out and either collected for analysis or discarded. The placenta was rapidly rinsed with ice-cold saline solution (0.9%) with gentle squeezing in order to rid it of as much blood content as possible. It was then cut into large portions and kept under ice-cold oxygenated Krebs-Ringer solution (KRS) until all of it (except the tissue adjacent to the chorionic plate) was manually converted into a mince with scissors. The mincing was carried out as rapidly as possible on an enamel plate kept over ice and the cut tissue was immediately suspended in ice-cold KRS. This mixture was made homogeneous by thorough stirring and as much blood and KRS as possible was filtered off prior to weighing of the portions. Samples usually 3 or 4, weighing approximately 100 gm. each were used.

b) Incubation conditions

The incubations were carried out in 1000 ml. beakers in a constant temperature water bath at 37°C. Oxygenated Krebs-Ringer bicarbonate solution (266) containing 200 mgm. % glucose was

used for the incubations in amount of 500 ml. per approximately 100 gm. of tissue and 95% O_2 plus 5% CO_2 was bubbled through the medium continuously. The incubation periods varied from 1 to 3 hours; the total length of incubation for each portion of placenta ranged from 3 to 6 hours. To avoid bacterial contamination penicillin was added when the incubation period was more than 3 hours. The tissue was kept in motion by the oxygen stream and by manual stirring.

c) Addition of hormones to incubation media

The trophic hormones, chorionic gonadotrophin, ACTH and prolactin, were dissolved in 1 ml. of 1/2 % acetic acid solution prior to their addition to the medium.

Progesterone and hydrocortisone were first dissolved in propylene glycol, then added. Cholesterol and pregnenolone, each in 0.5 ml. ethanol solution, were diluted with 2 ml. of propylene glycol prior to their addition to the medium and a fine suspension of the steroid in the incubation medium resulted. The final alcohol /KRS concentration was 1/1000.

2. Extraction of Steroids

a) Free steroids from incubation medium and cord blood

The incubation media were extracted with at least twice their volume of redistilled ethyl acetate. Cord blood was diluted with an equal volume of water prior to extraction with this solvent. The extract was evaporated in vacuo below 50°C to a volume of 400 ml. and then washed twice with 100 ml. of 1/5 N Na2 C03 solution and twice with 50 ml. of distilled water. The solvent was then evaporated off and the extract was usually partitioned

between 70% aqueous methanol and petroleum ether for further purification. The petroleum ether phase was discarded and the aqueous phase was evaporated in vacuo until all the methanol was removed. The aqueous residue was diluted with more distilled water and the free steroids were extracted with 6 volumes of redistilled chloroform. This solvent was evaporated off under reduced pressure and the steroid residue was transferred to test tubes with micropipettes using about 6 ml. of a solution of chloroform-methanol (1/1). The latter was evaporated off under nitrogen below 50°C and the extract was ready for paper chromatography.

b) Extraction of steroids from hydrolysed tissue

In experiment 9 an hydrolysis of the incubated tissue was carried out by allowing the tissue to remain in 5% NaOH solution at room temperature under an equal volume of redistilled ether. The mixture was allowed to stand with periodic stirring until all the tissue appeared to be in solution. The steroids were extracted by the ether phase. This was done according to the method described by Pearlman and Cerceo (158) in order to increase the recovery of progesterone from placental tissue but corticosteroids would be destroyed.

The ether layer was decanted twice and replaced with a fresh volume of solvent over a 48 hour period. All fractions were combined and the ether evaporated off. The residues were purified by alcohol-petroleum ether partitioning and chloroform extraction as described in section (a).

c) Extraction of steroids from homogenized tissue

In experiments 10 and 11 homogenization of placenta in 100 ml. distilled water was carried out in a Potter-Elvehjem apparatus following partial homogenization of the tissue for 1 minute in a Waring Blendor. This was done to ensure more complete recovery of corticosteroids as well as of progesterone from the incubated placental tissue.

The homogenates were mixed with excessive quantities of redistilled acetone to ensure precipitation of the proteins and extraction of the steroids into the solvent. The acetone was then filtered off. The residue from the tissue was thoroughly rinsed with warm acetone. This aqueous-acetone filtrate was evaporated down to remove all acetone. The aqueous residue was diluted with water, then extracted with ethyl acetate and treated according to the procedure described in section (a).

d) Extraction of conjugated steroids

In experiment 10 the media of the various samples were first extracted to remove the free steroids as described above. The remaining solution was adjusted to pH5.0 ,beta-glucuronidase was added (200 units per ml. of Ketodase from Warner-Chilcott lab.) and the mixture was hydrolysed at 37°C for 48 hours. The incubation medium was then reextracted with ethyl acetate and an extract was prepared in the manner described under section (a).

3. Paper chromatography of Steroids

The chromatographic methods used are presumed to be familiar and also, they have been described fully in a Masters thesis (268).

Each of the partially purified extracts was first applied on

wide strips (8 to 15 cm.) of paper and were run for a 4 hour period in the heptane-propylene glycol system (269). In this system all steroids except progesterone remain at the starting line, progesterone moves about one-half way down the paper while most of the fat and pigment moves close to the solvent front.

The progesterone zones were eluted and reapplied in the same system but on narrow strips of paper (2 to 3 cm.) prior to their measurement.

The more polar steroids were eluted from the starting line area of the heptane-propylene glycol paper. The eluates were reapplied on 4 cm. wide strips and rerun in the chloroform-formamide system (270) for 4 hours. The chromatograms were divided into the following zones in order of their decreasing polarity.

Zone 1. might contain compound THF
" 2. " " compounds THE & F
" 3. " " " E & X_1 (also zone of aldosterone)
" 4. " " less polar steroids such as compounds B and A.

Each of the zones containing a mixture of steroids was separately eluted and rechromatographed, usually on approximately 1.5 cm. wide strips of paper, as follows:

1. Compounds THE and F were separated in the Bush C system (271).
2. Compounds E and X_1 were also separated in this system, in which X_1 has the same mobility as compound F and aldosterone.
3. Less polar steroids were reapplied in the toluene-propylene glycol system (272), undiluted, for a 16 hour run for compounds B and A. Compounds X_2, X_3 and X_4 were also separated in this

system in a 4 hour run with the stationary phase, propyleneglycol, diluted with 50% methanol.

4. Detection of Steroids on Paper Chromatograms

After the paper strips were developed they were dried at room temperature in a fume hood.

Compounds having a Δ 4-3-ketone grouping in ring A were detected visually by virtue of their absorption of UV light in the region of 240mμ given off by a mercury vapor hand lamp. Usually photoprints of paper chromatograms were made in order to have permanent records of the UV light absorbing compounds which appeared as white areas on a black background.

Compounds with no Δ 4-3-ketone grouping but with an alpha-ketolic structure were detected on the paper strips by the purple colour which they developed following treatment with blue tetrazolium reagent used for a soda fluorescence reaction described in section 6.

5. Elution of Steroids from Paper Chromatograms

The zones containing the various steroids were eluted from the paper chromatograms with alcohol with either of the two following methods depending upon the size of paper to be eluted.

a) Paper strips of small area (about 1.5 x 10 cm.) were attached to elution tubes filled with alcohol which slowly ran down over the paper, eluted the steroids and dripped into a container.

b) Paper strips of relatively large area were cut into approximately 0.5 sq.cm. portions and soaked in alcohol for 1 hour after which the alcohol was filtered off with suction. More alcohol was added to the paper and the procedure was repeated twice. The alcohol eluates were combined.

Either methanol or ethanol (both redistilled) were used for elution. The alcohol was evaporated off in vacuo below 50°C.

6. Assay of Steroids

a) Quantitative chemical reactions

(i) Steroids with Δ 4-3-ketone grouping

The dried steroid residue was dissolved in purified ethanol so that, if it were possible, the concentration of steroid would be in the range from 5 to 15 ug./ml. The UV light absorption of the solution was read in silica cells in a Beckman DU spectrophotometer against an appropriate paper blank solution over a range from 220 to 300 mμ. The maximal optical density (O.D.) of UV light absorption for Δ 4-3-ketones is in the region of 240 mμ. This O.D. was used in the following formula to calculate the concentration of the steroids and is expressed as ug./ml. solution.

Concentration x E x 1 x 1000 = M.W. x O.D. max: M.W. = molecular weight of steroid.

1 = 1 = 1 cm. light path

E = molar extinction = 15,800 for compounds E and F

= 16,700 " " B

Isonicotinic acid hydrazide raction (INH)

The modification of Weichselbaum and Margraf (273) of the original method of Umberger (274) was used. The following reagents were made up.

No. 1 0.625 ml. conc. HCl diluted to 1000 ml. with purified ethanol.

No. 2. 25 mgm. INH dissolved in 100 ml. of reagent No. 1.

Three ml. of reagent No. 2. were added to the steroid residue which was dried under nitrogen, mixed, and allowed to stand at room temperature for 1 hour. The optical density of the yellow-colour which was developed was read at 380mμ in silica cells versus reagent and appropriate paper blanks.

The concentration was estimated from standard curves obtained with 5 to 25 ug. quantities of appropriate standard compounds.

(ii) Steroids with the alpha-ketol group

The blue tetrazolium (BT) reaction as described by Nowaczynski et al (275) was used. The reagents used were the following:

No. 1. 1 ml. Tetramethyl ammonium hydroxide solution (10% in water) was diluted to 10 ml. with ethanol.

No. 2. 50 mgm. BT dissolved (as much as possibly) in 10 ml. ethanol.

The dried steroid residue was dissolved in 1.5 ml. ethanol, then 0.25 ml. of reagents 1 and 2 were added in turn. After mixing the solution was allowed to stand in the dark at room temperature for 20 minutes. After the colour was developed (purple-pink) 1 ml. of conc. acetic acid was added to stop the reaction.

The O.D. of the solution was read at 525mμ in corex cells versus reagent and appropriate paper blanks. The concentration was estimated from standard curves obtained with 5 to 25 ug. quantities of appropriate standard compounds.

b) Semiquantitative chemical reaction

The soda fluoresence method described by Neher and Wettstein (276) was used. The reagent consisted of a mixture of 1 part of

0.1% aqueous solution of BT plus 9 parts 2 N NaOH solution.

Alpha-ketolic steroids developed a purplish colour within 5 minutes after the paper strips were dipped in this reagent. The sensitivity limit for the BT reaction was about 0.2 ug./ 1 cm^2. The strips were then dried at 90°C in an oven for 15 to 20 minutes after which they were observed under a UV light from a mercury vapor lamp with a filter which gave a radiation at of approximately 320. Steroids with a Δ 4-3-ketone grouping showed a yellow fluoresence and as little as 0.1 ug/cm^2 could be detected.

The measurement of BT positive and soda fluorescent spots of steroids on the chromatograms was made by comparing those obtained with from 0.1 to 5.0 quantities of standard hydrocortisone.

c) Bioassay for Na-retaining hormone (aldosterone)

The aldosterone zones on the paper chromatograms were in a few instances eluted with purified ethanol and assayed for Na-retaining activity with the method of Venning and Dyrenfurth (25). This bioassay could detect a minimum of 2 ug. of aldosterone.

7. Characterization of Steroids

a) UV light absorption

Steroids with the Δ 4-3-ketone grouping absorb UV light maximally in the region of 240 mμ in alcohol solution. Progesterone and certain corticosteroids are Δ 4-3-ketonic steroids.

Oestrogens (phenols), on the other hand, have maxima in alcohol solution in the region of 270 to 280 mμ.

b) H_2SO_4 chromogen spectrum

These were performed according to the method of Burton et al (272). The dried steroid residues were dissolved in conc. H_2SO_4

for 2 hours at room temperature. The UV light absorption spectrum from 200 to 600mμof the chromogens that had developed were read in silica cells in the Beckman DU spectrophotometer against an appropriately prepared paper blank.

It has been shown that individual steroids have a characteristic chromogen spectrum in H_2SO_4 (278).

c) Infra-red spectrophotometry

Material from the progesterone zone of the paper chromatograms was eluted and analysed for its infra-red spectrum. The latter was performed by Mr. W. Zaharia in the biochemistry department at McGill University.

B. The Urinary Excretion of Corticosteroids and Metabolites of Progesterone in Pregnant and Non-pregnant women

The following experiments were carried out when urine specimens collected from 2 adrenalectomized pregnant women on corticosteroid replacement therapy became available for study through the courtesy of Dr. V. Pollak. They were received in a frozen state from Chicago and unfortunately it was not possible to control the accuracy of the collections. The specimens were assayed at the endocrine laboratory of the University clinic of the Royal Victoria Hospital for aldosterone, pregnandediol and pregnanetriol content. At the same time it was of interest to investigate the urinary corticosteroid patterns of these patients and compare them with those obtained from normal pregnant and non-pregnant individuals. This was done according to a technique which was previously developed and used by the author of this thesis for

the study of urinary adrenal cortical hormones in patients with abnormal adrenal function. The latter experiments were reported in an MSc thesis (268).

Clinical Material

1. Non-pregnant subjects

a) Normal - One female M.E. was studied. Some previously reported data on normal female (L.C.) and male (H.B.) is included in the results (268). The age of the normal subjects ranged from 25 to 35 years.

Adrenalectomized - Case S.K. (60 years old) was totally adrenalectomized for Cushing's syndrome. Her urinary corticosteroid pattern (when on 50 mgm. cortisone therapy/day) is included in the results and has been previously presented (268).

2. Pregnant subjects

a) Normal - A single case, E.W. (32 years old) was studied in the 7th and 8th months of her first pregnancy.

b) Adrenalectomized - Case A.O. A diagnosis of Cushings disease has been made on this woman and in January, 1954, an hyperplastic left adrenal was completely removed; the patient was 23 years old at that time. There was a temporary improvement for a short time, however, she again became ill and progression of her disease occurred. In December, 1954, a right total adrenalectomy was performed and following this she was maintained on 37.5 mgm. cortisone per day. She married in August, 1955, and became pregnant in March, 1956. Urines were collected on October 9th, December 5th, 1956 and on January 23rd, 1957. She delivered on

February 3rd, 1957 and 4 days later another 24-hour urine specimen was collected. On each occassion she received 37.5 mgm. cortisone per day in divided doses, the last dose being given at 10:00 pm. on the day prior to the urine collection. On the day of collection she was maintained on 0.75 mgm. of 9α-fluorohydrocortisone given in 3 divided doses.

Case H.M. was a 30 year old housewife who had been married for 9 years and was childless. She showed a classical picture of Cushing's syndrome and underwent a two stage total adrenalectomy in 1955. In June, 1956, examination showed evidences of pregnancy of approximately 6 month duration. She was receiving as maintenance therapy at this time, 40 mgm. hydrocortisone, 0.25 mgm. 9-α- fluorohydrocortisone and a daily salt intake of 3 gm. Her pregnancy was uneventful, the features of Cushing's syndrome completely disappeared and except for increased pigmentation there was no suggestion of adrenal insufficiency. Twenty-four hour urine collections were made in the 7th and 9th month of her pregnancy and on the 6th day post partum.

METHODS

1) Hydrolysis and Extraction of Urines

Six or twelve hour aliquots of a 24 hour collection of urine were adjusted to pH 5.0 and were incubated at 37°C for 24 hours with beta-glucuronidase; Ketodase, a preparation of Warner-Chilcott lab. was used in amount of 300 units/ml. of urine. At the end of 24 hours the urine was extracted three times with 1/2, 1/4, 1/4 of its volume with chloroform. The urine specimens were then reincubated for a further 24 hours of hydrolysis after which they were acidified to pH 1.0 and extracted with chloroform immediately. The two extracts were combined.

In 3 instances (urine samples of case A.O. 8th, 9th months of pregnancy and 4 days post partum) the specimens were each extracted with an equal volume of benzene (3 times 1/3 the volume of urine) after 48 hours treatment with beta-glucuronidase and the benzene was evaporated off. Benzene was used to ensure more complete extraction of the metabolites of progesterone. The urine samples were then acidified to pH 1.0 with conc. H_2SO4 and after 24 hours of acid hydrolysis at room temperature were extracted with chloroform; the two extracts were combined.

In all cases, the crude extracts, in 200 ml. chloroform solvent, were washed with 3 x 50 ml. cold 0.1N NaOH solution and 2 x 25 ml. distilled water. The chloroform was then evaporated off in vacuo below 50° C. The steroid residues were transferred to test tubes and prepared for paper chromatography in the usual way.

2. Paper chromatography of Steroids

The urinary corticosteroid patterns were obtained in a similar way as that described in an M.Sc. thesis (268).

The dried extracts contained the neutral steroids which were applied on paper for separation by chromatographic method into 3 major groups of steroids depending upon their polarity.

The steroids were first fractionated in the chloroform-formamide system (27). All compounds more polar than, and including cortisone, ($C_{21}O_5$) were eluted separately from the faster moving material. The latter was applied in the benzene-formamide (272) system for further purification; all steroids more polar than and including compound A ($C_{21}O_4$) were eluted separately from the faster moving material, i.e. $C_{21}O_3$ and $C_{21}O_2$ compounds.

Group I: Most polar steroids

The most polar $C_{21}O_5$ compounds were reapplied in the toluene-propylene glycol system, diluted, (T-PGd) for a period, about 7 days, that would permit a separation of compounds THF, THE, F and E.

Group 2: Medium polar steroids

The overflow (containing cortisone) from the 7 day run was chromatographed in T-PG,d, for 3 days together with the $C_{21}O_4$ steroid fraction for good separation of cortisone from corticosterone.

Group 3: Least polar steroids

Material with the polarity of corticosterone came off in the overflow of the 3 day T-PG run. It was then combined with the least polar compounds, $C_{21}O_3$ and $C_{21}O_2$, and chromatographed in T-PG, undiluted, for 15 hours to obtain the pattern of steroids with

polarity intermediate between compounds B and DOC, if any such were present.

The same concentration of corticosteroids, expressed as number of hours urine extract /cm. width of paper strip, was applied on each of the 3 T-PG chromatograms which comprise a single urinary corticosteroid pattern. For urines of normal subjects 0.8 hrs. urine extract /cm. width of strip was an adequate concentration according to previous studies (268).

3. Scanning Patterns of T-PG Chromatograms

Strips one to 1.5 cm. wide were cut from each T-PG chromatogram after it had been dried. The strips were scanned every 0.5 cm. at 240mμ (UV light) in a Photovolt optical densitometer, for the pattern of compounds with the Δ 4-3-ketone grouping. Following this the same strips were treated with blue tetrazolium (BT) reagent (method described below) and then scanned at 525mμ (visible light) in an identical manner for the pattern of compounds with the alpha-ketol grouping at the carbon 17 side chain. The plotting of optical densities recorded was carried out with UV and BT patterns being superimposed. These urinary corticosteroid patterns (1) established the position of the separated steroids on the paper chromatograms and made immediately evident the qualitative character of each of the patterns. (2) They gave a rough estimation of the relative quantities of the separated compounds.

BT reaction on paper strips

Reagents: A 2% aqueous BT solution
B 10% " NaOH "
C 60% " Methanol "
D 2N - sulphuric acid

The paper strips were dipped in BT solution (a mixture of 25 c.c. A, 37.5c.c. B and 37.5c.c. of C) for about 2 minutes for development of purple-pink colour at the zones containing alpha-ketols. They were then immersed in solution D for a few seconds in order to stop the reaction and finally washed in distilled water. The strips were dried in an oven at 90°C for about 10 minutes prior to scanning.

4. Quantitative Studies

a) Estimation of compounds THF, THE, F and E

After the position of compounds THF, THE, F and E was determined by the methods just described the area on the paper strips containing these compounds was eluted with alcohol. The eluates were then reapplied on paper strips and rerun in the Bush C system (271) or chloroform-formamide system (270) in order to completely separate the steroids and to achieve further purification. The appropriate steroid zones were then eluted with alcohol and assayed for Δ4-3-ketone and/or alpha-ketolic steroid content by UV light absorbing intensity and blue tetrazolium reaction, respectively. These methods are described fully in part A of METHODS, section 6.

b) Endocrine assays

The following endocrine assays were done in the routine laboratory of the University Clinic of the Royal Victoria Hospital and not by the author of this thesis.

i) Aldosterone determinations

Aldosterone was assayed biologically for sodium-retaining activity in adrenalectomized rats by the method of Venning and

Dyrenfurth (25).

(ii) Pregnanediol and pregnanetriol determinations

Aliquots corresponding to 100 ml. volumes of urine previously hydrolysed with beta-glucuronidase were assayed for the content of these steroids in conc. H_2SO_4 after their isolation by a modification of the column chromatographic method of Eberlein and Bongiovanni (279)

(iii) Total 17-hydroxycorticosteroid excretion

The total 17-hydroxycorticosteroids were measured by a modification of the Porter-Silber (280) reaction on chloroform extracts of about 30 ml. urine that had been incubated with beta-glucuronidase.

c) Method of expressing results

It has been established in this laboratory that in pregnant (and non-pregnant) individuals the creatinine excretion per 24 hours, approximately 1 gm., remains constant from day to day (100). There was, however, a considerable variation, from 0.68 to 1.22 gm., in the creatinine excretion of supposedly 24 hour urine specimens received from case A.O. that indicated that some of the samples may not have been properly collected. It was decided, consequently, to express all quantitative results for each of the urine samples investigated per unit of creatinine excretion i.e. as mgm. (or ug.) of steroid excreted per gram of creatinine.

PART II: METHODS USED IN EXPERIMENTAL WORK ON PREGNANCY IN THE RAT

INTRODUCTION

As previously mentioned certain studies were carried out on the rat primarily because it was impossible to do them on human subjects. For this reason and because it was also of interest to see if in the rat pregnancy involved changes in the rate of corticosteroid production and metabolism as it apparently does in the human the following experiments were carried out.

1) The rate of corticosteroid secretion by adrenal glands of both pregnant and non-pregnant female rats was compared in both 'in vitro' and 'in vivo' studies including the incubation of adrenal glands and collection of adrenal venous blood, respectively.

2) The possibility that alterations in the rate of corticosteroid metabolism could be demonstrated directly at the level of liver function was investigated with incubations of liver tissue obtained from pregnant and non-pregnant female rats under 'in vitro' conditions.

3) The role of the rat placenta was also investigated in a series of incubations with consideration of the possibilities of showing a) a secretion of corticosteroids, b) a direct influence of the placenta on adrenal function and c) an interaction of placental tissue with corticosteroids, 'in vitro'.

All the animals used were hooded rats from the colony at the Royal Victoria Hospital. This colony has been inbred for 30 years. The rats were fed on a regular purina fox chow diet and given tap

water to drink.

A. Incubations of Adrenal Glands

Adrenal glands from female and male rats were incubated under 'in vitro' conditions and their rate of steroid production was measured.

The weights of the male rats used in these experiments ranged from 170 to 180 gm. Those of non-pregnant females ranged from 157 to 314 gm., average of 128 rats was 223 gm; the weights of pregnant rats ranged from 186 to 404 gm. (average of 112 rats was 277 gm.)

1) Preparation of Adrenal Glands for Incubation

The animals were kept in a room maintained at a constant temperature and humidity for 2 days prior to operation. The adrenalectomy was performed under ether anaesthesia except in 2 experiments where nembutal was used; 0.3ml. of a preparation (Abbott lab.) containing 60 mgm. pentobarbital sodium was administered subcutaneously. The excised adrenals were placed into ice-cold oxygenated Krebs-Ringer solution (KRS). Each adrenal was dissected free from fat with care taken to retain the capsule. It was then cut into 2 parts with fine scissors and each half added to one of 2 beakers containing ice-cold incubation medium. This procedure was carried out on a piece of filter paper wetted with KRS. It was kept cold on a petri dish over ice. The adrenalectomy and the cutting of the adrenals took about 3 hours in all. The tissue was then drained from its medium, blotted quickly on filter paper and weighed on a torsion balance.

2. Incubation Procedure

The weight of the adrenal tissue (approximately 500 mgm. per sample) varied depending upon the number of rats available. It was transferred to 100 ml. beakers containing 30 ml. of oxygenated Krebs-Ringer bicarbonate solution (p^H7.4) with added 200 mgm.% glucose (as for human placental incubations). The incubations were carried out under a 95% O_2, 5% CO_2 gas phase at 37°C in a Dubnoff metabolic shaking incubator for up to 5 hours.

Usually there were three incubation periods; (1) 0 to 1 hour, (2) 1 to 3 hours and (3) 3 to 5 hours. The medium was changed at the end of each period. It was warmed to 37°C prior to its addition to the tissue for the second and third incubation periods.

3) Incubation of Adrenal Glands with other Tissue

In a few experiments freshly removed rat placental tissue (cut into small pieces with scissors) and in 1 experiment freshly halved pituitaries were added to the incubations. These tissues were kept in ice-cold KRS prior to incubation.

4) Extraction of Steroids from Incubation Media

After the incubations were completed the medium was decanted off, the beaker content was rinsed with distilled water and this washing was added to the medium. This aqueous solution was then extracted for its corticosteroid content with 3 x 100 ml. redistilled chloroform and the solvent subsequently evaporated off in vacuo below 50°C.

5) Paper Chromatographic Procedure

The chloroform extracts were applied first in the chloroform-formamide system. The cortisone-aldosterone zone was eluted and

reapplied either in the Bush C or Bush B5 systems for isolation of aldosterone. The corticosterone zone of the first chromatogram was eluted and reapplied in the toluene-propylene glycol system for 15 hours to achieve better separation and a further purification of this steroid.

6) Measurement of Corticosteroids

Corticosterone was estimated by 3 different methods, INH and BT reactions and UV light absorption, aldosterone by UV light absorption. In cases where there was not sufficient material to be measured by this procedure the soda fluorescence method was used. All these procedures are described in detail in part I of METHODS (A. Human Placental Incubation, section no. 6).

B. Adrenal Venous Blood Collections

Adrenal venous blood was obtained from non-pregnant female rats ranging in weight from 165 to 240 gm. (average of 29 was 197 gm.) and pregnant rats with weight range from 186 to 310 gm. (average of 29 was 248 gm.). Pregnant rats were generally close to term (21 days) when used.

1) Preparation of Rats for Operation

Non-pregnant rats were anaesthesized with 0.2 to 0.3 ml/rat of nembutal given s.c. in a single dose. As pregnant rats did not tolerate these amounts of nembutal smaller quantities - 0.15 ml rat were given s.c. Fifteen minutes after the nembutal injection, each rat received 1 ml. (1000 units) of a solution of heparin (Connaught lab) in distilled water, s.c. and fifteen minutes later the surgical procedure was carried out. At this time some pregnant rats needed more nembutal and 0.1 ml, s.c. was admin-

istered if necessary.

2) Procedure for Collection of Blood

The adrenal venous blood collections were performed according to the modification of Giroud and Das Gupta (100) of the original method of Bush (185). The abdomen was cut open and 2 ml. of warm 0.9% saline injected into the gut. The viscera were gently moved to one side, covered with cotton pads wetted with warm saline and the left adrenal and kidney region exposed. All blood flow through the renal vein (except that coming from the adrenal vein) was stopped by ligations at the appropriate areas, i.e. blood supply from left kidney and ovarian vein and to the vena cava. A glass cannula rinsed with heparin solution was then inserted into the renal vein through which the adrenal venous blood could flow. The abdominal contents were covered with cotton pads and the rats were kept warm under lamps. This entire procedure took from 15 to 30 minutes to perform.

Blood samples were kept only if rats remained alive with continuous blood flow for a one hour period after the operation. Samples of less than 1.5 ml/rat were discarded.

At the end of the blood collection, the left adrenal glands were removed and weighed on a torsion balance.

3. Extraction of Blood

All blood samples were kept refrigerated and extracted within 48 hours after collection. Blood from 4 or 5 rats was pooled and laked with distilled water prior to extraction. Ethyl acetate extraction, alcohol-petroleum ether partitioning and subsequent

chloroform extraction were done exactly as described for methods used in human placental incubations, in part I of METHODS, section A, no. 2.

4. Paper Chromatography and Steroid Estimation

The extracts were chromatographed in the Bush B5 system. The aldosterone zone (between standard compounds E and F) was eluted with ethanol and submitted for assay according to the Simpson-Tait method (281) on 25 gm. adrenalectomized albino rats. This assay was very kindly carried out by Drs. Giroud and Das Gupta at the Montreal Childrens Hospital.

The corticosterone zone of the Bush B5 paper was eluted and reapplied in the toluene-propylene glycol system for 15 hours. The corticosterone zone was eluted and measured by UV light absorption and INH and BT reactions in the usual way, (Part I of METHODS, section A no. 6).

C. Incubations of Liver Tissue with Added Steroids

1) Source of Liver Tissue Used

Liver tissue was obtained from pregnant and non-pregnant female rats. One pregnant rat (from 18 to 21st day of gestation) and one non-pregnant were used for each experiment. The rats chosen were of similar age, approximately 3 months. The range of weights for pregnant rats was from 230 to 310 gms. (average, 270 gm.) for non-pregnant animals, from 185 to 250 gms. (average, 216 gm.). The increased weight in pregnant rats was accounted for by the products of gestation.

The animals were killed immediately by a blow on the head, their livers were quickly removed and added to ice-cold Krebs phosphosaline buffer (267), the medium for these incubations. Lobes of the same shape and position were used for all the incubations.

2) Incubation Procedure

The liver tissue was cut into slices with a razor blade and from 200 to 250 mgm. (weighed on a torsion balance) was used per sample. The cutting was done on petri dishes kept over ice. Usually 4 samples from different areas of each lobe were incubated with added corticosteroids in 6.2 ml. of phosphosaline buffer (pH 7.4) containing 100 mgm. % glucose. Controls consisted of pregnancy and non-pregnancy tissue incubated without steroid and, also, of samples of steroid incubated alone. In 10 experiments corticosterone (162 to 338 ug/sample) was used as the steroid substrate and, in 2 others, 200 ug/sample of d.l. aldosterone-21-monoacetate was used. These steroids, in solution in ethanol, were added to 20 ml. incubation beakers. The alcohol was evaporated off under nitrogen and the dry steroid residue was then completely dissolved in 0.2 ml. propylene glycol prior to its dilution with incubation medium. This was done to avoid the presence of alcohol in the incubation medium but at the same time to ensure complete solution of the steroid substrate therein. The incubations were carried out for 3 hours under a 100% O_2 gas phase at 37°C in a Dubnoff metabolic shaking incubator.

3. Extraction of Total Free Steroids

After the incubations were completed the medium for each

sample was decanted, the beaker content washed thoroughly with distilled water and this washing added to the medium. The steroids were extracted from the aqueous solution (50 ml.) with 3 x 100 ml. redistilled chloroform and the solvent then evaporated off in vacuo below 50°C.

4. Measurement of Steroids

The dried chloroform extracts were ready for chemical assay without further purification being necessary. Each sample was dissolved in an appropriate amount of purified ethanol. The Δ4-3-ketone content of this alcohol solution was measured by its UV light absorption. Aliquots of the ethanol solution were then estimated by the INH and BT reactions which are described in detail in part I of METHODS (human placental incubation, section A, no. 6.)

D. Incubations of Rat Placentae with Added Steroids

1) Preparation of Tissue

In 4 experiments placentae were taken from pregnant rats after removal of their livers and suspended in ice-cold Krebs phosphosaline buffer (medium). Each placenta was cut with fine scissors into 2 to 4 portions (as equal as possible) depending upon the number of samples desired for incubation. Each of these portions was then separately cut into smaller pieces which were dropped into beakers containing ice-cold medium. All cutting was done on petri dished kept over ice. After all the tissue was cut each of the samples was drained from its medium, quickly blotted

and weighed on a torsion balance. The weighed tissue was transferred to beakers containing buffer and, in some samples, added corticosterone for a 2 hour incubation period.

2. Incubation Procedure, Extraction of Steroids from Media and their measurement

The conditions for these placental incubations were identical to those described for the liver experiments. The extraction of the steroids from the media and their measurement was similarly performed as for the studies on liver, (section C.)

3. Homogenates of Tissue

In 1 experiment after the incubation was completed the samples of tissue after removal of the medium were transferred to Potter Elvehjem tubes with 5 ml. of distilled water for homogenization. This was done to recover any steroid retained within the tissue. The homogenates were then treated with 150 ml. of redistilled acetone and filtered after 3 hours standing with occassional stirring. The filtrate was evaporated in vacuo below 50° C to remove all acetone. The aqueous residue was extracted with chloroform as usual (see part C of methods) and the extracts were chromatographed in the Bush B5 system. The zone of corticosterone was eluted and this steroid estimated by INH and BT reactions and by UV light absorption in the usual way. See METHODS part I, Section A, no. 6.

RESULTS

PART 1: EXPERIMENTAL WORK ON HUMAN PREGNANCY

A. Incubations of Human Placentae

Eleven experiments were carried out with placentae obtained at caesarian section. They were converted into a mince and incubated in Krebs-Ringer solution with the purpose of demonstrating an 'in vitro' production of aldosterone and other corticosteroids. Efforts were also made to stimulate steroidogenesis by the addition of the trophic hormones, chorionic gonadotrophin, ACTH and prolactin to the incubation medium of samples of tissue; cholesterol and pregnenolone which are known precursors of adrenal cortical hormones were similarly added. The possibility of showing an 'in vitro' production of progesterone under these conditions was simultaneously investigated. It was also considered that the placental tissue although producing corticosteroids and, especially, progesterone was not freely releasing them into the incubation medium due to protein binding or other phenomena. For this reason in one experiment a NaOH hydrolysis of the incubated tissue was carried out for a more complete recovery of progesterone and in two other experiments the incubated placental tissue was homogenated to liberate all steroids from within the cells.

1. Compounds isolated from human placental incubations.

Seven UV light absorbing compounds were isolated from the blood content and/or incubation medium of human placentae. They include cortisone, hydrocortisone, progesterone and 4 unidentified steroids designated as compounds X_1, X_2, X_3, and X_4 (Table 1).

a) Compounds more regularly isolated

(i) Progesterone

This steroid was obtained from the incubation medium of each of the 11 placentae and was also isolated from the cord blood content of one of them in amount of 0.38 ug./ml. Progesterone is the only steroid which has been definitely identified. (1) Its chromatographic mobility in heptane-propylene glycol was similar to that of authentic progesterone. (2) It had a UV light absorbing maximum at 240 mμ which is characteristic of $\Delta 4$-3- ketonic steroids and (3) reacted positively with isonicotinic acid hydrazide which measures the same grouping. (4) The maximum of UV light absorption in conc. H_2SO_4 was at 292 mu which is similar to that obtained for standard progesterone and (5) the infra-red spectrum of the steroid isolated was the same as for authentic progesterone, Fig. 1.

(ii) Cortisone

Cortisone was isolated from the 9 placentae in which its presence was investigated. Cortisone was identified on the basis of (1) its chromatographic mobility in the chloroform-formamide, Bush C and Bush B5 systems, (2) its UV light absorbing maximum in ethanol at 239 mu, (3) its reactions with blue tetrazolium which establishes the presence of the alpha-ketol group and (4) by its maxima at 280, 340, 420 mu in conc. H_2SO_4 which were similar to those obtained for authentic cortisone.

(iii) Hydrocortisone

Hydrocortisone was obtained from 6 out of 7 placentae in which its presence was investigated. Its characterization was

TABLE I

COMPOUNDS ISOLATED FROM HUMAN PLACENTAL INCUBATIONS

Compound	UV absorption in ethanol maxima (mu)	BT reaction	UV absorption in conc.H_2SO_4 maxima (mu)	Chromatographic mobility of unknown compounds
THF + THE (zone)	- -	+		
Hydrocortisone	+ 240	+		
Cortisone	+ 239	+	280, 340, 420	
Progesterone	+ 240	-	292	
X_1	+ 238	-		similar to aldosterone in $CHCl_3$-form., Bush C and Bush B5 systems
X_2	+ 250		below 220, 245-250, 335	Toluene-propylene glycol = 0.18 (Rt DOC)
X_3	+ 272		235, mainpeak at 265	" 0.35
X_4	+ 238		285, 420	" 0.74

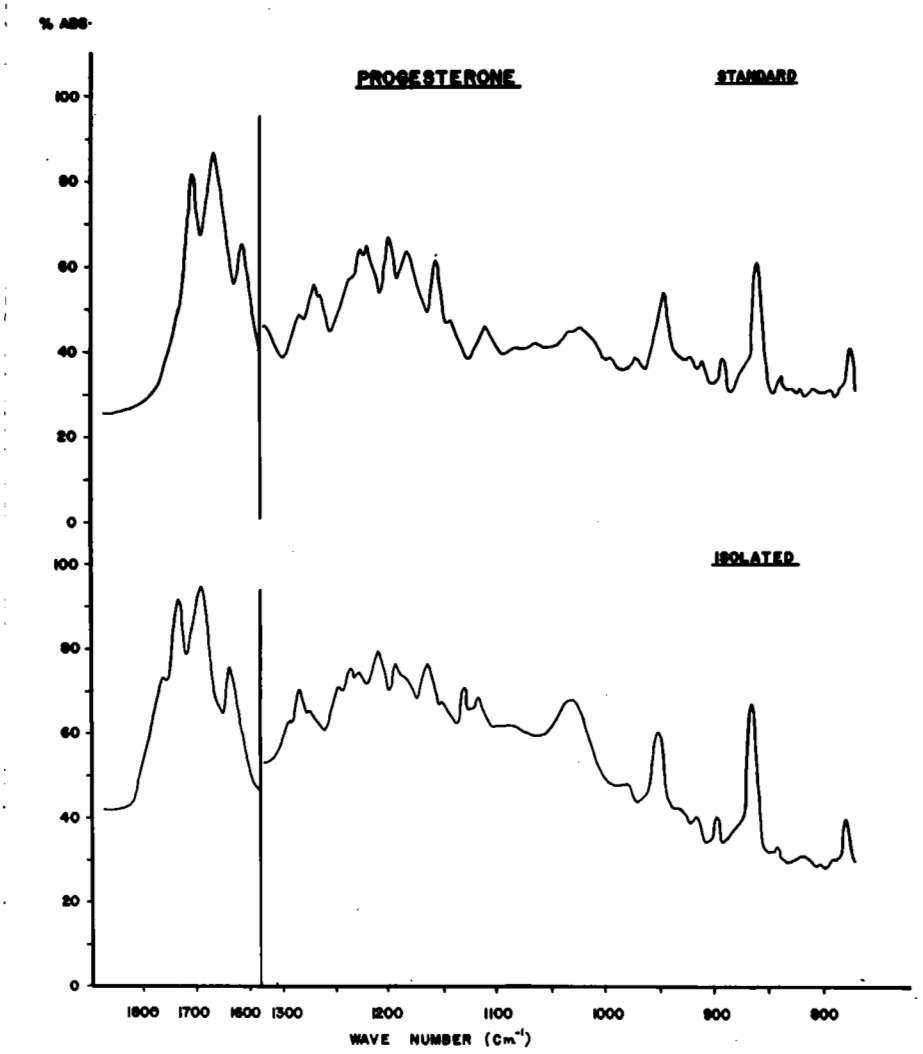

FIGURE 1:

Infrared spectrum of progesterone isolated from human placenta and of standard progesterone (KB_r Crystal).

based on (1) mobility in the chloroform-formamide, Bush C and Bush B5 systems, (2) its UV light absorbing maximum at 240 mu in ethanol and (3) its reaction with BT solution. The amounts present were too small to permit further identification by H_2SO_4 chromogen spectrum.

(iv) Compound X_1

A compound, X_1, was obtained from 7 out of 11 placentae. It has chromatographic mobility similar to that of aldosterone in the chloroform-formamide, Bush C and Bush B5 systems of chromatography. It does not appear to be aldosterone, however, as 5 and 3 ug. samples (estimated by UV light absorption) did not show Na-retaining activity in a bioassay that can detect a minimum of 2 ug. of aldosterone. In another experiment 7 and 8 ug. quantities of this compound did not give a positive BT reaction as would aldosterone which possesses an alpha-ketolic group. The zone of compound X_1, may, however, be a mixture which consists predominantly of the unknown steroid but in which aldosterone may also be present in quantity too small in the amounts of tissue used to be detectable.

b) Other compounds isolated from human placentae

(i) Compound X_2

The compound X_2 was isolated from the cord blood of one placenta. It was also obtained in amount of 11.25 ug. (hydrocortisone equivalents) in the first hour of incubation of 268 gm. of tissue from another placenta but could not, however, be detected in the medium of the following period of incubation.

Compound X_2 absorbed UV light maximally at 250 mu and appears, therefore, not to have been a $\Delta 4$-3-ketonic steroid. The maxima in conc. H_2SO_4 were at 245-250, 335 and below 220 mu. In the toluene-propylene glycol system it was more polar than compound DOC and had the same mobility as corticosterone.

(ii) Compound X_3

This compound isolated from 3 placentae was present in largest amount of all the unidentified compounds. It was obtained in greatest quantity from the blood content of one placenta while the tissue, 225 gm., yielded a much lesser amount into the incubation medium according to comparative intensity on paper chromatograms. Compound X_3 does not appear to be a corticosteroid as it showed a UV light absorbing maximum at 272 mu which is close to that shown by oestrogens. Its maxima in conc. H_2SO_4 were at 235 and 265 (main peak) mu. In toluene-propylene glycol it is more polar than desoxycorticosterone and moves between compounds A and B.

(iii) Compound X_4

A compound X_4 was obtained from 2 placentae and no attempt was made to isolate it in other experiments. It was obtained from the blood content of one placenta in amount corresponding to 14.25 ug. of hydrocortisone. Compound X_4 was also present in trace quantities in the incubation medium of the first hour of incubation of 268 gm. of tissue but not in detectable quantities in that of a succeeding incubation period. Its UV light absorption maximum at 238 mu indicates that it was a $\Delta 4$-3-ketonic steroid. Maxima in conc. H_2SO_4 were at 285 and 420 mu.

In toluene-propylene glycol it was slightly more polar than compound DOC and moved faster than both compounds A and B.

(iv) Compounds THF and THE

Blue tetrazolium reactive material with the same mobility as standard compounds THF and THE in the chloroform-formamide system was isolated from one placenta. It was not characterized further but presumed to consist of a mixture of these steroids.

2. Quantity of various steroids isolated from incubation media of the different placentae.

Table II shows the quantity of steroids isolated from the incubation medium of the first period of incubation of the different placentae studied. The values are expressed in ug. of steroid per 100 gm. of incubated tissue.

Of all the steroids in the incubation media progesterone was always present in the highest concentration. It was isolated in amounts ranging from 77.00 to 185.00 ug./100 gm. of tissue with the average of 10 determinations being 114.2 ug. Of the corticosteroids, cortisone was present in greatest quantity ranging from 6.70 to 21.60 ug/100 gm. of tissue - the average of 8 measurements was 13.71 ug. Hydrocortisone was isolated in quantity ranging from 0.00 to 9.0 ug/100 gm. of tissue, the average being 4.48 ug. Compound X_1 was always the least of the regularly measured steroids ranging from 0.00 to 7.50 ug/100 gm. of incubated tissue and the average of 10 determinations was 2.31 ug.

There appeared to be no correlation between the number of hours of the first period of incubation of the different

TABLE II

STEROID CONTENT OF MEDIA DURING FIRST PERIOD OF INCUBATION OF DIFFERENT PLACENTAE

Experiment	No. hours	ug. steroid/100 gm. tissue			
		Progesterone	Cortisone	Hydrocortisone	Compound X_1
1	5	122.37			6.75
2	3	125.00			3.13
3	1	103.00	21.00		1.96
4	3	185.00	6.70		1.27
5	3	129.40	11.26	8.24	1.52
6	1	90.54	21.60	0.00	0.00
7	3	136.00	14.30	3.20	0.95
8	3	79.97	12.30	3.48	0.00
9	3	74.00	7.20	2.96	0.00
10	3	93.75	15.36	9.00	7.50
Average		114.20	13.71	4.48	2.31

placentae and the corresponding quantity of progesterone and compound X_1 yielded into the incubation media (Table II). On the other hand, the highest concentrations of cortisone, 21.00 and 21.60 ug/100 gm., were obtained in the 2 experiments when the first period of incubation was only 1 hour. In one of the latter instances no detectable amount of hydrocortisone could be obtained but this steroid was always isolated in measurable quantity when the first incubation period was of 3 hours duration. The results suggest that the amounts of compounds E and F isolated from these incubations of placenta were to some extent related to time and this possibility is dealt with more thoroughly in a following section.

3. Effect of addition of chorionic gonadotrophin, adrenocorticotrophin and prolactin to the incubation medium.

a) Chorionic gonadotrophin (HCG)

The hormone HCG was used on the basis of its luteotrophic activity in humans. The administration of HCG stimulates the production of progesterone by the corpus luteum in women who show subnormal pregnanediol excretion in early pregnancy. Furthermore, when chorionic gonadotrophin is given to women in the luteal phase of the menstrual cycle there is a delay of bleeding while pregnanediol continues to be excreted in the urine (100). There has been speculation, therefore, that HCG may act to stimulate the production of progesterone and oestrogens by placental tissue after the corpus luteum becomes nonfunctional. For this reason HCG was added to the incubation medium of 3 placentae (Table III) in an attempt to stimulate steroidogenesis 'in vitro'. It was used in experiment 5 in amount

TABLE III

ADDITION OF HUMAN CHORIONIC GONADOTROPHIN (HCG) TO THE INCUBATION MEDIA OF HUMAN PLACENTAE

Experiment	Sample	Steroids isolated from media of 0 to 3 hours incubation of tissue ug./100 gm. tissue			
		Progesterone	Cortisone	Hydrocortisone	Compound X_1
5	Control (1)	129.40	11.26	8.24	1.52
	" (2)	119.30	-	7.37	1.20
	7 U HCG/gm.	119.70	12.50	7.83	1.69
6	Control (1)	131.32	23.15	0.00	0.00
	" (2)	131.08	24.08	0.00	0.00
	10.4 U HCG/gm. (after first hour)	134.05	18.54	0.00	0.00
7	Control	136.00	14.30	3.20	0.95
	32.7 U HCG/gm.	137.19	13.70	3.81	1.01

of 7 U/gm. of tissue from 0 to 3 hours of incubation; in experiment 6 in quantity of 10.4 U/gm after the first hour of incubation (from 1 to 3 hours) and in experiment 7 in amount of 32.7 U/gm. from 0 to 3 hours of the incubation. In none of these experiments did the steroid content isolated from the incubation media differ significantly in HCG stimulated tissue in comparison with the controls. For example, in experiment 7, where the greatest amount of HCG was used, the control sample showed levels of compounds E, F, X_1 and progesterone amounting to 14.30, 3.20, 0.95 and 136.00 ug./100 gm., respectively. When 32.7 U HCG/gm. were added to the incubation medium of another portion of tissue the steroid content/100 gm. of tissue included 13.70 ug. of cortisone, 3.81 ug. of hydrocortisone, 1.01 ug. of compound X_1 and 137.19 ug. of progesterone. Similar findings were obtained in the other 2 experiments.

The results indicate that HCG did not stimulate a production of any of the measured steroids during incubation of placental tissue.

b) Adrenocorticotrophin (ACTH)

Adrenocorticotrophin stimulates the secretion of corticosteroids by the adrenalcortex both in vivo and in vitro. If the placenta can produce adrenal-like hormones in vitro, by analogy such activity might be stimulated by the addition of ACTH to the incubations. This was the purpose for the use of ACTH in 2 experiments. Amounts of 4.9 and 8.0 U/gm. of tissue were added to the incubation medium of one portion of tissue (Table IV). The ACTH preparation used was previously shown to stimulate the secretion of aldosterone by adrenal glands which were

TABLE IV

ADDITION OF ADRENOCORTICOTROPHIN (ACTH) TO THE INCUBATION MEDIA OF HUMAN PLACENTAE

Experiment	Sample	Steroids isolated from media of 0 to 3 hours incubation of tissue ug./100 gm. tissue			
		Progesterone	Cortisone	Hydrocortisone	Compound X_1
7	Control	136.00	14.30	3.20	0.95
	4.9 U ACTH/gm.	130.00	14.35	3.50	0.94
8	Control	79.97	12.30	3.48	0.00
	8.0 U ACTH/gm.	76.67	12.15	3.67	0.00

incubated 'in vitro' under similar conditions to those used for the placental incubations. It was made by Nordic Biochemical Co. from hog pituitary tissue.

In experiment 8 the following steroid concentration /100 gm. of tissue was obtained from the incubation medium of the control sample: 12.30 ug. of cortisone, 3.48 ug. of hydrocortisone, 0.00 ug. of compound X_1 and 79.97 ug. of progesterone. The portion of tissue incubated with 8 U ACTH/gm. yielded similar quantities of steroid into the incubation medium; compounds E, F, X_1 and progesterone were present in amounts of 12.12 3.67, 0.00 and 76.6 ug./100 gm., respectively. Similar results were obtained in the other experiment where ACTH was used.

Adrenocorticotrophin did not stimulate the production of the corticosteroids which were measured or of progesterone when added to incubations of placental tissue.

c) Prolactin (luteotrophin)

Prolactin is used clinically to enduce lactation in some women following termination of pregnancy (282). This hormone is identical to luteotrophin which is necessary for the secretion of progesterone by the corpus luteum in hypophysectomized rats which have been properly primed with FSH and LH hormones (100). Prolactin was, therefore, used in one experiment in an attempt to stimulate a production of progesterone and of corticosteroids by placental tissue 'in vitro'. The prolactin (NIH-SP-1) used was a highly purified sheep preparation estimated to contain 20 I.U./mg., being distributed by the National Institutes of Health. It was added in amounts of 1.48 U/100 gm. of tissue for both a 0 to 3 hour and a subsequent 3 to 6 hour

TABLE V

ADDITION OF PROLACTIN TO THE INCUBATION MEDIA OF HUMAN PLACENTAE

Incubation period (hrs)	Sample	Steroids isolated from incubation media of tissue and from tissue hydrolysate ug./100 gm. tissue			
		Progesterone	Cortisone	Hydrocortisone	Compound X 1
0 - 3	Control	74.00	7.20	2.96	0.00
	1.48 U Pro./gm.	74.00	6.68	2.96	0.00
3 - 6	Control	41.90	0.74	0.00	0.00
	1.48 U Pro./gm.	36.40	0.74	0.00	0.00
Tissue hydrolysed after incubation	Control	90.50			
	Prolactin added	89.50			

incubation period (Table V). In the first period of incubation the steroid content in the incubation medium of the control sample was 7.20 ug of cortisone, 2.96 ug. of hydrocortisone, 0.00 ug. of compound X_1 and 74.00 ug. of progesterone, all values per 100 gm. of tissue. The portion of tissue incubated with prolactin yielded similar amounts 6.68, 2.96, 0.00 and 74.00 ug. of compounds E, F, X_1 and progesterone/100 gm. of tissue, into the medium. A similar pattern of values was obtained in the 3 to 6 hour period of incubation.

After the incubation was completed the residual tissue was hydrolysed with 5% NaOH solution. This procedure according to Pearlman and Cerceo (158) results in a greater recovery of progesterone from placental tissue. (It would, however, result in destruction of corticosteroids). Virtually the same quantity of progesterone was thus obtained from the control sample and from the one which had been incubated with prolactin for 6 hours, 90.50 and 89.50 ug. progesterone/100 gm., respectively. This showed that the addition of prolactin did not increase the total progesterone content of placental tissue during incubation.

4. <u>The influence of time on the quantity of steroids isolated from the incubation media.</u>

Table VI shows the influence of time on the quantity of cortisone and progesterone isolated from the incubation media of human placentae. The media were changed after each period of incubation.

The values for cortisone/100 gm. of tissue were always the highest in the first period of incubation and then were con-

TABLE VI

INFLUENCE OF TIME ON QUANTITY OF STEROIDS ISOLATED FROM INCUBATION MEDIA OF HUMAN PLACENTAE

Exp.	Period of incubation	Duration (hours)	Steroids isolated			
			Cortisone		Progesterone	
			ug./100 gm.	ug./100 gm./hr	ug./100 gm.	ug./100 gm/hr
3	First	1	21.00	21.00	103.00	103.00
	Second	2	5.32	2.66	50.37	25.19
6	First	1	21.60	21.60	90.42	90.42
	Second	2	2.15	1.08	40.90	20.45
	Third	2	1.08	0.54	33.90	16.95
8	First	3	12.30	4.10	79.97	26.66
	Second	3	1.74	0.58	51.46	17.15
9	First	3	7.20	2.40	77.00	25.67
	Second	3	0.74	0.25	41.50	13.83

siderably less in the following period. For instance in experiment 3 they decreased from 21.00 to 5.32 ug./100 gm. tissue and in experiment 6 from 21.60 ug. in the first period to 2.15 ug/100 gm. in the second; a further decrease to 1.08 ug. in the third period of incubation was observed. Similar findings were obtained in 2 other experiments. When these cortisone values are expressed as ug./100 gm./hr., in experiment 3 they decreased from 21.00 ug in the first period to 2.66 ug. in the second; in experiment 6 they decreased from 21.60 to 1.08 ug./100 gm./hr., and similar observations were made in the other experiments. The other steroids with polarity similar to that of cortisone, hydrocortisone and compound X_1, were always present in lesser quantities than those of cortisone in the first incubation period and usually were no longer detectable in a succeeding one. In experiment 9 the concentration of hydrocortisone/100 gm. tissue decreased from 2.96 ug. in the lst period to 0.00 ug. in the second; these values per/100 gm./hr., were 0.99 and 0.00 ug., respectively.

Progesterone, also, was always isolated in greatest amount from the medium during the first period of incubation. But these values did not decrease to the same extent as those of cortisone neither in the second nor third periods of incubation, (Table VI). For instance, in experiment 6 the quantities of progesterone/100 gm. tissue were 90.42, 40.90 and 33.90 ug. in the 3 succeeding periods of incubations; when expressed as ug. progesterone/100 gm. of tissue/hr. they were 90.42, 20.45 and 16.95, respectively. Similar findings were obtained in 3 other experiments.

5. Possibility of 'in vitro' production of progesterone during incubations of human placentae.

It would appear that the placenta continues to yield progesterone into the incubation medium after the 1st period at a relatively high rate in comparison with that of cortisone. The following suggestions could be made to explain this phenomenon:

(1) The placenta is producing progesterone during the incubation.

(2) The placenta is not producing progesterone during the incubation but its progesterone content is diffusing into the incubation medium at a limited rate. The latter might be due to the relative insolubility of progesterone in aqueous solution or to a binding of this steroid to a placental protein. The retention of progesterone within the cell might result from some other, unknown, phenomenon.

These possibilities were investigated in studies with NaOH hydrolysed and homogenated tissue.

a) NaOH hydrolysis of placental tissue

In experiment 9, Table V, as previously mentioned, two samples of tissue both yielded 74.00 ug. progesterone/100 gm. into the incubation medium during the first period and then 41.90 and 36.40 ug/100 gm. tissue into the medium during the second period of incubation. After a total of 6 hours of incubation each of the samples of tissue was removed from the medium, hydrolysed with NaOH solution and then extracted for progesterone content. With this procedure an additional 90.50 and 89.50 ug. progesterone/10^0 gm. of tissue was obtained from the 2 samples.,

this means that 43.84 and 44.77% of the total progesterone recovered from the two portions of tissue remained in the placenta even after prolonged incubation. This experiment did not indicate whether or not any progesterone was produced during the incubation periods but did demonstrate that this steroid was not freely passing into the medium since hydrolysis of the tissue resulted in the release of more progesterone.

b) Homogenated placental tissue

Breakdown of the cell wall by homogenization was found to release the progesterone content of the placental tissue and was at the same time a safe procedure for the recovery of corticosteroids which were also measured. The possibility of an 'in vitro' production of progesterone by placental tissue in the experimental system used was investigated in the following experiment with use of homogenates.

In experiment 10, Table VII, one portion of tissue was not incubated but homogenized at zero hour of the experiment; it yielded 255.00 ug. progesterone/100 gm. Another equal portion of tissue was first incubated for 3 hours and then homogenized. The progesterone content of the incubation medium and homogenate were separately determined and were 93.75 and 150.00 ug./100 gm., respectively, a total of 243.75 ug./100 gm. of tissue. The difference in the total progesterone content of the incubated and non-incubated portions of tissue, less than 5%, is not considered to be significant. If progesterone had been produced during the incubation it is reasonable to assume that the total amount isolated from the incubated tissue would have

TABLE VII

THE EFFECT OF INCUBATION ON STEROID CONTENT OF HUMAN PLACENTAE

Sample	ug. steroid isolated/100 gm. of tissue				
	Progesterone	Cortisone	Hydrocortisone	Compound X_1	Zone of cmpds. THF + THE
Non-incubated tissue (homogenate).	255.00	33.00	1.86	8.25	11.25
Incubated tissue 0 - 3 hours medium	93.75	15.36	9.00	7.50	16.50
homogenate.	150.00	1.14	1.50	1.14	13.25

been greater than the amount obtained simply by extraction of the tissue without prior incubation. This indicates that progesterone was not being produced during these incubations of placental tissue from any endogenous precursors of this steroid. The progesterone which was regularly isolated from the placentae was, rather, present in the tissue at the time of caesarian section.

In a second experiment, No. 11 (Table VIII), one portion of tissue was immediately homogenized at zero hour. A second portion was incubated with 5.0 mgm. cholesterol for a 3 hour incubation period and a third portion was similarly incubated with 2.5 mgm. Δ 5-prenene-3β-ol-20-one, pregnenolone. After the incubations were completed the samples of tissue were removed from the media and homogenized.

An amount of 183.00 ug. progesterone/100 gm. of tissue was obtained from the non-incubated sample. The incubation medium and homogenate of the tissue incubated with cholesterol yielded 127.84 and 149.14 ug. progesterone/100 gm., respectively - a total of 276.98 ug./100 gm. tissue. An amount of 670.39 ug. progesterone/100 gm. was obtained from the incubation medium and 586.59 ug/100 gm. from the homogenate of the tissue incubated with pregnenolone; the sum was 1256.98 ug. progesterone /100 gm. of tissue.

The total progesterone content/100 gm. of tissue in the samples incubated with cholesterol and pregnenolone was increased above the control level by 51.36 and 586.87%, respectively. This increase above the amount of progesterone obtained

merely by extraction of the tissue amounted to a production of progesterone at a rate of 31.33 ug./100 gm./hr. in the sample incubated with cholesterol while 357.99 ug. of progesterone/100 gm./hr. was produced in the sample incubated with pregnenolone.

The placental tissue in the incubation system used although not capable of producing progesterone from endogenous precursors did have the capacity to convert cholesterol and Δ5-pregnene-3β-ol-20-one to progesterone. The conversion of the pregnenolone to progesterone occurred very readily as it merely involves oxidation of the 3 carbon hydroxyl group and a subsequent shift of the double bond from the Δ5 to the Δ4 position (74). The conversion of cholesterol to progesterone, however, occurred much less rapidly for it is a more complex process (283). It involves first the loss of 6 carbon atoms from the side chain at carbon 17 of cholesterol and an oxidation of the 20 carbon to form the intermediate Δ5-pregnene-3B-ol-20-one; once the pregnenolone is formed it is rapidly converted to progesterone as described above. It would appear as if the transformation of cholesterol to pregnenolone occurred only slowly in the incubation system used and presumably was the rate-limiting step in the conversion of cholesterol to progesterone.

6. Possibility of 'in vitro' production of corticosteroids during incubations of human placentae.

In 4 experiments the cortisone levels/100 gm. of tissue were always the highest in the first period of incubation and then decreased sharply in the suceeding period(s) as shown

in Table VI. The results suggested that if cortisone were produced after the 1st period of incubation the quantity was negligible. The same could be said of the other compounds with similar polarity, hydrocortisone and X_1, which were usually not present in measurable quantity in the incubation medium after the first period.

The relatively high levels of cortisone during the first incubation periods and the rapid decline thereafter may have been due to a relatively rapid diffusion into the media of the cortisone present in the placentae at the time of caesarian section rather than to any 'in vitro' production. This would seem to be borne out with the following experiments.

In experiment 10, 'Table VII), each of the samples was extracted twice - once, as usual, for free corticosteroids and, then, for any conjugated steroids that might be present. These extracts were combined prior to chromatography and assay.

The control sample of tissue was not incubated but homogenized immediately at zero hour of the experiment; it contained compounds E, F and X_1 in amounts of 33.00, 1.86 and 8.25 ug./100 gm., respectively, and 11.25 ug./100 gm. of alpha-ketolic material with polarity of compounds THF plus THE. Another portion of tissue was first incubated for 3 hours then homogenated. The quantities of steroid obtained from the incubation medium were 15.36 ug. of cortisone, 9.0 ug. of hydrocortisone, 7.50 ug. of compound X_1, and 16.50 ug. of more polar BT reactive steroid, all per 100 gm. of tissue. The homogenate of the incubated tissue yielded an additional amount of compounds E, F and X_1 in

in quantities of 1.14, 1.50 and 1.14 ug./100 gm., respectively, and also 13.25 ug. more of alpha-ketolic compound with polarity of compounds THF plus THE were obtained.

In comparison with the amount of steroid obtained simply by extraction of the tissue there was a lesser total quantity of cortisone, 16.50 as compared with 33.00 ug./100 gm. but a greater quantity of hydrocortisone, 10.50 as compared with 1.86 ug./100 gm., was obtained from incubated tissue. A larger amount of more polar alpha-ketolic material also was found as a result of incubation. This is an indication that about one-half of the cortisone content of the tissue was converted during the 3 hour incubation period, some to hydrocortisone by hydrogenation of the 11-ketone group of cortisone, and another portion to ring A reduced, BT reactive material, presumably consisting of compounds THF and/or THE.

The total concentration of compound X_1 obtained from incubated tissue, 8.64 ug./100 gm., was the same as that isolated from tissue which was not incubated prior to extraction, 8.25 ug./100 gm.

An attempt was made to isolate alpha-ketolic steroids less polar than cortisone ($C_{21}O_4$) from each of the samples. No such material could be detected on 3.0 cm. wide strips of those areas of chromatograms which would contain compounds B and A and their ring A reduced metabolites. The paper strips were treated with a BT reagent which could detect a minimum of about 0.2 to 0.5 ug. of alpha-ketolic steroid per sq. cm. of paper chromatogram.

In experiment 11 the control tissue was similarly

TABLE VIII

THE ADDITION OF CHOLESTEROL AND PREGNENOLONE TO INCUBATIONS OF HUMAN PLACENTA

Sample	ug. steroid isolated/100 gm. of tissue			
	Progesterone	Cortisone	Hydrocortisone	Compound X_1
Non-incubated tissue homogenate	183.00	12.80	0.00	0.00
Tissue incubated with 5.0 mgm. cholesterol (0 - 3 hrs.)				
- medium	127.84	7.65	<*2.00	0.00
- homogenate	149.14	1.68	0.00	0.00
Tissue incubated with 2.5 mgm. Δ5-pregnene-3β-ol-20-one (0 to 3 hrs)				
- medium	670.39	9.75	<*2.00	0.00
- homogenate	586.59	2.27	0.00	0.00

* Soda fluorescence test was positive on 1.5 cm. wide strips of paper.

homogenized without incubation and showed a content of 12.80 ug./100 gm. of cortisone. No hydrocortisone or compound X_1 could be detected by soda fluoresence on 1.5 cm. wide paper strips. A second portion of tissue was incubated for 3 hours with 5.0 mgm. of cholesterol and then homogenized. The incubation medium contained 7.65 ug. of cortisone, traces of hydrocortisone (less than 2.0 ug), and no detectable quantity of compound X_1 per 100 gm. of tissue; the homogenate of the incubated tissue yielded an additional 1.68 ug. of cortisone/100 gm. but no hydrocortisone or compound X_1 could be detected. A third portion of tissue was first incubated for 3 hours with 2.5 mgm. of Δ 5-pregnene-3β-ol-20-one and then homogenized. The incubation medium contained 9.75 ug. of cortisone/100 gm., no detectable compound X_1 and traces, less than 2.0 ug, of hydrocortisone/100 gm. of incubated tissue. The tissue homogenized after incubation contained an additional 2.27 ug. cortisone/100 gm. but neither of the other two steroids in detectable quantity.

These results suggest a conversion of cortisone to hydrocortisone during the incubation period although it occurred to a much lesser extent in this particular placenta than in the one used in experiment 10. This conversion appeared to depend on the initial concentration of cortisone in the placenta, being greater when the cortisone content of the tissue was larger.

The concentration of cortisone and of compound X_1 was, therefore, not increased during incubations of human placentae even when known precursors of adrenal cortical hormones were added to the medium. This is in contrast to the finding of

greater quantities of progesterone during incubation with added cholesterol and pregnenolone although the amount of this steroid similarly did not increase during incubation of placental tissue alone. The quantity of compound X_1 was not influenced by incubation but that of cortisone was decreased as a result of conversion to hydrocortisone and, perhaps, to the ring A reduced metabolites, compounds THF and THE.

There is further indication for the conversion of cortisone to hydrocortisone which has been mentioned previously. In a number of experiments shown in Table II the highest concentrations of cortisone/100 gm. of tissue were obtained in the 2 instances when the first period of incubation was only of 1 hour duration while no hydrocortisone could be detected in one of these cases. When, however, the first period of incubation was of 3 hours duration the concentrations of cortisone were lower but hydrocortisone could always be detected. In the light of the findings obtained in the later experiments the above results suggest that there may have been a slow conversion of cortisone present in placental tissue to hydrocortisone during prolonged incubation.

7. <u>In vitro conversion of exogenous hydrocortisone to cortisone during incubations of human placental tissue</u>.

Repeated experiments indicated that cortisone was the major corticosteroid that could be obtained from placental tissue. During incubation there was no production of cortisone but rather a conversion of this steroid to other compound(s). This suggests that the cortisone isolated from the different

TABLE IX

CONVERSION OF HYDROCORTISONE TO CORTISONE BY HUMAN PLACENTAL TISSUE IN VITRO

Incubation period (hours)		ug. cortisone isolated/100 gm. tissue	
		0 - 3	3 - 6
Exp.	Sample		
8	Control	12.30	1.74
	Hydrocortisone added, 182 ug./100 gm.	39.33	16.06
9	Control	7.20	
	Hydrocortisone added, 364 ug./100 gm.	65.00	

placental incubations was present in the tissue at the time of caesarian section. This presence of cortisone could be due either to (1) production of cortisone by placental tissue, 'in vivo', or (2) to a concentration of cortisone from the maternal circulation by the placental tissue. Since hydrocortisone is the major blood 17-hydroxycorticosteroid in late pregnancy (46) one might expect to find more hydrocortisone than cortisone in placental extracts if it is only a question of concentration of corticosteroid unless a conversion of hydrocortisone to cortisone occurs in the placenta, 'in vivo'. An interconversion of these steroids has been demonstrated in non-pregnant human subjects and the liver is presumed to be the site of this activity (60) (61). It was of interest to see whether conversion of hydrocortisone to cortisone could be shown with placental tissue 'in vitro' as it apparently occurs 'in vivo'. For this reason in 2 experiments hydrocortisone was added to the incubation medium of placental tissue, (Table IX). In experiment No. 8, 182 ug. of hydrocortisone/100 gm. of tissue was added to the incubation medium of both an 0 to 3 hour and a subsequent 3 to 6 hour period of incubation of one portion of tissue. The quantities of cortisone/100 gm. of tissue were 39.93 and 16.06 ug. during the first and second periods of incubation, respectively; the cortisone content in the incubation media of the control sample was significantly lower, 12.30 in the first period and 1.74 ug/100 gm. tissue in the second period.

In experiment No. 9, a still greater conversion of hydrocortisone to cortisone was observed when a larger concen-

tration of hydrocortisone, 364 ug./100 gm. tissue, was added to the incubation medium of one portion of tissue. The quantity of cortisone isolated/100 gm. was 65.00 ug. as compared with 7.20 ug. cortisone/100 gm. obtained from the incubation medium of tissue incubated with nothing added.

The placental tissue, therefore, showed a capacity to convert hydrocortisone to cortisone by oxidation of the 11 carbon hydroxyl group 'in vitro'. If a similar conversion occurs 'in vivo' it might explain the finding of considerably greater quantities of cortisone than of hydrocortisone in placentae about two weeks before termination of pregnancy, even though the major 17-hydroxycorticosteroid in the maternal peripheral circulation at this time is hydrocortisone.

8. Summary of findings.

a) Seven UV light absorbing compounds were isolated from the incubation media of 11 human placentae obtained by caesarian section. These included cortisone, hydrocortisone, progesterone and 4 unidentified compounds designated as X_1, X_2, X_3, and X_4. Compound X_1 had the same mobility as aldosterone in all the chromatographic systems used but no detectable amounts of aldosterone could be isolated from the quantities of tissue used. Alpha-ketolic material with polarity of compounds THF and THE was obtained in one experiment where the incubation media had been extracted for both free and conjugated steroid content.

b) Compounds E, F, X_1 and progesterone were more regularly isolated than the others; they were present in average

amounts of 13.71, 4.48, 2.31 and 114.20 ug./100 gm. tissue respectively, in the medium during the first period of incubation of the different placentae.

c) The addition of the trophic hormones, chorionic gonadotrophin, adrenocorticotrophin and prolactin to the incubation medium of placental tissue did not alter the concentrations of any of the steroids which were subsequently measured. These trophic hormones, therefore, did not appear to stimulate the production of progesterone or of corticosteroids during the incubations.

d) The quantity of all steroids isolated from the incubation media decreased with time of incubation. This decline was marked in the case of the predominant corticosteroid, cortisone, but placental tissue continued to yield progesterone into the medium at a more constant rate. This steady secretion of progesterone into the medium was not, however, necessarily due to 'in vitro' production as one experiment with NaOH hydrolysed tissue showed that even after prolonged incubation approximately 50% of the total progesterone content of the placenta was still retained within the tissue.

e) The total quantity of progesterone was not increased during incubation above the amount which could be obtained simply by extraction of tissue without any incubation. In the incubation system used, therefore, progesterone was not produced from any precursors present in the placentae at time of caesarian section. When, however, known precursors of proges-

terone, cholesterol and Δ5-pregnene-3β-ol-20-one were added to the incubations the progesterone content was increased above control levels. These increases corresponded to an 'in vitro' production of progesterone at the rate of 31.33 and 357.99 ug. /100 gm. tissue/hr., respectively.

f) The more polar steroids, cortisone and compound X_1, were not increased in quantity during incubation above the amount which could be obtained simply by extraction of tissue without any incubation. Unlike progesterone, however, there was still no increase above control levels upon the addition of cholesterol and Δ5-pregnene-3β-ol-20-one to the incubation medium. This means that there was no 'in vitro' production of cortisone or of compound X_1 in any of the experimental conditions employed. While the concentration of compound X_1 was unchanged during a 3 hour incubation period in comparison with the amount present in a non-incubated control sample of tissue that of cortisone actually decreased. This decrease of cortisone as a result of incubation was, however, associated with the appearance of hydrocortisone in the incubation medium in quantity increased above the amount obtained from the non-incubated control sample of tissue. Some cortisone present in the placenta at the time of caesarian section was apparently converted to hydrocortisone during the 3 hour incubations. The extent of this conversion appeared to depend upon the initial concentration of cortisone since it was greater when the placental content of cortisone was larger. In one experiment there appeared also to be a metabolism of cortisone to more polar

alpha-ketolic steroids.

g) The conversion of cortisone to hydrocortisone involves reduction of the carbon 11-ketone to the β-hydroxyl group. This is a reversible reaction which according to the Law of Mass Action would go either way depending upon the relative concentractions of the 2 steroids. Upon the addition of relatively large quantities of hydrocortisone to incubations of 2 placentae a significant conversion of this steroid to cortisone was observed. If the placentae is not producing corticosteroids 'in vivo' the presence of larger quantities of cortisone than of hydrocortisone in the placenta could be due to a similar conversion of hydrocortisone, which is the predominant 17-hydroxycorticosteroid in the maternal circulation in late pregnancy, to cortisone, 'in vivo'.

B. The Urinary Excretion of Corticosteroids and of Metabolites of Progesterone in Pregnant and Non-Pregnant Women

Results obtained for various endocrine assays carried out on normal pregnant and non-pregnant subjects, on two totally adrenalectomized pregnant women receiving corticosteroid replacement therapy and on a similar but non-pregnant patient are reported. These assays include the determination of aldosterone, total 17-hydroxycorticosteroids, pregnanediol and pregnanetriol. The corticosteroid excretion patterns in these patients were investigated and the excretion of the major free corticosteroids, hydrocortisone and cortisone, and of their ring A reduced metabolites was also determined.

1. Normal subjects

The creatinine value on the urine collection of subject M.E. was 1.0 gm. indicating a proper 24 hour sample. The two other normal subjects H.B. and L.C. worked in the laboratory and their urine collections are considered to be reliable.

The range of values for total 17-hydroxycorticosteroid excretion as estimated by Porter-Silber method is from 2.0 to 5.5 mgm./day in normal women and from 2.0 to 8.6 mgm./day in normal men according to routine determinations in the Endocrine laboratory (Table X). M.E. excreted 3.7 mgm./24 hours.

The urinary corticosteroid pattern of M.E. is shown in fig. 2. The concentration of steroid applied was 0.8 hrs. urine per cm. width of paper strip. The pattern of the most polar (Group I) steroids showed the presence of alpha-ketolic

TABLE X

INFORMATION ON URINE SAMPLES STUDIED

Subject	Month of pregnancy	Urine vol.(ml)	Creatinine excretion gm./total vol.	Total 17-OHCS excretion *Porter-Silber test	
				mgm./total vol.	mgm/gm. creatinine
M.E.	-	1080	1.00	3.70	3.70
E.W.	8		1.00	5.10	5.10
** Case H.M.	9	2225	1.07		
** Case A.O.	6	3050	1.22	2.50	2.05
" "	8	2340	0.70	1.50	2.04
" "	9	2100	0.68	1.50	2.20
" "	4 days post-partum	1160	1.04	0.00	0.00
Case S.K.	-			9.20	

* Normal range of PS values is for females from 2.0 to 5.5 mgm./day.
** Urine specimens received in a frozen state.

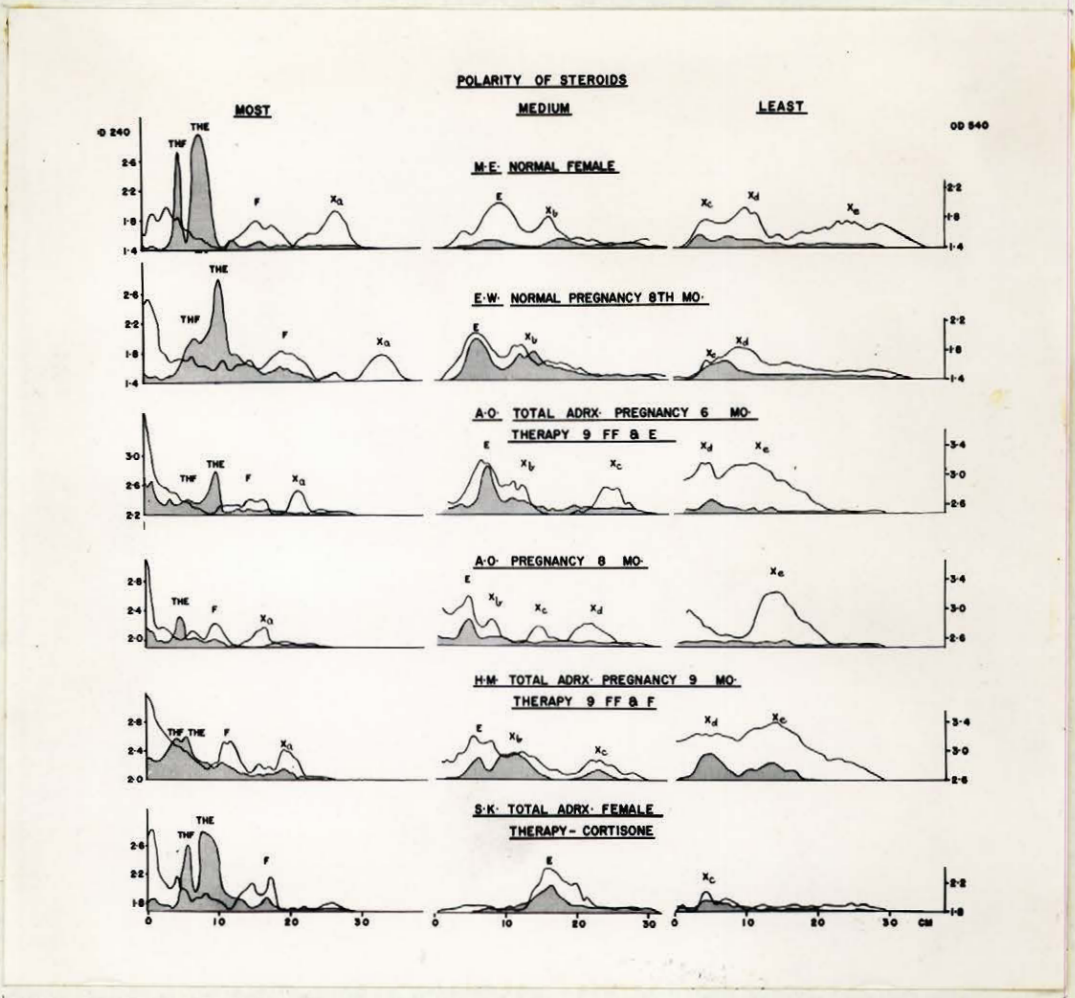

FIGURE 2:

Urinary corticosteroid pattern of pregnant and non-pregnant women.

Aliquot of urinary extract applied (no./hrs/cm. width of paper strip) : M.E. (0.8 hrs/cm), E.W. (0.8 hrs/cm), Case A.O. 6th mo.preg. (1.96 hrs/cm) and 8th mo.preg. (1.12 hrs/cm), Case H.M. (1.6 hrs/cm., and Case S.K. (0.8 hrs/cm).

steroid material in the region of tetrahydrocortisone (THF) and of much smaller amounts of hydrocortisone. The major peak of blue tetrazolium positive steroid was at the region of tetrahydrocortisone (THE). A UV light absorbing steroid with no alpha-ketol group, designated as X_a, and slightly less polar than hydrocortisone, was also present. The pattern of the medium polar (Group II) of steroids showed the presence of small amounts of alpha-ketolic steroid material at the region of cortisone which was associated with larger quantities of UV light absorbing compound of similar polarity. Blue tetrazolium reactive steroid was also seen which was slightly less polar than cortisone, designated as X_b. The pattern of the least polar steroids (Group III) showed the presence of traces of alpha-ketolic steroid material in the region designated as X_c associated with larger amounts of UV light absorbing compound. There were also peaks of UV light absorbing material, but with no alpha-ketol group, in the areas designated as X_d and X_e. This pattern of M. E. was virtually identical to those obtained in another normal female, L.C. and normal male H.B. which have been previously reported except that the male subject excreted somewhat greater amounts of compound X_b. Previous studies have shown that compound THB (tetrahydrocorticosterone) is the predominant metabolite in the zone of X_b (277).

Compounds THF, THE, F and E were excreted at the rate of 0.75, 1.96, 0.16 and 0.17 mgm./day, respectively, in the urine of M.E, (Table XI). The excretion of compounds THF plus

TABLE XI

URINARY EXCRETION OF ALPHA-KETOLIC STEROIDS WITH CHROMATOGRAPHIC MOBILITY OF COMPOUNDS THF, THE, F AND E BY PREGNANT AND NON-PREGNANT INDIVIDUALS

Subject	Condition	Month preg.	mgm. steroid excreted/gm. creatinine						
			THF	THE	(THF + THE)	F	E	(F + E)	THF+THE / F + E
M.E.	normal fem.	-	0.75	1.96	(2.71)	0.16	0.17	(0.33)	8.10
L.C.	" "	-			(1.60)	0.05	0.08	(0.13)	12.30
H.B.	" male	-			(2.20)	0.10	0.16	(0.26)	8.50
E.W.	" preg.	8	0.42	1.99	(2.41)	0.19	0.40	(0.59)	4.08
Case H.M.	preg.(adrenal-ectomized)	9	0.68	0.59	(1.27)	0.36	0.27	(0.63)	2.02
Case A.O.	preg.(adrenal-ectomized)	6	0.12	0.39	(0.51)	0.09	0.79	(0.88)	0.58
"		8	0.03	0.25	(0.28)	0.07	0.39	(0.46)	0.61
"		9	0.11	0.46	(0.57)	0.16	0.65	(0.82)	0.70
"	4 days post-partum		0.05	0.14	(0.19)	0.07	0.28	(0.35)	0.70

THE in subjects L.C. and H.B. was 1.60 and 2.20 mgm./day, respectively. Compound F was present in amount of 0.05 and 0.10 mgm. and compound E in quantity of 0.08 and 0.16 mgm. /24 hours in the urines of L.C. and H.B., respectively. The ratio of the amounts of compounds THF plus THE excreted to those of compounds F plus E were 8.10, 12.30 and 8.50 in subjects M.E., L.C. and H.B. Thus, in these normal individuals the free biologically active sterids, hydrocortisone and cortisone, were excreted in amounts from 1/8th to 1/12th of those of their reduced metabolites, compounds THF and THE.

The range of aldosterone excretion in normal female subjects is from 2 to 7 ug./day, (Table XII).

2. Normal pregnant subject.

One urine specimen from a normal woman, E.W. in the 8th month of her first pregnancy had a urinary creatinine value of 1.0 gm./ day and a total 17-hydroxycorticosteroid excretion of 5.10 mgm/24 hours. This was in the higher range of normal (Table X).

The urinary corticosteroid pattern of E.W. is shown in figure 2. The concentration of steroid applied, 0.8 hrs. urine/cm. width of strip, is the same as for the pattern of the normal subject. The pattern of the most polar (Group I) steroids showed the presence of alpha-ketolic steroid material in the region of compound THF and of smaller amounts in the area of hydrocortisone. The major peak of blue tetrazolium positive steroid was at the region of compound THE; the UV light absorbing material, compound X_a, was also present.

The pattern of the medium polar (Group II) of steroids showed the presence of alpha-ketolic compound in the region of cortisone; the amount was greater than that observed in patterns of normal non-pregnant subjects. There was also a definite zone of BT reactive material slightly less polar than cortisone which in previous studies was shown to consist partly of compound THB. The pattern of the least polar (Group III) of steroids indicates the presence of an alpha-ketolic compound in the area of X_d which was associated with a UV light absorbing material. The least polar UV light absorbing compound, X_e, appeared to be absent from the pattern; it may have come off in the overflow of the chromatogram.

Compounds THF, THE, F and E were present in the urine sample of E.W. in amounts of 0.42, 1.99, 0.19 and 0.40 mgm./24 hours, (Table XI). The ratio of the quantity of the reduced metabolites to that of the 2 free corticosteroids was 4.08. This was below the range from 8.10 to 12.30 observed in the normal subjects and indicates that this pregnant individual was excreting relatively more of the biologically active steroids, hydrocortisone and, especially, cortisone, than were the non-pregnant normal subjects.

The aldosterone excretion in case E.W. was 50.88 ug. day in the 6th month of gestation. The range for aldosterone excretion in normal pregnancy is from 12 to 65 ug./24 hrs. according to many determinations in the Endocrine laboratory of the University Clinic, (Table XII).

TABLE XII

URINARY EXCRETION OF NA-RETAINING HORMONE IN PREGNANT (NORMAL AND ADRENALECTOMIZED) AND NON-PREGNANT INDIVIDUALS

Subject(s)	ug. aldosterone/24 hours.	
	Single determination	Range
Normal, non-pregnant		2 - 7
" , pregnant (3rd - 9th mo.)		12 - 65
Case S.K., non-pregnant (adrenalect.)	1.0	
* Case H.M, pregnant (adrenalect.), 9th mo.	13.0	
* Case A.O, " " 6th mo.	1.0	
" 8th mo.	1.5	
" 9th mo.	1.5	
" 4 days post-partum	1.0	

* Values expressed as ug./gm. creatinine excreted.

A urine specimen of E.W. collected in the 7th month of pregnancy showed values of pregnanediol and pregnanetriol amounting to 51.30 and 12.30 mgm./day, respectively, (Table XIII). These are within the range for normal pregnancy (100).

3. Non-pregnant adrenalectomized patient.

The corticosteroid excretion pattern of case S.K. was studied following total adrenalectomy for Cushing's disease at a time when she was receiving 50 mgm. of cortisone therapy/day. The total 17-hydroxycorticosteroid content of the urine specimen investigated, 9.10 mgm. per day, was slightly above the range for normal subjects. The aldosterone excretion was less than 1.0 ug./day.

The urinary corticosteroid pattern of case S.K. is shown in figure 2; the concentration of steroid applied, 0.8 hrs. urine/cm. paper strip was the same as the one used for the normal pattern. The pattern of the most polar steroids (Group I) showed the presence of compound THF and of a small amount of hydrocortisone. Compound THE was the predominant alpha-ketolic steroid. There was no detectable amount of the UV light absorbing compound X_a. The pattern of the medium polar steroids (Group II) showed an amount of cortisone greater than the quantity observed in patterns of normal non-pregnant subjects. Compound X_b (THB) was absent from the pattern but the UV light absorbing material characteristically present in the area of X_b was seen. The pattern of the least polar steroids (Group III) failed to show any definite areas of either alpha-ketolic or UV light absorbing compounds. There appeared, how-

TABLE XIII

URINARY EXCRETION OF PREGNANEDIOL AND PREGNANETRIOL IN PREGNANT (NORMAL AND ADRENALECTOMIZED) WOMEN

Subject	Condition	Month preg.	mgm. excreted/gm. creatinine	
			pregnanediol	pregnanetriol
E.W.	pregnant, normal	7	51.30	12.30
Case H.M.	pregnant, adrenalectomized	9	35.40	3.54
Case A.O.	" "	6	21.30	1.00
"	" "	8	66.75	9.45
"	" "	9	66.20	1.93
"	4 days post-partum		2.78	0.00

ever, to be traces of BT reactive steroid in the region of X_C associated with similarly small amounts of UV light absorbing material.

4. Pregnant adrenalectomized patients.

The urinary excretion of corticosteroids and of metabolites of progesterone was studied in two pregnant women who were previously totally adrenalectomized for Cushing's disease.

The four urine specimens obtained from one patient, case A.O., were collected in the 6th, 8th and 9th month of gestation and 4 days postpartum. On each occasion she received as maintenance therapy 37.5 mgm. cortisone per day in divided doses, the last dose being given at 10:00 p.m. on the day prior to the urine collection. On the day of collection she was given 0.75 mgm. of 9-α-fluorohydrocortisone in 3 divided doses. A 24 hour urine specimen was obtained from the other patient, case H.M., in the 9th month of pregnancy when she was receiving as maintenance therapy 40 mgm. hydrocortisone, 0.25 mgm. 9-α-fluorohydrocortisone and a daily intake of 3 gm. of salt.

The total urine volumes received from case A.O. were 3050, 2340, 2100 and 1160 ml. with the corresponding creatinine content being 1.22, 0.70, 0.68 and 1.04 gm., respectively. This variation in creatinine levels in supposedly 24 hour samples suggests an improper collection of urine. For this reason all quantitative studies on case A.O. are expressed in terms of amount of steroid excreted/gm. of creatinine for the purpose of comparison with results obtained from other individuals.

Determinations carried out on the urine of case H.M. are also expressed as amount of steroid excreted/gm. of creatinine although the creatinine excretion per total volume of urine received, 1.07 gm., was normal.

The total 17-hydroxycorticosteroid excretion/gm. of creatinine in case A.O. was 2.05, 2.04, 2.20 and 0.00 mgm. in samples of the 6th, 8th, and 9th months of pregnancy and 4 days after delivery, (Table X).

The urinary corticosteroid pattern of case A.O. in the 6th and 8th months of pregnancy is shown in figure 2; the patterns of the 9th month and 4 days postpartum specimens are not shown but were virtually identical to the other two. The concentrations applied per cm. width of paper strip were 1.96 and 1.12 hrs. urine excreted for the 6th and 8th month patterns, respectively.

The pattern of the most polar steroids (Group I) shows the presence of traces of alpha-ketolic material in the region of compound THF in the urine of the 6th month of pregnancy but none could be seen in that of the 8th month; this was probably due to the lesser concentration of steroid applied in the latter pattern. Nevertheless, the amounts of compound THF were less than in any of the other patterns shown even though greater concentrations of steroid were applied on the chromatograms. There were traces of BT reactive material in the zone of hydrocortisone in both patterns while the largest amount of alpha-ketolic steroid was in the region of compound THE in both cases. The UV light absorbing steroid X_a, slightly

less polar than hydrocortisone was obtained from both samples of urine. The patterns of the medium polar (Group II) steroids showed a relatively large area of alpha-ketolic material in the cortisone zone, especially in the one of the 6th month of pregnancy. The latter pattern also appeared to contain a small amount of BT reactive material in the region of X_b (compound THB) which was associated with a characteristically present UV light absorbing compound. No similarly detectable amounts of compound X_b could be detected in the pattern of the 8th month of pregnancy; this was probably due to the lesser concentration applied on the paper strips. The pattern of the least polar (Group III) of steroids indicated the presence of traces of BT positive compound in the region of X_d in the urine of the 6th month of pregnancy of case A.O.; the UV light absorbing compounds X_c, X_d, and X_e were seen in both.

The steroid concentration applied on the chromatograms for case H.M. was 1.6 hrs. urine/cm. of paper strip, figure 2. The pattern of the most polar group (I) of steroids showed the presence of alpha-ketolic steroid material in the area of compounds THF and THE; a relatively large amount of BT reactive compound was seen in the region of hydrocortisone and traces were associated with the UV light absorbing compound X_a. The pattern of the medium polar group of steroids showed a considerable excretion of cortisone; there was a surprisingly large quantity of alpha-ketolic steroid in the zone of X_b but only a small amount in the area of X_c. Both of the latter zones contained the UV light absorbing compounds usually found in

normal urines. The pattern of the least polar steroids (Group III) showed, also, relatively large amounts of BT positive material in the regions of X_d and X_e; these were associated with the UV light absorbing compounds of similar polarity.

The single urine specimen of case H.M. had a content of compounds THF, THE, F and E amounting to 0.68, 0.59, 0.36 and 0.27 mgm./gm. creatinine, respectively, (Table XI). In case A.O., compounds THF, THE, F and E were excreted at the rate of 0.12, 0.39, 0.09 and 0.79 mgm./gm. creatinine, respectively, in the 6th month of pregnancy; at the rate of 0.03, 0.25, 0.07 and 0.39 mgm./gm.creatinine, respectively, in the 8th month of pregnancy and at the rate of 0.11, 0.46, 0.16 and 0.65 mgm/gm. creatinine, respectively, in the 9th month of gestation. Four days after delivery compounds THF, THE, F and E were excreted at the rate of 0.05, 0.14, 0.07 and 0.28 mgm./gm. of creatinine, respectively.

It would perhaps be more correct not to refer to the above determinations of alpha-ketolic steroids as estimations solely of compounds THF, THE, and F and, especially, of compound E. Characterization studies by H_2SO_4 chromogen spectra established the BT reactive steroid in the zone of compound THF in the 6th month pattern of case A.O. to be compound THF. The H_2SO_4 chromogen spectra of the alpha-ketolic material with polarity of compound THE in each of the patterns was also similar to that of standard THE. Material from the cortisone zone observed in the pattern of the 6th month of pregnancy separated into 3 spots upon reapplication in the chloroform-formamide system; one

spot continued to have the same mobility as authentic cortisone and had a similar H_2SO_4 chromogen spectrum, a second compound was more polar and a third one less polar than standard cortisone. A similar observation was made with steroid obtained from the cortisone region of the pattern of the 9th month of pregnancy. It must be kept in mind that 9-α-fluorohydrocortisone was administered to both of the adrenalectomized pregnant patients and it is reasonable to assume that this steroid and its metabolite(s) were components of one or more of the BT reducing zones eluted from the paper chromatograms. The free compound is however much faster moving than cortisone in the toluene-propylene system; its runs off the chromatogram in 3 days while cortisone remains at the upper half of the paper. According to Dorfman (277) the fluorinated derivative of hydrocortisone is metabolized to 17α, 21-dihydroxy-9-α-fluoropregnane-3, 11, 20-trione and some is recovered unchanged. Although the free compound would not have contributed to the measurement of any of the compounds THF, THE, F or E the metabolite(s) may have. This contribution would be unimportant in the case of the patient H.M. who received 40 mgm. of hydrocortisone at the same time as 0.25 mgm. of the fluorinated compound on the day of urine collection. The patient A.O. however, received 0.75 mgm. of 9-α-fluorohydrocortisone and a final dose of cortisone only at 10:00 p.m. prior to each of the days of urine collection. The amount excreted of the former compound and its metabolite(s) would presumably comprise a larger proportion of the total quantity of alpha-ketolic steroids appearing in the urine of case A.O.

With the above reservations in mind, the ratio of the amount of compounds THF plus THE excreted to that of compounds F plus E was 2.02 in the case of the patient H.M. This ratio was lower for all the samples of urine of the other, similar, patient A.O., being 0.58, 0.61, 0.70 and 0.70 for the specimens obtained in the 6th, 8th and 9th months of pregnancy and 4 days after delivery, respectively. The variance between the 2 patients is most probably due to the different therapy administered. In every case, however, this ratio was well below the range from 8.10 to 12.30 observed in normal subjects.

Characterization studies were carried out on the alpha-ketolic steroids which were faster moving than cortisone on the toluene-propylene glycol chromatograms of case H.M., Table XIV. These were in the zones designated as X_b, X_c, X_d, and X_e; all were reapplied in the chloroform-formamide system. In this system X_b separated into 2 spots and their amount was 0.20 and 0.22 mgm/gm. creatinine excreted. The former steroid had maxima at 295, 360 and 500 mu in conc. H_2SO_4. The other spot when further rechromatographed in the benzene-formamide system separated in turn into 3 distinct spots. One steroid had maxima at 310, 410 mu in conc. H_2SO_4; its chromatographic mobility and H_2SO_4 chromogen spectrum suggest that this steroid was compound THB. The other two steroids showed maxima of UV light absorption at 335 and 450 mu and at 305, 400 and 440 mu in conc. H_2SO_4. Compound X_c also separated into 2 distinct spots following

TABLE XIV

CHARACTERIZATION STUDIES ON LESS POLAR STEROIDS EXCRETED BY A PREGNANT ADRENALECTOMIZED PATIENT*

Zone eluted from T-PG chrom.	Compound(s) isolated after reapplic'n, $CHCl_3$-Form	Amount excreted mgm./gm. creatinine BT	H_2SO_4 chromogen spectra maxima (mu)
Xb	XbI " II	0.20 0.22	295, 360, 500 Separates into 3 spots in Benzene-Form. (i) 310, 410 (THB?) (ii) 335, 450 (iii)305, 400, 440
Xc	XcI II	0.09 0.02	
Xd	XdI II	0.16 0.23	300, 365-370, 420 & 470
Xe	Xe	0.22	Separates into 2 spots in Benzene-Form. (i) 295, 360, 420 (ii) 320, 345, 420

* Case H.M., in 9th mo. of pregnancy, totally adrenalectomized and receiving 0.25 mgm. 9-α-fluorohydrocortisone plus 40 mgm. hydrocortisone per 24 hours.

rechromatography in the chloroform-formamide system. These were excreted at the rate of 0.09 and 0.02 mgm/gm. creatinine, by BT estimation. Compound X_d simularly separated into 2 components, X_{dI} and X_{dII}, which were excreted at the rate of 0.16 and 0.23 mgm./gm. creatinine; X_{dI} showed maxima of UV light absorption at 300, 365, 370, 420 and 470 mu in conc. H_2SO_4. Compound X_e remained a single entity in the chloroform-formamide system and was present in the urine in amount of 0.22 mgm./gm. creatinine. Upon rechromatography in the benzene-formamide system, however, it separated into 2 spots; one showed maxima at 295, 360 and 420 mu in conc. H_2SO_4, the other spot, at 320, 345 and 420 mu. These studies showed that each of the zones designated as X_b, X_c, X_d and X_e in the corticosteroid excretion pattern of case H.M. were certainly not homogeneous.

The aldosterone content in all the samples of urine obtained from case A.O. was less than 1.5 ug./gm of creatinine excreted, Table XII. This indicated that there was little or no aldosterone produced in the 6th, 8th and 9th months of pregnancy and 4 days postpartum. In the single specimen obtained from case H.M., however, the aldosterone determination was 13.0 ug./gm. of creatinine, slightly below the range observed in normal pregnant women.

In the 9th month of pregnancy case H.M. excreted 35.40 and 3.54 mgm./gm of creatinine of pregnanediol and pregnanetriol, respectively. The other totally adrenalectomized pregnant patient, case A.O., excreted 21.31, 66.75 and 66.20 mgm./gm creatinine of pregnanediol in the 6th, 8th and

9th months of her pregnancy and much less, 2.78 mgm., 4 days after delivery. The pregnanetriol excretion was 1.00, 9.45 and 1.93 mgm./gm. creatinine in the 6th, 8th and 9th months of gestation and decreased to 0.00 mgm. 4 days postpartum, (Table XIII).

5. Summary.

(a) Two totally adrenalectomized patients receiving corticosteroid replacement therapy and one normal woman were studied in the last trimester of gestation. Each case showed total urinary 17-hydroxycorticosteroid levels within the range for normal non-pregnant women.

(b) The pattern of urinary corticosteroids with respect to both the Δ 4-3-ketonic and alpha-ketolic steroid components was in the normal pregnant subject qualitatively similar to those obtained for non-pregnant normals. The adrenalectomized women also showed corticosteroid excretion patterns which with respect to the C_{21} O_5 alpha-ketolic steroids were qualitatively similar to those observed for normal subjects. It would be reasonable to assume that the steroids present in the urine of these patients were derived from the hormone therapy administered. One case, however, also excreted relatively large amounts of less polar alpha-ketolic steroids in the 9th month of pregnancy. The latter compounds may have been derived from regenerated adrenal tissue since they did not appear in comparable quantities in the urine of other similarly treated adrenalectomized patients.

(c) Three normal subjects excreted quantities of cortisone plus hydrocortisone which ranged from 1/8th to 1/12th of those of compounds THF plus THE while a normal pregnant woman

excreted relatively more of these free steroids, the amount being 1/4th of those of the reduced metabolites. Even greater proportions of the free 17-hydroxycorticosteroids relative to those of their ring A reduced derivatives appeared in the urine of the adrenalectomized patients; this further change from the normal non-pregnancy pattern, however, may have not been due entirely to the condition of pregnancy but in part to the character of steroid therapy administered. The studies tended to suggest that a reduced rate of reduction of the Δ 4-3-ketone grouping in ring A of 17-hydroxycorticosteroids occurs during pregnancy. One of the adrenalectomized women continued to show a similar pattern of corticosteroid metabolism 4 days after delivery.

(d) There appeared to be no extra-adrenal source of aldosterone in one adrenalectomized pregnant woman studied in the 6th, 8th, and 9th months of gestation. Four days post-partum she continued to excrete only less than 1.5 ug. of Na-retaining hormone per gm. of creatinine. A similarly negligible quantity was excreted by a non-pregnant adrenalectomized patient. But a determination on another adrenalectomized woman in her 9th month of pregnancy showed the presence of 13 ug. of Na-retaining hormone per gm. of creatinine, in the urine. This value although well below the amount observed in normal pregnancy urine in the last trimester is still appreciable being more than the quantity excreted by non-pregnant normal individuals. This excretion of aldosterone as well as the urinary corticosteroid pattern in this particular patient suggest the presence of regenerated adrenal tissue.

(e) Pregnanediol determinations in the 2 adrenalectomized pregnant patients were within the range observed for normal pregnancy. The sharp decrease in pregnanediol excretion observed in one of these cases 4 days post-partum is also seen after termination of normal gestation.

(f) There was considerable variation in the excretion of pregnane-3α, 17α, 20α- triol in the latter patients during pregnancy. But the failure to detect this metabolite in the urine 4 days after delivery in one case suggests that it was, as well as the pregnanediol, derived from the progesterone which was produced during gestation.

PART II: EXPERIMENTAL WORK ON PREGNANCY IN THE RAT

A. The Adrenal Secretion of Corticosteroids in Pregnant and Non-Pregnant Female Rats

The rate of secretion of corticosteroids by adrenal glands of pregnant and non-pregnant female rats was investigated in order to determine whether a difference during pregnancy could be demonstrated.

In vitro incubations of excised adrenal glands in Krebs-Ringer solution were first carried out and then an 'invivo' study was made involving collection of adrenal venous blood over a one hour period from pregnant and non-pregnant female rats under anaesthesia. The major drawback to both these experiments is their limited number. This was due to the difficulty of obtaining pregnant and non-pregnant rats of similar age.

1. Incubations of adrenals glands.

a. Total body and adrenal weights of rats.

Adult female rats of similar age (approximately 4 months) and weight were divided into 2 groups and one group was placed in the breeding cages. Incubations were carried out when a sufficient number of rats became pregnant and were in the last week of gestation, from 15th to 21st day. Six groups of pregnant and non-pregnant rats were studied, each group containing from 11 to 30 animals. The range of weight and the average weight for each group are shown in Table XV. The average weight for the non-pregnant groups varied from 174 to 249 gm. and that for the pregnant rats from 236 to 328 gm. The difference in weight between the two groups was accounted for predominantly by the products of conception.

The adrenal glands of these rats were excised and

TABLE XV

BODY AND ADRENAL WEIGHTS IN PREGNANT AND NON-PREGNANT FEMALE RATS

Exp.	Rats		Total body weight (gm)		Adrenal weight (mgm.)	
	type	No.	range	aver.	aver. per adr.	aver./100 gm. body wt.
1	Non-preg	20	193-309	246	31.7	25.8
2	"	30	192-288	243	30.8	25.3
3	"	25	214-281	242	22.5	18.6
4	"	11	194-314	249	24.7	19.8
5	"	20	165-190	174	22.6	26.0
6	"	22	157-280	185	25.0	27.0
Mean ± S.E.					26.2 ± 1.64	24.0 ± 1.40
1	Preg.	20	264-404	328	29.8	18.2
2	"	26	214-357	276	30.9	22.4
3	"	25	214-365	294	22.7	15.4
4	"	11	228-337	285	25.0	17.5
5	"	19	186-300	236	23.9	20.2
6	"	23	190-350	254	22.0	17.4
Mean ± S.E.					25.7 ± 1.54	18.0 ± 1.00

weighed. The average weight of the adrenal glands in each group of the non-pregnant varied from 22.6 to 31.7 mgm. per adrenal; the mean value of all the groups was 26.2 mgm. The values found in the groups of pregnant rats ranged from 22.0 to 30.9 and the mean weight was 25.7 mgm. These findings indicate that there is no significant difference between the weight of the adrenal glands in the pregnant and non-pregnant rats.

When these values are expressed in terms of body weight, the non-pregnant rats showed adrenal weights of 24.0 mgm./100 gm. body weight and the pregnant rats 18.0 mgm./100 gm. This difference was statistically significant and indicates that the rapid gain in weight in pregnancy, particularly during the last week of gestation is not accompanied by a corresponding gain in adrenal weight.

Male rats of the same colony have average adrenal weights of 12 mgm; a value approximately one-half of that observed in female rats.

b. In Vitro Secretion of Corticosterone and Aldosterone by Adrenal Glands from Pregnant and Non-Pregnant Female Rats

(i) Corticosterone

Although it was not possible to demonstrate any significant difference between the weights of the adrenals from pregnant and non-pregnant rats, the secretion of corticosteroids under 'in vitro' conditions was investigated.

Six groups of pregnant and non-pregnant rats were adrenalectomized. The number of rats used in each experiment is shown in Table XV. In 4 experiments ether anaesthesia was used and in two, nembutal. It has been reported (284) that induction of anaesthesia by ether stimulates the release of

TABLE XVI

IN VITRO SECRETION OF CORTICOSTERONE BY INCUBATED ADRENAL GLANDS OF PREGNANT (P) AND NON-PREGNANT (N) FEMALE RATS

Incubation period (hrs)		ug. corticosterone secreted/gm. tissue/hr.					
		0-1		1-3		3-5	
Expt.	Anaesth.	N	P	N	P	N	P
1	Ether	34.80	47.90	-	-	-	-
2	"	41.80	32.80	16.03	11.98	7.60	5.00
3	"	38.93	34.20	8.05	9.10	4.78	3.10
4	"	28.84	43.02	17.62	15.54	3.53	2.16
Mean ±	S.E.	36.09±2.80	39.48±3.60	13.90±3.00	12.21±1.86	5.30±1.20	3.42±0.84
5	Nembut-al.	18.64	14.01	10.80	6.69	7.56	3.47
6	"	14.92	6.36	15.79	5.55	6.14	3.59
Mean ±	S.E.	16.78±1.86	10.19±3.82	13.29±2.50	6.12±0.76	6.85±1.00	3.53±0.70

of adrenocorticotropin. The present studies appeared to show this effect since the secretion of corticosterone was considerably higher in the first period of incubation, from 0 to 1 hour, than it was in the two succeeding periods, from 1 to 3 and from 3 to 5 hours. The results are shown in Table XVI. In 4 groups of rats the adrenals of the non-pregnant animals secreted an average of 36.09 ± 2.80 ug. corticosterone /gm. tissue/hr. while those of the pregnant animals secreted 39.48 ± 3.60 ug. In the 2nd and 3rd periods of incubation there was a rapid decrease in the secretion rate of corticosterone in both groups; for non-pregnant rats it decreased to an average of 13.90 ± 3.00 and 5.30 ± 1.20 ug./gm./hr. while for pregnant rats it was an average of 12.21 ± 1.86 and 3.42 ± 0.84 ug./ gm./hr.

When nembutal was used as the anaesthetic in 2 groups each of non-pregnant and pregnant rats for the removal of the adrenals, the average secretion rate for corticosterone in the initial period was 16.78 ± 1.86 and 10.19 ± 3.82 ug./gm./ hr., respectively. Adrenal glands of pregnant rats therefore secreted 39.3% less corticosterone than those of non-pregnant animals. In both groups, however, the secretion of corticosterone in the first period of incubation was considerably lower than that observed for ether. In the 2 succeeding periods, the secretion rates of corticosterone were more similar to those observed under ether anaesthesia. In periods 2 and 3 the adrenals of the non-pregnant rats secreted 13.29 ± 2.50 and 6.85 ± 1.00 ug./gm./hr. while those of the pregnant rats secreted.

6.12±0.76 and 3.53±0.70 ug. Adrenal glands of pregnant rats, therefore, secreted 54.0 and 48.5% less corticosterone/gm./hr. than those of non-pregnant animals in the 2nd and 3rd periods of incubation.

When one compares the total average output of corticosterone during the 5 hour period of incubation (Table XIX) it was found that the adrenals of the non-pregnant and pregnant rats secreted 74.49 and 70.74 ug./gm., respectively, when ether was used as anaesthetic. With nembutal the value for adrenals of non-pregnant rats was 57.06 ug./gm. and that for adrenals of the pregnant group was 29.69 ug./gm. Thus when ether was used for removal of the adrenals there was no significant difference, 5.03%, between the average total output of corticosterone of the two groups. When nembutal was used, however, the adrenals of the pregnant animals appeared to secrete considerably less corticosterone, by 48.32%, than those from the non-pregnant rats over a 5 hour period. It was realized that a larger series of animals should be studied with nembutal before any conclusions could be drawn regarding different rates of secretion of corticosterone in adrenals from non-pregnant and pregnant rats; there was, however, a tendency for the secretion rates of corticosterone to decrease more rapidly with time in the adrenals of the pregnant rats,

(ii) Aldosterone.

The adrenal secretion rates of aldosterone were measured in the same groups of rats. The results are shown

TABLE XVII

IN VITRO SECRETION OF ALDOSTERONE BY INCUBATED ADRENAL GLANDS OF PREGNANT (P) AND NON-PREGNANT (N) FEMALE RATS

Incubation period (hrs.)		ug. aldosterone secreted/gm.tissue/hr.					
		0-1		1-3		3-5	
Expt.	Anaesth.	N	P	N	P	N	P
1	Ether	1.92	3.48				
2	"	2.44	6.07	2.85	3.24	2.85	3.31
3	"	3.40	2.99	1.86	1.86	1.86	1.86
4	"	2.72	2.95	1.73	1.69	0.72	0.69
Mean ± S.E.		2.62±0.31	3.87±0.74	2.15±0.35	2.26±0.49	1.81±0.61	1.95±0.75
5	Nembutal	4.99	5.79	4.05	6.00	4.05	5.25
6	"	4.12	5.92	6.50	9.30	5.85	7.20
Mean ± S.E.		4.56±0.44	5.86±0.10	5.28±1.22	7.65±1.65	4.95±0.90	6.23±0.97

in Table XVII.

When ether anaesthesia was used, the average secretion rates of aldosterone in the adrenals of the non-pregnant rats were 2.62±0.31, 2.15±0.35 and 1.81±0.61 ug./gm. tissue /hr. in the 3 succeeding incubation periods. Those for the pregnant rats were 3.87±0.74, 2.26±0.49 and 1.95±0.75 ug./gm. /hr. The average rate of secretion of aldosterone by adrenal glands of pregnant rats was 47.6% greater than that of adrenal tissue from non-pregnant rats in the 1st period of incubation but,thereafter, the differences between both groups were negligible. The average total secretion of aldosterone for the 5 hour period was 10.54 ug./gm. for the adrenals of the non-pregnant rats and 12.33 ug./gm for the pregnant rats, i.e. 16.63% more, (Table XIX).

When nembutal was used in the 2 groups of each of non-pregnant and pregnant rats for the adrenalectomy, the aldosterone secretion of the adrenals in both groups was higher and appeared to increase slightly with time in contrast to the previous experiments using ether. This was most pronounced in experiment 6. The rates for each incubation period are shown in Table XVII. In these studies with nembutal the average secretion rate of aldosterone in the 1st. period was 4.56±0.44 and 5.86±0.10 ug./gm. tissue/hr. for adrenals of non-pregnant and pregnant groups, respectively. In the 2 succeeding periods of incubation the adrenals of non-pregnant rats secreted an average of 5.28±1.22 and 4.95±0.90 ug./gm./hr. while those of the pregnant rats secreted an average of 7.65±1.65 and 6.23±0.97 ug./gm./hr. The adrenal glands of pregnant rats,therefore,

secreted average amounts of aldosterone which were increased by 28.5, 44.9 and 25.8% above those secreted by glands of non-pregnant rats in the 3 succeeding periods of incubation. The average total secretion of aldosterone for the 5 hour period was 25.02 ug./gm. for the adrenals of the non-pregnant rats and 33.62 ug./gm. for the pregnant rats, i.e. 34.37% greater, (Table XIX).

(iii) Ratio of Corticosterone to Aldosterone Secretion under in vitro conditions

A comparison of the ratio of corticosterone to aldosterone secreted by incubated adrenal glands of pregnant and non-pregnant rats is shown in Table XVIII.

In the 3 succeeding incubation periods the average ratios for the non-pregnant group were 13.03±2.05, 6.71±1.78 and 3.38±0.76 when ether was used as anaesthetic for adrenalectomy; the corresponding average ratios for pregnant rats were 10.47± 2.70, 5.93±1.70 and 2.10±0.52. With ether, therefore, the average rate of secretion of aldosterone relative to that of corticosterone by adrenal tissue of pregnant rats was only slightly greater than that observed with adrenal glands of non-pregnant rats.

When nembutal was used for anaesthesia this difference between both groups was more pronounced. The average ratios of the quantity of corticosterone to aldosterone secreted in the 1st, 2nd and 3rd periods were 3.68±0.06, 2.55±0.12 and 1.46± 0.41, respectively in the non-pregnant group; the corresponding values for the pregnant rats were 1.75±0.68, 0.86±0.26 and 0.58± 0.08.

Adrenal tissue of the two groups of rats showed a

TABLE XVIII

COMPARISON OF THE RATIO OF CORTICOSTERONE TO ALDOSTERONE SECRETED BY INCUBATED ADRENAL GLANDS OF PREGNANT (P) AND NON-PREGNANT (N) FEMALE RATS

	Incub. adr.tissue wt.(mgm.)		Secretion of corticosterone (ug./gm./hr.) secretion of aldosterone (ug./gm./hr.)					
			0-1 hr.incub.period		1-3 hr.incub.period		3-5 hr.incub.period	
Exp.	N	P	N	P	N	P	N	P
2	924	793	17.10	5.40	5.61	3.70	2.66	1.51
3	604	604	11.40	11.40	4.33	4.89	2.57	1.65
4	284	290	10.60	14.60	10.20	9.20	4.90	3.14
Ether anaesthesia	:Mean±S.E.		13.03±2.05	10.47±2.70	6.71±1.78	5.93±1.70	3.38±0.76	2.10±0.52
5	463	504	3.74	2.42	2.67	1.12	1.87	0.66
6	578	522	3.62	1.07	2.43	0.60	1.05	0.50
Nembu-tal anaesthesia	:Mean±S.E.		3.68±0.06	1.75±0.68	2.55±0.12	0.86±0.26	1.46±0.41	0.58±0.08

TABLE XIX

STEROID SECRETION DURING PROLONGED INCUBATION OF ADRENAL GLANDS OF PREGNANT AND NON-PREGNANT FEMALE RATS

Experiments	No.	Total average secretion of steroids for 5 hour period of incubation ug./gm. adrenal tissue			
		Aldosterone		Corticosterone	
		Non-pregnant	Pregnant	Non-pregnant	Pregnant
Ether anaesthesia	4	10.54	12.29	74.49	70.74
Nembutal "	2	25.02	33.62	57.06	29.49

greater secretion of aldosterone relative to that of corticosterone in each period of incubation when nembutal was used for anaesthesia rather than ether. Both pregnant and non-pregnant groups also showed a progressively greater secretion of aldosterone relative to that of corticosterone with increasing time of incubation; this effect was observed when either nembutal or ether were used for removal of the adrenals.

C. Summary

(i) There was no significant difference between the average weights of adrenal glands obtained from pregnant and non-pregnant rats. The adrenal weights of the female rats were approximately double those observed in male rats of the same colony.

(ii) In 4 experiments when adrenals were removed under ether anaesthesia the average secretion of corticosterone in the 1st period of incubation was considerably higher than in the 2 succeeding periods in both pregnant and non-pregnant rats. The total average output of corticosterone during the 5 hour period of incubation per gm. adrenal tissue of pregnant and non-pregnant animals differed only by 5.03%.

When nembutal was used as the anaesthetic in 2 experiments the secretion rate of corticosterone in the initial period was considerably lower than that observed for ether in both groups of rats. In the 2 succeeding periods the average secretion rates of corticosterone approximated those observed under ether. In each period of incubation this rate was less for adrenal tissue of pregnant rats than for that of non-pregnant ones. The average total secretion of corticosterone for the 5 hour period of incubation was 48.32% less per gram of tissue for

adrenals of pregnant rats than for those of non-pregnant animals.

(iii) The secretion rates of aldosterone were measured in the same group of rats. When ether was used as anaesthetic the average secretion rate of aldosterone by adrenal tissue of pregnant rats was 47.6% greater in the 1st period of incubation but thereafter the difference between both groups of rats was negligible. The average total secretion of aldosterone for the 5 hour period of incubation was 16.63% greater for the adrenals of the pregnant rats than for those of non-pregnant rats.

When nembutal was used as anaesthetic for adrenalectomy the aldosterone secretion rate of the adrenals in both groups was higher than that observed under ether. Adrenal glands of pregnant rats secreted amounts of aldosterone per gm. per hour which were increased by 28.5, 44.9 and 25.8% above those secreted by adrenal tissue of non-pregnant rats in the 3 succeeding periods of incubation. The average total secretion of aldosterone per gm. tissue for the 5 hour period of incubation was 34.37% greater for adrenals of pregnant rats than for those of non-pregnant ones.

(iv) The average rate of secretion of aldosterone relative to that of corticosterone for adrenal tissue of pregnant rats was only slightly greater than that observed for adrenals of non-pregnant groups when ether was used for adrenalectomy but this difference between both groups was more marked with nembutal. Adrenal tissue of both groups of rats, however, showed a greater rate of secretion of aldosterone relative to that of corticosterone when it was removed under nembutal rather than ether anaesthesia. This quantitative change in the pattern of

corticosteroid secretion became progressively greater with time of incubation of the adrenal glands obtained from both pregnant and non-pregnant rats in every experiment carried out.

2. Collections of Adrenal Venous Blood

a. Total body weights of rats and blood volumes collected

Adrenal venous blood was collected from 6 groups each of pregnant and non-pregnant rats. In each experiment blood from 5 animals was pooled, (Tables XX, XXI).

The mean total body weights of the non-pregnant rats in the 6 groups varied from 181 to 224 gm. while those of the pregnant rats ranged from 230 to 280 gms. The difference in the average weights was accounted for by the products of conception in the pregnant animals.

The average volume of blood collected per adrenal per hour per rat was similar in both groups, 2.7 ± 0.09 and 2.8 ± 0.07 ml., for non-pregnant and pregnant rats, respectively. When the volume of blood collected was related to body weight there was a difference in both groups of animals; the mean value for the non-pregnant rats was 13.7 ± 0.40 ml., that for the pregnant rats was 11.2 ± 0.20 ml./adrenal/kg./hr.

b. Aldosterone and Corticosterone in adrenal venous blood of pregnant and non-pregnant female rats.

(i) Aldosterone

Aldosterone was measured by the bioassay method of Simpson and Tait (281). The secretion rates of aldosterone are expressed in DOCA equivalents since the latter compound served as the standard in the tests. Aldosterone is approximately one hundred times as active as DOCA in this assay.

The aldosterone secretion in ug. DOCA equivalents /adrenal/hr. has been expressed in 4 ways; (1) per rat, (2) per kg. total body wt., (3) per 100 mgm. adrenal wt. and (4) per

TABLE XX

ALDOSTERONE IN ADRENAL VENOUS BLOOD OF PREGNANT AND NON-PREGNANT FEMALE RATS

Gp	No. rats in pool	Mean Wt. g	Mean vol. blood collected ml./adr./hr. /rat	/kg.BW	Aldosterone (as DOCA equivalents) ug./adrenal/hour /rat	/kg.BW	/100 mgm. adr.	/10 ml blood coll'd
1	5	200	2.7	13.6	23	115	89	85
2	4	224	3.0	13.5	23	103	85	76
3	5	196	2.9	14.7	14	72	55	50
4	"	194	2.5	12.7	26	134	116	106
5	"	189	2.4	12.7	18	95	73	67
6	"	181	2.7	15.1	20	111	-	73
Mean ± S.E.		Non-pregnant rats			21±1.8	105±8.5	84±10.1	76±9.0
1	5	280	2.9	10.4	20	71	45	69
2	"	242	2.6	10.9	12	50	36	45
3	"	235	2.6	11.2	14	60	39	53
4	"	230	2.7	11.9	18	78	61	66
Mean ± S.E.		Pregnant rats			16±1.8	65±6.3	45±5.6	58±5.62

10 ml. blood collected. These values are listed in Table XX.

In each case the rate of secretion of aldosterone by non-pregnant rats was greater than that observed for pregnant rats. The mean values for aldosterone secretion (in DOCA equiv.)/adrenal/hour/rat were 21±1.8 and 16±1.8 ug. for non-pregnant and pregnant rats respectively; in terms of kg. body weight they were 105±8.5 ug. in non-pregnant rats and 65±6.3 ug. in pregnant ones. Aldosterone secretion (in DOCA equiv.) /adrenal/hour/100 mgm. adrenal wt. averaged 84±10.1 in non-pregnant rats and was 45±5.6 ug. in pregnant groups. The average aldosterone secretion per adrenal/hr./10 ml. blood collected was 76±9.04 and 58±5.62 ug. in non-pregnant and pregnant rats, respectively. This difference in aldosterone secretion between the 2 groups was, however, statistically significant only when related to unit body weight ($P<0.01$ and >0.001) and to unit adrenal weight ($P<0.02$ and >0.01).

(ii) Corticosterone

The 'in vivo' secretion of corticosterone was studied in the same groups of animals. The rate of secretion of this steroid by pregnant rats was less than that observed for the non-pregnant rats, Table XXI. The average secretion of corticosterone was 30±2.5 and 25±2.2 ug./adrenal/hr./rat in non-pregnant and pregnant groups, respectively. When related to body weight it was 156±12.7 ug./adrenal/hr./kg. body weight in non-pregnant animals as compared with 103±9.1 ug. in the pregnant ones. The average rate of corticosterone secretion per unit adrenal weight was 119±8.6 and 93±6.0 ug./ adrenal/hr./100 mgm. adrenal weight in non-pregnant and pregnant groups, respectively. Non-pregnant

TABLE XXI

CORTICOSTERONE IN ADRENAL VENOUS BLOOD OF PREGNANT AND NON-PREGNANT FEMALE RATS

Gp	No. rats in pool	Mean Wt.g	Mean vol. blood collected ml./adr./hr /rat	/kg.BW	Corticosterone secretion ug./adrenal/hour /rat	/kg.BW	/100 mgm. adr.	/10 ml. blood coll'd
3	5	196	2.9	14.7	34	171	128	116
4	"	194	2.5	12.7	24	121	102	96
5	"	189	2.4	12.7	34	178	128	140
6	"	181	2.7	15.1	28	155	-	102
Mean ± S.E.		Non-pregnant rats			30±2.5	156±12.7	119±8.6	113±9.8
2	5	242	2.6	10.9	22	91	81	83
3	"	235	2.6	11.2	21	87	68	78
4	"	230	2.7	11.9	30	130	118	109
5	"	240	2.7	11.1	21	87	96	79
6	4	261	3.0	11.5	31	120	104	104
Mean ± S.E.		Pregnant rats			25±2.2	103±9.1	93±6.0	91±6.6

rats secreted an average of 113±9.8 ug. corticosterone/adrenal/hr./10 ml. blood as compared with 91±6.6 ug. secreted by pregnant rats. These differences in the average rate of secretion of corticosterone between pregnant and non-pregnant groups were, however, statistically significant only when related to unit body weight ($P<0.02$ and >0.01) and to unit adrenal weight ($P<0.05$ and >0.02).

c. Summary

The concentration of aldosterone and corticosterone was less in the adrenal venous blood of pregnant rats than in that of non-pregnant ones. The differences in both aldosterone and corticosterone content between the 2 groups were statistically significant when the steroid secretion rates were related to unit total body and adrenal weights. The average rate of secretion of these 2 steroids when expressed merely per rat or per unit blood volume collected was also less, from 17 to24%, in pregnant rats than in the non-pregnant ones but these decreases were not of statistical significance.

d. Comparison of corticosteroid secretion under 'in vitro' and 'in vivo' conditions in pregnant and non-pregnant female rats

It is realized that both types of experiments carried out were too limited in number to establish conclusively whether or not adrenal function is altered in pregnancy in the rat. Nevertheless, a decrease in the secretion rate of corticosterone in pregnant rats was observed in the 'in vivo' study and also under 'in vitro' conditions when the adrenal tissue had been removed under nembutal anaesthesia. This latter difference between pregnant and non-pregnant rats,was, however, slight when ether anaesthesia was used for adrenalectomy. The aldosterone content

in adrenal venous blood of the pregnant animals was also decreased in comparison with that present in blood collected from non-pregnant rats. The adrenal glands of pregnant rats under 'in vitro' conditions showed a tendency, however, to secrete higher average amounts of aldosterone than those from non-pregnant rats when the adrenals were removed under nembutal anaesthesia. This effect was less pronounced with ether anaesthesia. Under 'in vitro' conditions there appeared, therefore, a tendency for the average rate of secretion of aldosterone relative to that of corticosterone to be greater in the pregnant group of rats than in the non-pregnant ones. The latter effect was not, however, observed in the 'in vivo' study, perhaps, because of the condition of acute stress under which adrenal venous blood is collected. This stress would result in increased secretion of corticosterone to a greater extent than that of aldosterone.

B. The Metabolism of Corticosteroids During Incubation with Liver Tissue from Pregnant and Non-Pregnant Female Rats

The purpose of these experiments was to determine whether a difference in the rate of corticosteroid metabolism could be demonstrated in pregnancy at the level of liver activity. Liver tissue obtained from pregnant and non-pregnant female rats was incubated under 'in vitro' conditions with corticosterone and aldosterone-21-monoacetate and the metabolism of these hormones was studied.

1. Total body and liver weights of rats.

Pregnant rats between the 18th to 21st day of gestation were used. Their weights ranged from 230 to 310 gm, the average being 270 gm. The weights of the non-pregnant rats ranged from 185 to 250, aver.216 gm; the difference in the mean weight of both groups of rats was 54 gm. and was accounted for by the products of conception in pregnant rats. All weights are listed in Table XXll.

It was noted that the livers of pregnant rats were generally larger than those of non-pregnant ones; the mean weights were 11.2±0.44 and 8.6±0.64 gm., respectively, and this difference was found to be statistically significant, ($P<0.01$ and >0.001). The weight of the particular lobe used for incubations was also noted to be significantly heavier in pregnant rats than in those non-pregnant, ($P<0.001$).

When total liver weights were related to total body weights average values of 4.1 and 4.3 gm./100 gm. body weight in non-pregnant and pregnant rats were observed. The corresponding means of the weights of the lobe incubated/100 gm.

TABLE XXII

LIVER AND TOTAL BODY WEIGHTS (TBW) OF PREGNANT AND NON-PREGNANT FEMALE RATS

Rats	Non-pregnant rats					Pregnant rats				
	TBW gm.	Liver wt., gm. total	Liver wt., gm. /100g TBW	Lobe incubated, gm. total	Lobe incubated, gm. /100g TBW	TBW gm.	Liver wt., gm. total	Liver wt., gm. /100g TBW	Lobe incubated, gm. total	Lobe incubated, gm. /100g TBW
1	220	-	-	-	-	300	-	-	-	-
2	250	-	-	3.2	1.3	310	-	-	4.7	1.5
3	235	-	-	2.7	1.2	295	-	-	4.8	1.6
4	230	-	-	-	-	280	-	-	-	-
5	230	-	-	-	-	280	-	-	-	-
6	195	-	-	2.3	1.2	230	-	-	4.1	1.8
7	190	7.2	3.8	2.5	1.3	255	12.0	4.7	4.5	1.8
8	224	8.7	3.9	2.7	1.2	280	10.2	3.7	3.7	1.3
9	203	7.1	3.4	2.2	1.1	250	9.5	3.8	3.2	1.3
10	210	10.2	4.9	3.7	1.8	260	10.6	4.1	5.3	2.0
11	185	6.7	3.6	2.0	1.1	230	12.1	5.3	4.3	1.9
12	220	11.0	5.0	4.5	2.0	280	11.0	3.9	5.5	2.0
13	222	9.6	4.3	3.3	1.5	270	12.7	4.7	4.8	1.8
Mean ±S.E.	216	8.6± 0.64	4.1	2.9± 0.24	1.4	270	11.2± 0.44	4.3	4.5± 0.22	1.7

body weight were 1.4 and 1.7 gm., respectively. In contrast, therefore, to the findings observed with adrenal glands, liver weight increased in pregnancy in relation to the gain in body weight.

2. Method of expressing results.

Liver tissue of pregnant and non-pregnant rats was incubated in Krebs phosphosaline buffer solution with and without added corticosteroids. Corticosterone and aldosterone-21-monoacetate were the steroid substrates used. The compounds measured were the total free corticosteroids having a $\Delta 4$-3-ketone grouping in ring A and an alpha-ketolic group at carbon 17 present in the incubation media at the end of the experiment. These values for recovered steroids were subtracted from the amount of steroid added to the samples and the difference represents, therefore, the total disappearance of steroid. To correct for the variation in amount of steroid used in the different experiments, the results were expressed as the per cent total disappearance. In addition these values are also related to the weight of liver tissue which varied from 200 to 250 mgm. per sample.

As it is necessary to make a correction for loss of steroid due merely to technique, the recovery of corticosteroids incubated without liver tissue in this system was determined. When expressed as % disappearance of the $\Delta 4$-3-ketone grouping the values ranged from 9 to 13% using the INH reaction. When estimated by BT reaction values obtained for % disappearance of the alpha-ketol group ranged from 4 to 6%. The appropriate values were, therefore, subtracted from the % total disappearance

observed with samples of steroid incubated with tissue. The difference was corrected for variations in liver weight in the different samples incubated and this value is the appropriate one for comparison of activity of liver tissue obtained from pregnant and non-pregnant rats.

3. Incubations of liver tissue from pregnant and non-pregnant female rats with corticosterone.

(a) Disappearance of Δ 4-3-ketone grouping

Nine experiments were carried out with liver tissue from non-pregnant rats. In each experiment 3 to 5 samples of the same lobe of liver (a major, single-lobed portion) were used making a total of 36 determinations on 9 rats. The amount of added corticosterone varied from 220 to 338 ug. per sample. The results are charted in Table XXIII. Following incubation the average loss of Δ 4-3-ketone ranged from 105 to 192 ug./sample in the 9 experiments. When expressed as a percentage of the original amount this varied from 47 to 71%, mean 57±1.51%. When this value was corrected for the loss of the Δ 4-3-ketone grouping which occurs in the absence of liver tissue the mean value was 44±1.45%. These results were also expressed as a percentage disappearance of Δ 4-3-ketone grouping/100 mgm. liver tissue; the averages in 9 experiments ranged from 16 to 26% and the mean value of 36 samples was 19±0.66%/100 mgm.

Similar experiments with liver tissue of pregnant rats were carried out. In each experiment usually 4 aliquots of the same lobe of liver as that obtained from non-pregnant rats were used making a total of 39 determinations on 10 rats. The results are shown in Table XXIV. Following incubation the average loss

TABLE XXIII

REDUCTION OF Δ 4-3-KETONE GROUPING OF CORTICOSTERONE DURING INCUBATION WITH LIVER OF NON-PREGNANT FEMALE RATS

Disappearance (Dis.) of Δ 4-3-ketone grouping of added corticosterone.

Exp.	Amount Compd B Added	No. Samples	Total amount Dis. ug. range (mean)	Total % Dis. of amount added range (mean)	Total % Dis. /100 mgm. tissue range (mean)	% Dis. corr'd for loss of steroid incub. alone (11%) range (mean)	% Dis. (corr'd) /100 mgm. tissue range (mean)
1	225	4	95-120 (110)	42-54 (49)	17-24 (20)	31-43 (38)	12-19 (16)
2	"	"	135-155 (147)	60-69 (66)	22-31 (27)	34-45 (41)	13-19 (16)
3	"	"	100-125 (113)	45-56 (50)	20-23 (22)	34-45 (40)	15-18 (17)
4	338	"	168-213 (192)	50-64 (57)	23-28 (24)	37-51 (45)	17-22 (19)
5	225	5	120-165 (143)	54-73 (64)	23-30 (27)	43-62 (52)	18-26 (22)
7	"	4	95-115 (105)	42-51 (47)	20-25 (22)	31-40 (36)	15-20 (17)
8	223	3	113-128 (121)	51-57 (54)	25-26 (26)	40-46 (43)	20-20 (20)
9	220	4	145-165 (156)	66-75 (71)	28-34 (31)	55-64 (60)	23-29 (26)
10	258	"	123-148 (132)	48-57 (51)	21-25 (23)	37-46 (40)	16-20 (18)
Mean ± S.E., 36 determinations				57 ± 1.51	25 ± 0.65	44 ± 1.45	19 ± 0.66

TABLE XXIV

REDUCTION OF Δ 4-3-KETONE GROUPING OF CORTICOSTERONE DURING INCUBATION WITH LIVER OF PREGNANT RATS

Disappearance (Dis.) of Δ4-3-ketone grouping of added corticosterone

Exp.	Amount Cmpd B Added	No. Samples	Total amount Dis. ug. range (mean)	Total % Dis. of amount added range (mean)	Total % Dis. /100 mgm. tissue range (mean)	% Dis. corr'd for loss of steroid incub. alone (11%) range (mean)	% Dis. (corr'd) /100 mgm. tissue range (mean)
1	225	4	145-175 (155)	65-77 (69)	27-29 (28)	54-67 (58)	22-25 (24)
2	"	"	148-175 (160)	66-78 (71)	27-32 (27)	43-54 (48)	15-22 (19)
3	"	"	130-160 (143)	58-71 (64)	23-30 (27)	47-60 (52)	18-25 (21)
4	338	"	203-223 (212)	60-66 (63)	25-30 (27)	44-54 (47)	18-25 (20)
5	225	"	145-150 (149)	65-67 (66)	26-31 (28)	65-67 (55)	21-26 (24)
6	"	"	115-140 (124)	51-63 (55)	21-29 (24)	40-52 (44)	17-24 (20)
7	"	3	95-120 (110)	42-53 (49)	18-22 (21)	31-42 (38)	13-17 (16)
8	223	4	123-160 (140)	55-72 (63)	27-36 (30)	44-61 (52)	21-30 (24)
9	220	"	110-135 (120)	50-61 (55)	22-29 (25)	39-50 (44)	17-24 (20)
10	258	"	108-148 (127)	42-57 (51)	19-27 (22)	31-46 (38)	14-22 (18)
Mean ± S.E., 39 determinations				61 ± 1.42	26 ± 0.57	48 ± 1.25	21 ± 0.59

of the Δ 4-3-ketone ranged from 110 to 212 ug./sample in the 10 experiments. When expressed as a percentage of the amount added this varied from 49 to 71%, mean 61±1.42%. The latter value upon correction for the loss of the Δ 4-3-ketone grouping which occurs in the absence of liver tissue was 48±1.25%. The results when expressed as a percentage disappearance of Δ 4-3-ketone grouping/100 mgm. liver tissue gave a mean value of 21±0.59% for 39 samples.

Liver tissue of pregnant rats was, therefore, slightly more active than that of non-pregnant rats in the reduction of the Δ 4-3-ketone grouping in ring A of corticosterone during 3 hour incubations. This increased activity per 100 mgm. liver tissue amounted only to a 10.5% difference but was statistically significant, ($P < 0.05$ and > 0.02 at 72 degrees of freedom).

(b) Disappearance of alpha-ketol group.

The extracts of the above described experiments were also examined for alpha-ketolic steroid content using the blue tetrazolium reaction. The results are shown in Table XXV. In 9 experiments following incubation with liver of non-pregnant rats the average loss of alpha-ketol ranged from 38 to 84 ug./sample. When expressed as a percentage of the original amount this varied from 17 to 38%, mean 28±1.42%. The latter value upon correction for the loss of the alpha-ketol group which occurs in the absence of liver tissue was 23±1.36%. The results were furthermore expressed as a percentage disappearance of alpha-ketol group/100 mgm. liver tissue; the averages in 9 experiments ranged from 6 to 12% and the mean value of 34 determinations was 10±0.48%/100 mgm.

TABLE XXV

REDUCTION OF ALPHA KETOL GROUP OF CORTICOSTERONE DURING INCUBATION WITH LIVER OF NON-PREGNANT FEMALE RATS

Disappearance (Dis.) of Alpha-ketol group of added Corticosterone.

Exp.	Amount Cmpd B Added	No. Sample	Total amount Dis. ug. range (mean)	Total % Dis. of amount added range (mean)	Total % Dis. /100 mgm. tissue range (mean)	% Dis. corr'd for loss of steroid incub. alone (4%) range (mean)	% Dis. (corr'd) /100 mgm. tissue range (mean)
1	225	4	33-50 (44)	15-22 (20)	6-10 (8)	10-18 (15)	4-8 (7)
2	"	3	60-105(84)	27-47 (38)	11-17 (14)	23-43 (34)	9-16 (12)
3	"	4	60-70 (63)	27-31 (28)	12-13 (12)	23-27 (24)	10-11 (10)
4	338	"	66-98 (81)	19-29 (24)	9-13 (10)	15-25 (20)	7-11 (9)
5	225	5	50-95 (74)	22-42 (33)	9-17 (14)	17-36 (27)	7-15 (12)
7	"	4	53-60 (57)	23-27 (25)	11-13 (12)	19-23 (22)	9-11 (10)
8	223	3	33-43 (38)	15-19 (17)	7-9 (8)	11-15 (13)	5-7 (6)
9	220	4	38-60 (54)	17-29 (25)	7-14 (11)	13-25 (21)	6-12 (9)
10	258	"	75-83 (80)	29-32 (31)	13-15 (14)	25-28 (27)	11-13 (12)
Mean ± S.E., 34 determinations				28 ± 1.42	12 ± 0.53	23 ± 1.36	10 ± 0.48

TABLE XXVI

REDUCTION OF ALPHA-KETOL GROUP OF CORTICOSTERONE DURING INCUBATION WITH LIVER OF PREGNANT RATS

Disappearance (Dis.) of Alpha-ketol group of added Corticosterone.

Exp.	Amount Cmpd B Added	No. Samples	Total amount Dis. ug. range (mean)	Total % Dis. of amount added range (mean)	Total % Dis. /100 mgm. tissue range (mean)	% Dis. corr'd for loss of steroid incub. alone (4%) range (mean)	% Dis. (corr'd) /100 mgm. tissue range (mean)
1	225	4	70-88 (83)	31-39 (37)	14-17 (15)	27-35 (33)	12-15 (13)
2	"	"	80-100(88)	34-44 (39)	12-17 (15)	32-44 (38)	12-17 (15)
3	"	"	60-88 (75)	27-39 (33)	10-16 (14)	23-35 (30)	9-15 (13)
4	338	"	71-93 (81)	21-27 (24)	10-11 (10)	17-23 (20)	8-9 (9)
5	225	"	70-95 (85)	31-42 (38)	14-18 (16)	27-38 (34)	12-16 (15)
6	"	"	58-65 (63)	26-29 (28)	11-13 (12)	22-25 (25)	9-11 (11)
7	"	"	50-78 (61)	22-35 (27)	11-15 (12)	18-31 (24)	9-14 (10)
8	223	3	33-43 (36)	15-19 (16)	7-10 (8)	11-15 (12)	5-8 (6)
9	220	4	45-55 (49)	20-25 (22)	9-12 (10)	16-21 (18)	7-10 (8)
10	258	3	65-93 (80)	25-36 (31)	11-17 (14)	21-32 (27)	10-15 (12)
Mean ± S.E., 38 determinations				30 ± 1.30	13 ± 0.49	26 ± 1.60	11 ± 0.50

In 10 experiments following incubation of liver obtained from pregnant rats the average loss of alpha-ketol ranged from 36 to 88 ug./sample, (Table XXVI). When expressed as a percentage of the amount added to incubations this varied from 16 to 39%, mean 30±1.30%. When this value was corrected for the loss of the alpha-ketol group which occurs in the absence of liver tissue the mean value was 26±1.60%. The average % disappearance of alpha-ketol group/100 mgm. tissue ranged from 6 to 15% in 9 experiments and the mean value of 38 samples was 11±0.50%.

Liver tissue of pregnant rats was, therefore, slightly more active than that of non-pregnant rats in the reduction of the alpha-ketol group of corticosterone during 3 hour incubations. This increased activity per 100 mgm. liver tissue amounted only to a 10.0% difference but was statistically significamt ($P<0.05$ and >0.02 at 70 degrees of freedom).

C. Comparison of extent of reduction of Δ 4-3-ketone and alpha-ketol groups of corticosterone.

The mean % disappearance/100 mgm. incubated tissue of the Δ 4-3-ketone grouping of corticosterone, 21±0.59%, was 91% greater than that of the alpha-ketol group, 11±0.50%, following 3 hour incubations with liver tissue of pregnant rats. A similar difference, of 90%, was observed after incubations of liver tissue obtained from non-pregnant rats.

4. Incubations of liver tissue from pregnant and non-pregnant female rats with aldosterone-21-monoacetate.

In 2 experiments d.l. aldosterone-21-monoacetate was used as the substrate. In all other respects the incubation conditions were similar to those used with corticosterone.

(a) Disappearance of Δ 4-3-ketone grouping

Following incubation with liver of non-pregnant rats the average loss of Δ 4-3-ketone was 65 and 62 ug./sample in the 2 experiments, (Table XXVII). When expressed as a precentage loss of the original amount the mean value for 7 determinations was $32 \pm 1.40\%$. When this value was corrected for the loss of Δ 4-3-ketone grouping which occurred in the absence of liver tissue the mean was $19 \pm 1.92\%$. These results were also expressed as a % disappearance of the Δ 4-3-ketone grouping/100 mgm. liver tissue; the mean of 7 determinations was $8 \pm 0.93\%$ /100 mgm. tissue.

The percentage disappearance of the Δ 4-3-ketone grouping/100 mgm. of liver tissue obtained from pregnant rats was similarly determined. The mean value for 8 samples was $12 \pm 0.27\%$/100 mgm. tissue as compared with $8 \pm 0.93\%$ for liver tissue of non-pregnant rats, (Table XXVII).

Liver tissue obtained from pregnant rats was, therefore, on the average, 50% more active in reducing the Δ 4-3-ketone grouping of aldosterone 21-monoacetate than was that from non-pregnant rats. This difference was statistically significant, ($P < 0.01$ and > 0.001 at 13 degrees of freedom).

(b) Disappearance of alpha-ketol group.

When the alpha-ketolic group was measured in the same extracts of incubations of liver from non-pregnant rats the BT determinations showed a mean value of $22 \pm 1.18\%$ total disappearance of the original amount. When this value was dorrected for the loss of alpha-ketol group which occurred in the absence of

TABLE XXVII

REDUCTION OF Δ_4-3-KETONE GROUP OF ALDOSTERONE - ACETATE DURING INCUBATION WITH LIVER OF PREGNANT AND NON-PREGNANT FEMALE RATS

Disappearance (Dis.) of Δ_4-3-ketone grouping of added aldosterone-21-monoacetate (200 ug. per sample).

Exp.	No. samples	Rats	Total amount Dis. ug. range (mean)	Total % Dis. of amount added range (mean)	Total % Dis. /100 mgm. tissue range (mean)	% Dis.corr'd for loss of steroid incub. alone (13%) range (mean)	% Dis.(corr'd) /100 mgm. tissue range (mean)
11	4	Non-preg.	58±73 (65)	29±36 (32)	12±16 (14)	15±22 (18)	6±10 (8)
12	3	"	50±73 (62)	25±36 (31)	11±15 (14)	11±27 (20)	5±12 (9)
Mean±S.E.,7 determinations.				32 ± 1.40	14 ± 0.68	19 ± 1.92	8 ± 0.93
11	4	Pregnant	75±88 (80)	38±44 (40)	17±19 (18)	24±30 (26)	11±12 (12)
12	"	"	75±85 (83)	38±43 (41)	17±19 (18)	24±29 (28)	11±13 (12)
Mean±S.E.,8 determinations				41 ± 0.92	18 ± 0.27	27 ± 0.90	12 ± 0.27

TABLE XXVIII

REDUCTION OF ALPHA-KETOL GROUP OF ALDOSTERONE-ACETATE DURING INCUBATION WITH LIVER OF PREGNANT AND NON-PREGNANT FEMALE RATS

Disappearance (Dis.) of Alpha-ketol group of added Aldosterone-21-monoacetate (200 ug. per sample).

Exp.	No. Samples	Rats	Total amount Dis. ug. range (mean)	Total % Dis. of amount added range (mean)	Total % Dis. /100 mgm. tissue range (mean)	% Dis. corr'd for loss of steroid incub. alone (6%) range (mean)	% Dis.(corr'd) /100 mgm. tissue range (mean)
11	4	Non-preg.	40-53 (47)	20-26 (23)	9-12 (10)	14-20 (18)	6-9 (8)
12	"	"	33-48 (42)	16-24 (21)	7-11 (10)	10-18 (15)	5-8 (7)
Mean ± S.E., 8 determinations				22 ± 1.18	10 ± 0.60	16 ± 1.18	7 ± 0.50
11	4	Pregnant	50-65 (56)	25-33 (28)	11-13 (13)	19-27 (23)	9-11 (10)
12	3	"	45-50 (47)	23-25 (23)	10-11 (11)	17-19 (18)	7-8 (8)
Mean ± S.E., 7 determinations				26 ± 1.35	12 ± 0.56	20 ± 1.36	9 ± 0.60

liver tissue the mean value of 8 samples was 16±1.18%. These results expressed as a % disappearance of the alpha-ketol group /100 mgm. liver tissue gave a mean value of 7±0.50% for 8 samples.

The percentage disappearance of the alpha-ketol group /100 mgm. of liver tissue obtained from pregnant rats was similarly determined; the mean value for 7 samples was 9±0.60%/100 mgm.tissue.

Liver tissue obtained from pregnant rats was, on the average, 28.6% more active in the reduction of the alpha-ketol group of aldosterone-21-monoacetate, than was that from non-pregnant rats. This difference was of statistical significance, ($P<0.05$ and >0.02 at 13 degrees of freedom, (Table XXVIII).

(c) Comparison of extent of reduction of Δ4-3-ketone and alpha-ketol groups of aldosterone-21-monoacetate.

The mean % disappearance/100 mgm. incubated tissue of the Δ4-3-ketone grouping of the aldosterone derivative, 12± 0.27%, was 25% greater than that of the alpha-ketol group, 9± 0.60%, following 3 hour incubations with liver tissue of pregnant rats. This difference is statistically significant, ($P<0.001$) while that observed with liver tissue of non-pregnants is not.

5. Comparison of the extent of metabolism of corticosterone and aldosterone-21-monoacetate

As shown in Table XXIX the average extent of metabolism of both the Δ4-3-ketone and alpha-ketol groups of aldosterone-21-monoacetate was less than that observed for corticosterone following incubations with liver tissue obtained from either pregnant or non-pregnant rats. The mean percentage disappearance of

TABLE XXIX

INCUBATION OF LIVER OF PREGNANT AND NON-PREGNANT FEMALE RATS WITH CORTICOSTERONE AND ALDOSTERONE-MONOACETATE

Percentage disappearance of steroid substrate (corr'd)* /100 mgm. tissue average values

Grouping measured	Corticosterone		Aldosterone-21-monoacetate	
	Δ4-3-ketone	alpha-ketol	Δ4-3-ketone	alpha-ketol
Liver, non-pregnant rats	19±0.66(36)**	10±0.48(34)	8±0.93(7)	7±0.50(8)
" , pregnant "	21±0.59(39)	11±0.50(38)	12±0.27(8)	9±0.60(7)

* Corrected for loss of steroid which occurs with incubations of steroid substrate alone.

** Values in brackets are total number of determinations which were carried out.

the alpha-ketol group of corticosterone/100 mgm. tissue was 10±0.48% for liver of non-pregnant rats and 11±0.50% for that of pregnant rats; the corresponding mean values for the reduction of the alpha-ketol group of the aldosterone derivative were 7±0.50 and 9±0.60%, respectively. The difference between the extent of metabolism of these 2 steroids was, however, more apparent when the reduction of the Δ4-3-ketone grouping is considered. The mean percentage disappearance of the Δ 4-3-ketone grouping of corticosterone/100 mgm. tissue was 19±0.66 for liver tissue from non-pregnant rats and 21±0.59% for that of pregnant rats; the corresponding mean values for the reduction of the Δ 4-3-ketone grouping of aldosterone-21-monoacetate were 8± 0.93 and 12±0.27%/100 mgm. tissue, respectively. All these observed differences were of statistical significance, ($P < 0.001$ or $P < 0.02$ and > 0.01).

6. Summary

a. When corticosterone was used as substrate in the incubations the liver of pregnant rats was about 10% more active in the 'in vitro' reduction of both the Δ4-3-ketone and alpha-ketolic groups than was liver tissue of non-pregnant rats. These differences although slight were statistically significant. The extent of metabolism of the Δ4-3-ketone grouping was approximately twice that of the alpha-ketol group during incubations with liver from both groups of rats.

b. Pregnancy liver tissue was also 50 and 28% more active in the metabolism of the Δ4-3-ketone and alpha-ketol groups, respectively, of aldosterone-21-monoacetate than was similarly

incubated liver tissue from non-pregnant rats. The extent of metabolism of the Δ4-3-ketone grouping was significantly greater than that of the alpha-ketol (by 25%) during incubations with liver tissue from pregnant rats but no significant difference was observed with liver from non-pregnant rats.

c. Both types of tissue metabolized corticosterone to a greater extent than the aldosterone derivative when extent of alteration of either the Δ4-3-ketone or alpha-ketol groups is considered.

d. Pregnancy liver tissue was, therefore, more active in the metabolism of corticosteroids under 'in vitro' conditions than was that of non-pregnant female rats. These differences per unit weight were small but statistically significant and may be more important in the intact animal when the increased liver weight in pregnancy (by 30%) is taken into consideration.

C. Incubations of Rat Placental Tissue Under Various 'In Vitro' Conditions.

1. Possibility of production of adrenal-like hormones by rat placenta 'in vitro'.

Placental tissue obtained from pregnant rats near term was quickly cut into small pieces and incubated in Krebs-Ringer bicarbonate solution in amounts ranging from 1.3 to 10.0 gm. Six experiments were carried out and in 5 of these the quantity of placenta incubated approximated 1½ times the amount of tissue present in one pregnant rat close to term. In none of these experiments one of which is shown in Table XXX was it possible to detect aldosterone, corticosterone or any other Δ4-3-ketonic steroids of similar polarity in the extracts of the incubation media. These experiments suggest that the rat placenta

does not produce corticosteroids in measurable amounts under 'in vitro' conditions.

2. Incubation of rat placenta with rat adrenal glands.

Rat placental tissue was also incubated with adrenal glands in order to determine whether a direct influence on adrenal function could be demonstrated under 'in vitro' conditions.

(a) Male rat adrenals.

Since male rats were more available than female ones their adrenals were used in 4 out of 5 experiments. The adrenal tissue was divided so that one-half was incubated as the control for 3 hours; the other half was incubated with placental tissue for a similar period of time.

In 3 preliminary experiments with male adrenal glands from 1.3 to 3.6 gm. of placental tissue were incubated with approximately 0.6 gm. of adrenal tissue while similar quantities of the latter tissue were incubated alone. In each case the addition of placental tissue to the incubation medium of adrenal tissue caused a decrease in the amount of aldosterone and/or corticosterone when compared to control values. This observed effect was most marked in a subsequent experiment when the greatest amount of placental tissue used, 12.15 gm., was added to the incubation medium of 1.96 gm. adrenal tissue. At the same time 2.27 gm. of adrenal tissue was incubated alone using an equal volume, 100 ml., of Krebs-Ringer bicarbonate solution. The incubation medium of both samples was extracted for total free steroid content. Aldosterone and corticosterone were

TABLE XXX

INCUBATION OF MALE RAT ADRENAL GLANDS WITH RAT PLACENTA

Sample*	ug. steroid secreted/gm. adrenal tissue/hr.			
	Corticosterone	Aldosterone	Compound X_5	Compound X_6
Adrenal tissue (2.27 gm.) incubated alone	36.50	3.68	4.30	5.10
Adrenal tissue (1.96 gm.) incubated with 12.15 gm. placenta.	17.30	1.99	2.29	2.76
Placental tissue (10.0 gm.) incubated alone	0.00	0.00	0.00	0.00

* All samples incubated in a total volume of 100 ml. Krebs Ringer bicarbonate solution.

were isolated and also 2 unidentified $\Delta 4$-3-ketonic compounds designated as X_5 and X_6 as shown in Table XXX. Compound X_5 was more polar than aldosterone while X_6 had polarity intermediate between that of aldosterone and corticosterone in the chromatographic systems used. In the sample of adrenal tissue incubated with placenta the amount of each of these steroids was approximately one-half of the quantity obtained from the incubation medium of the control sample. The secretion of compounds B, X_5, X_6 and of aldosterone in ug./gm. of adrenal tissue/hr. was 36.50, 4.30, 5.10 and 3.68 ug., respectively, when adrenal glands were incubated alone. Following addition of placental tissue the secretion rate of compound B decreased to 17.30 ug. and that of aldosterone to 1.99 ug./gm./hr. The secretion rates of compounds X_5, and X_6 were also reduced, to 2.29 and 2.76 ug./gm./hr. respectively.

(b) Female rat adrenals.

A similar effect was observed in one experiment when placental tissue was incubated with adrenal glands obtained from both pregnant and non-pregnant female rats.

The adrenal tissue obtained from 22 non-pregnant rats was divided into 2 parts with one part incubated as control over 3 successive incubation periods of 0 to 1, 1 to 3 and 3 to 5 hours. The other part was incubated in the 2nd period with halved pituitary glands which were freshly removed from 11 non-pregnant rats under nembutal anaesthesia. In the 3rd period of incubation freshly removed and cut placental tissue was also added. A parallel incubation with adrenal glands obtained from 23 pregnant rats was simultaneously performed exdept that the pituitary tissue

used was removed from pregnant rats.

The following observations were made with adrenal tissue obtained from non-pregnant rats. The secretion of corticosterone in the control sample decreased from 15.79 to 6.14 ug./gm./hr. from the 2nd to the 3rd incubation periods. In the 2nd period of incubation adrenal tissue in the presence of pituitary glands secreted 20.27 ug. of corticosterone/gm./hr. and this was reduced by 89% to 2.17 ug. in the 3rd period of incubation when 3.8 gm. placental tissue was added. This decrease was 28% greater than that observed in the control sample.

Similarly with adrenal tissue of pregnant rats, the rate of secretion of corticosterone decreased from 5.55 to 3.59 ug./gm./hr. in the 2nd to 3rd periods of incubation of the control sample. In the second period of incubation adrenal tissue in the presence of pituitary glands secreted 11.50 ug. of corticosterone/gm./hr. and this decreased to less than 1.50 ug. in the 3rd period when 3.7 gm. placental tissue was added. This decrease of approximately 100% was about 65% greater than that observed for the control samples from the 2nd to the 3rd periods of incubation.

The secretion of aldosterone by adrenal tissue obtained from both pregnant and non-pregnant rats was similarly decreased in the presence of placental tissue, by 37 to 47%, respectively, below the control values.

Three suggestions could be made for the cause of this effect of placental tissue on the rate of secretion of corticosteroids during incubations of adrenal glands. It may have been due to a non-selective inhibition of normal respiratory processes

of the adrenal tissue in the presence of relatively large amounts of foreign tissue. Another possibility to be considered was whether or not the corticosteroids secreted into the medium were being metabolized by placental tissue so that estimations of the Δ4-3- ketonic steroids showed a decrease. A third factor might be a possible concentration or adsorption of corticosteroids by placental tissue involving little or no metabolism of the steroids.

The last two possibilities were investigated in experiments in which placental tissue was incubated with corticosterone. These studies were done simultaneously with a series of liver incubations. The tissues were incubated in Krebs phosphosaline buffer solution with and without added steroid. The compounds measured were the total free corticosteroids having a Δ4-3-ketone grouping in ring A and an alpha-ketolic group at carbon 17 present in the incubation media at the end of the experiment. The recovery of steroid incubated without placental tissue in this system was also determined by the INH and BT reactions.

3. Incubations of rat placenta with corticosterone

In the first experiment 0.66 and 0.84 gm. of placental tissue were incubated with 110 ug. of corticosterone. There was a disappearance of the Δ4-3-ketone structure of this steroid amounting to 47 and 43%/gm. tissue in the two samples; the percentage disappearance of the alpha-ketol group being in the same samples 30 and 29%/gm. tissue, respectively. This indicated that the placental tissue was taking up corticosterone from the solution and the discrepancy in disappearance of the Δ4-3-ketone and alpha-ketol groups suggested, furthermore, that the placental

tissue was actively metabolizing the steroid.

To investigate whether this removal of corticosterone was an active process or due only to a passive adsorption of steroid by placental tissue experiments were carried out in which 1 or 2 samples of tissue were incubated in the usual way while others were kept at ice-cold temperature during the incubation period.

As shown in Table XXXI in 7 samples in which the tissue was incubated at 37°C in a solution of corticosterone the percentage disappearance of the Δ4-3-ketone group/gm. tissue ranged from 26 to 47%; the mean value was 39±2.90%. When placental tissue was not incubated in 4 samples the percentage disappearance of the Δ4-3-ketone group ranged from 12 to 21%/gm. tissue and the mean was 18±1.96%. The percentage disappearance of the Δ4-3-ketone group of corticosterone when estimated by the INH reaction was significantly greater ($P < 0.001$) when the placental tissue was incubated at 37°C.

The extracts were also examined for alpha-ketolic steroid content using the BT reaction and the results are charted in Table XXXII. In the 7 samples of placental tissue incubated at 37°C in a solution of corticosterone the percentage disappearance of the alpha-ketol group/gm. tissue ranged from 21 to 30%; the mean value was 27±1.51%. When placental tissue was kept at ice-cold temperature this percentage disappearance of the alpha-ketol group ranged from 10 to 24%/gm. tissue and the mean was 17±3.30%. The percentage disappearance of the alpha-ketol group of corticosterone was, therefore, significantly greater ($P < 0.05$ and > 0.02) when the placental tissue was incubated at 37°C.

TABLE XXXI

DISAPPEARANCE (DIS.) OF Δ 4-3-KETONE GROUPING OF CORTICOSTERONE IN THE PRESENCE OF RAT PLACENTA

Exp.	Tissue - wt(gm)	Amount Cmpd B added	Total Disug.	% Total Dis.	% Total Dis./gm.	% Dis.corr'd for loss of steroid inc. alone (9%)	% Dis./gm. (corr'd)
1	Incub. 0.66	113	46	40	61	31	47
"	" 0.84	"	51	45	53	36	43
2	" 0.64	111	36	32	51	23	36
"	" 0.76	"	36	32	43	23	30
3	" 0.96	110	47	43	45	34	36
"	" 0.84	"	45	41	49	32	38
4	" 1.11	258	97	38	34	29	26
Mean ± S.E.				39 ± 1.19	48 ± 3.21	30 ± 1.93	39 ± 2.90
2	Not inc 0.68	111	24	21	31	12	18
3	" 0.73	110	25	23	32	14	19
"	" 0.63	"	24	22	35	13	21
4	" 1.04	258	55	21	21	12	12
Mean ± S.E.				22 ± 0.50	30 ± 3.04	13 ± 0.50	18 ± 1.96

TABLE XXXII

DISAPPEARANCE (DIS.) OF ALPHA-KETOL GROUP OF CORTICOSTERONE IN THE PRESENCE OF RAT PLACENTA

Exp.	Tissue - wt(gm)	Amount Cmpd B added	Total Dis.ug.	% Total Dis.	% Total Dis./gm.	% Dis.corr'd for loss of steroid inc. alone (5%)	% Dis./gm. (corr'd)
1	Incub. 0.66	113	28	25	38	20	30
"	" 0.84	"	33	29	35	24	29
2	" 0.64	111	26	23	31	18	28
"	" 0.76	"	24	21	33	16	21
3	" 0.96	110	38	34	36	29	30
"	" 0.84	"	30	27	33	22	26
4	" 1.11	258	73	28	25	23	21
Mean ± S.E.				27 ± 1.62	33 ± 1.80	22 ± 1.62	27 ± 1.51
2	Not inc. 0.68	111	14	12	18	7	10
3	" 0.73	110	23	20	28	15	21
"	" 0.63	"	23	20	32	15	24
4	" 1.04	258	45	18	17	13	13
Mean ± S.E.				18 ± 1.92	24 ± 3.80	13 ± 1.91	17 ± 3.30

The difference between the average percentage disappearance of the alpha-ketol and Δ 4-3-ketone groups of the corticosterone substrate was significant only in the samples of placental tissue which were incubated, ($P < 0.01$ and > 0.001). The mean % disappearance of the Δ 4-3-ketone and alpha-ketolic groups was 39 ± 2.90 and 27 ± 1.51%/gm. tissue, respectively. This would suggest that a selective metabolism of the Δ 4-3-ketone group occurred during incubation of placental tissue with corticosterone.

In experiment 4 (Table XXXIII) a sample of placental tissue was incubated with 259 ug. of corticosterone while another sample similarly prepared was not incubated. After 2 hours of incubation the former sample contained 160 ug. of Δ 4-3-ketonic and 186 ug. of alpha-ketolic steroid in the medium. The medium of the other sample which was kept at ice-cold temperature contained 203 and 213 ug. of Δ 4-3-ketonic and alpha-ketolic steroid, respectively. The tissue in both cases was homogenized after separation from its medium. The homogenates were chromatographed and analysed for corticosterone content. An additional 50 ug. of corticosterone was recovered from incubated tissue while another 36 ug. was obtained from placental tissue not incubated. The percentage disappearance of the Δ 4-3-ketone group of the substrate corticosterone was thereby reduced from 26 to 8%/gm. incubated tissue and that of the alpha-ketol group was similarly decreased from 21 to 4%. If any metabolism of added corticosterone did occur the extent was, therefore, less than 10% gm. of incubated tissue. The recovery of corticosterone from the homogenate of the non-incubated tissue likewise reduced the extent of disappearance of the alpha-ketol and Δ 4-3-ketone groups of corticosterone from

TABLE XXXIII

DISAPPEARANCE (DIS.) OF CORTICOSTERONE

IN THE PRESENCE OF RAT PLACENTA

Tissue-wt.(gm)		△ 4-3-ketone grouping				alpha-ketol group			
		Total Dis.ug.	% Total Dis.ug	% Dis. corr'd for loss of steroid inc. alone (9%)	% Dis./gm. (corr'd)	Total Dis.ug	% Total Dis.	% Dis. corr'd for loss of steroid inc. alone (5%)	% Dis/gm. (corr'd)
Incub.	1.11	97	38	29	26	73	28	23	21
		(-50)*				(-50)*			
		48	18	9	8	22	9	4	4
Not incub.	1.04	55	21	12	12	45	18	13	13
		(-34)*				(-36)*			
		21	8	0	0	9	4	0	0

* Amounts of corticosterone recovered from homogenates of placental tissue after incubation or standing in solution of corticosterone.

13 and 12%/gm. of tissue to zero values.

4. Summary

A) Rat placental tissue did not secrete aldosterone, corticosterone or any other Δ4-3-ketonic steroids of similar polarity in measurable amounts when incubated under 'in vitro' conditions.

B) The addition of rat placental tissue to incubations of adrenal glands obtained from male and female rats resulted in a decrease in the quantities of Δ4-3-ketone steroids isolated from the incubation media. This effect was increased following addition of greater quantities of placental tissue.

C) Subsequent incubations of rat placenta with corticosterone suggest that the above effect of placental tissue may have been partly due to an adsorption or concentration of corticosteroids by this tissue. This adsorption appeared to be increased as a result of incubation. Any metabolism of corticosterone per unit weight of placental tissue which may have occurred was slight in comparison with that observed with rat liver tissue under similar incubation conditions.

DISCUSSION

There is an accumulation of evidence suggesting increased adrenal function in human pregnancy. Normal women show during gestation increased blood 17-hydroxycorticosteroids and urinary glucocorticoids (20)(21)(22) and a marked rise in aldosterone excretion (25)(27)(28). In earlier studies women with adrenal insufficiency were reported also to excrete greater amounts of corticoids in the last trimester of pregnancy (40)(45); the source of these steroids was refractory to ACTH stimulation and presumed to be the placenta (24)(40). Moreover both Addisonian women and patients with rheumatoid arthritis, a disease also associated with decreased adrenal function, were observed generally to have increased well-being while pregnant (31)(32)(44).

The observations in the normal pregnant women tended to indicate an increased adrenal activity; those in adrenal deficient women suggested that an extra-adrenal source of corticosteroids may exist during pregnancy. This source would presumably be either the placenta or fetal adrenals, or both.

The above findings served as the basis for experiments carried out in the present study. These were designed to investigate the source(s) of corticosteroids produced in pregnancy and special consideration was given to the placenta. Since levels of corticosteroids in blood and urine are dependent not only on rate of production but also on rate of metabolism and clearance (58)(59) it would be necessary to evaluate as well the role of metabolism

of corticosteroids in pregnancy. Within the past 3 years much information has been gained regarding the production and metabolism of corticosteroids in human pregnancy; very little is known about adrenal cortical function during gestation in the rat and other species of animals.

A. Investigations of placental function with respect to corticosteroid production under 'in vitro' conditions

The possibility that the placenta may elaborate adrenal cortical hormones has been investigated by a number of workers. Corticosteroids were first isolated from extracts of placental tissue by De Courcy et al (173) who obtained about 100 ug. of cortisone and traces of other Δ 4-3-ketonic steroids per placenta, and subsequently by others (174)(176). Recently Berliner et al (177) have isolated 9 UV light absorbing substances including compounds E, THE, A and F in amounts of 90, 6, 45 and 6 ug./kg. placental tissue, respectively.

Similarly, in the present study corticosteroids were obtained merely by extraction of portions of 2 placentae. From 1 placenta 33.00 ug. of cortisone, 1.86 ug. of hydrocortisone and 8.25 ug. of a compound X_4, were isolated /100 gm. tissue. No detectable amount of $C_{21}O_4$ corticosteroids could be found. Another placenta yielded 12.80 ug. cortisone /100 gm. tissue but not the other steroids of similar polarity. Aldosterone was not detected in these studies perhaps because too little tissue, approximately 100 gm., was extracted. This is supported by the findings of Berliner et al who obtained only 3 ug. aldosterone /kg. tissue (177). The unidentified compound X_1 was of interest because it had chromatographic mobility similar to aldosterone in the 3

systems used. It may have been a mixture containing relatively small amounts of aldosterone but the major component did not reduce blue tetrazolium and did not show Na-retaining activity when bioassayed. Compound X_1 may have been identical to either of compounds II or III which have been isolated from human urine by Nowaczynski et al (285); the latter compounds have similar chromatographic mobility and also do not possess an alpha-ketolic group. Since compound III has shown K excreting activity when assayed in rats (100) it would be of interest to determine whether compound X_1 has a similar effect.

Of the 3 other unidentified UV light absorbing compounds only X_4 appears to have been a Δ4-3-ketonic steroid by virtue of its maximum at 240 mμ in ethanol. Compound X_3 was present in greatest amount of all the unknown substances; its maximum of UV absorption was at 272 mμ in ethanol. This property and its H_2SO_4 spectrum were similar to those reported for a substance F_3B_2 described by Berliner et al (177). The latter group isolated it also from extracts of ovary and from plasma of pregnant and non-pregnant women. Its chemical properties and occurrence suggest that compound X_3 may have been an oestrogen.

Although in the present study and in those of other workers (127) there was variation in the quantity of corticosteroids present in different placenta it was apparent in the present experiments that the amounts of 17-hydroxycorticosteroids isolated from placental tissue were increased above the quantity which might be expected on the basis of blood content alone even when one considers that these steroids are elevated in the maternal

circulation. In the last trimester 17-hydroxycorticosteroids may approach 50 ug./100 ml. plasma and upon administration of ACTH a further increase to a range from 70 to 110 ug./100 ml. has been observed (289). A calculation of the amount of 17-hydroxycorticosteroids present in placental tissue assuming a high level of 100 ug./100 ml. blood and that 10% of the weight of placenta is due to its blood content (178) indicates that 100 gm. of tissue might contain as much as 10 ug. of these steroids derived from the maternal circulation. This would be true if no attempt were made to remove any blood content. But placental tissue in the present study was freed from its blood content as much as possibly and still 33 and 12 ug. quantities of cortisone /100 gm. of tissue were isolated simply by extraction. This suggests that if the 17-hydroxycorticosteroids are not derived from placental production 'in vivo' they must in some way be concentrated within the latter tissue from the maternal circulation. The latter theory has been tested by Salhanick et al (160). They administered radioactive cortisone to a pregnant woman just before delivery and found subsequently that the specific activity in placental blood was more than twice that in the maternal peripheral circulation. This suggests that a rather rapid mechanism exists for the concentration by placental tissue of corticosteroids administered to the mother and that the 17-hydroxycorticosteroids present in the placenta are not necessarily elaborated by this organ.

The latter observation seems to be in accordance with the results obtained in the present incubations of minced human plac-

ental tissue. In the experiments described no increase in the 'in vitro' production of cortisone, the predominant placental corticosteroid, or of that of a compound with similar polarity, X_1, could be detected during incubation of placental tissue alone. Neither were these compounds increased following the addition of various trophic factors such as human chorionic gonadotrophin, ACTH and prolactin, to the incubation media. The addition of similar quantities of ACTH, on the other hand, to incubations of adrenal tissue would have resulted in near maximal stimulation of steroidogenesis. Placental tissue, moreover, contains appreciable quantities of an endogenous substance with ACTH-activity (131)(132)(133). Even the addition of known precursors of adrenal cortical hormones, cholesterol and $\Delta 5$-pregnene-3β-ol -20-one to these incubations of human placenta did not stimulate a production of corticosteroids whereas the amounts of progesterone were increased under similar conditions. While the quantities of compound X_1 appeared to be unaltered during incubation those of cortisone actually decreased. This decrease of cortisone was apparently due in part to a conversion to hydrocortisone since the quantities of hydrocortisone in the same sample of incubated tissue were increased above the amount that could be extracted from the non-incubated control sample. In one experiment approximately one-half of the cortisone disappeared, some in part due to conversion to hydrocortisone and some was apparently metabolized to still more polar alpha-ketolic material consisting presumably of compounds THF and/or THE. In another experiment the conversion to hydrocortisone was only slight and

it appeared to be partly dependent upon the initial placental concentration of cortisone. A report that has just been published by Endroczi et al (286) gives findings which are similar in a number of respects to those obtained in the present study. These authors incubated homogenates of full term human placenta and also could not obtain an increase of corticosteroid content during incubation of tissue alone or following the addition of ACTH and chorionic gonadotrophin. At variance with the present observations Endroczi et al (286) were not able to isolate hydrocortisone from their incubations even when cortisone was added to the medium. This disagreement may have been due to differences in placental preparations or in the extent of removal of blood content prior to incubation or, perhaps, to the fact that caesarian section placentae were used in the present study but full term tissue in the other.

The present studies, nevertheless, offer an explanation for the finding of much larger amounts of cortisone in placenta than of hydrocortisone if it is only a question of concentration by placental tissue of maternal 17-hydroxycorticosteroids. Upon the addition of relatively large amounts of hydrocortisone to incubations a significant conversion to cortisone was observed. Similar observations were reported by Endroczi et al (286). Since placental tissue is capable of converting hydrocortisone to cortisone 'in vitro' it may also perform this conversion 'in vivo'.

The latter observation is not necessarily in conflict with the one previously described of conversion of cortisone to hydrocortisone. The interconversion of these compounds is under the

influence of a 11β-hydroxysteroid dehydrogenase and is a reversible reaction (74). The direction of conversion would depend, therefore, upon the relative concentrations of the 2 steroids in the particular system studied. This interconversion is, however, not unique to placental tissue. It has been previously demonstrated that administration of either of compounds E or F to non-pregnant humans results in the urinary excretion of the other steroid (43). The site of conversion is presumed to be the liver but according to reports of Berliner et al (287) other tissues may also be involved since they incubated loose connective tissue from rats with hydrocortisone and observed a transformation to cortisone.

Placental tissue when converted to a mince (or homogenate) does not appear, therefore, to have an innate capacity to produce corticosteroids, including aldosterone, when incubated under 'in vitro' conditions. Neither could ACTH or certain other trophic hormones induce corticosteroidogenesis when added to incubations of placental tissue. Certain transformations of corticosteroids present in the placenta may occur, however, as a result of incubation. Similar observations were made by Pincus and his coworkers (127) with control and ACTH-stimulated perfusions of human placentae. The addition of radioactive progesterone to the perfusions, moreover, did not result in the appearance of radioactivity in the hydrocortisone and more polar fractions. The latter observation, however, disagrees with the finding of Endroczi et al (286) of increased amounts of cortisone and other Δ4-3-ketonic steroids in the medium following incubation of

placental tissue homogenates with progesterone.

On the other hand, Little and Rossi (175) reported a production of Porter-Silber (PS) reactive material during incubations of minced human placental tissue alone. The experimental techniques employed would, however, cast doubt on the validity of their conclusions. Stronger evidence for elaboration of PS reactive substances by human placentae (full term) has been described in an abstract published by Troen (184) but details of these experiments have not yet been reported. The placentae were perfused in Tyrode's solution for up to 12 hours and a range from 0.75 to 14.9 (mean 8.2) mgm. of PS positive compound(s) was obtained per placentae. Extraction of placental tissue yielded less than 1/20th of the amount of this material obtained from perfusates. The PS reactive material consisted presumably of compounds E,F, THE, THF and S. This author considers that the human placenta can produce and conjugate corticosteroids. Since aldosterone does not give the Porter-Silber reaction it is not known whether it was also produced during these perfusions.

The latter results are certainly at variance with those obtained in the present study and those reported by others (127) (286). Can they be due to differences in the preparation of placental tissue used for study? Admittedly placental minces and homogenates would probably be weaker systems for steroidogenesis than intact perfused placenta. Adrenal tissue, however, whether minced, sliced, homogenated or perfused intact can produce cortical hormones under similar 'in vitro' conditions. But the texture of placental and adrenal tissue is very different. Pincus et al

(127) however, could not observe corticosteroidogenesis in their placental perfusion studies in contrast with the findings of Troen. They, however, used beef-blood for perfusion while the latter used an artificial medium and this difference may account for the conflicting results since a blood perfusion may have favoured metabolism rather than secretion of corticosteroids.

All the observations described above were made under 'in vitro' conditions and it is necessary to relate them to findings obtained in 'in vivo' studies since the latter would give a more physiological and more accurate picture of what is actually occurring in the human being during pregnancy.

B. The production of aldosterone in human pregnancy

The most recent studies on totally adrenalectomized or Addisonian women during pregnancy (30)(48) have demonstrated that the placenta is not a source of any significant quantities of aldosterone during human pregnancy and neither are the fetal adrenal glands. The greatly increased quantities of aldosterone in pregnancy urine, especially during the last trimester when they may approach 100 ug./24 hours whereas normal non-pregnancy urine usually contains less than 10 ug. /day, must rather be derived from maternal adrenal activity.

Similarly in the present study one totally adrenalectomized woman receiving corticosteroid replacement therapy did not excrete any significant quantities, less than 1.5 ug./ gm. creatinine, of Na-retaining hormone in the 6th, 8th and 9th months of her pregnancy and 4 days after delivery. A determination made in

the present study on another similar patient in her 9th month of pregnancy, however, showed the presence of 13 ug. Na-retaining hormone in the urine. This value although well below the amount observed in normal pregnancy urine in the last trimester is still appreciable, being more than the amount excreted by normal non-pregnant subjects. What could be the source of this Na-retaining substance which, presumably was aldosterone? It is not likely to be either the placenta or foetal adrenals since neither of these glands appears to be a source of similarly large quantities in other adrenal deficient pregnant women. Neither is it likely that the aldosterone would be derived from metabolism of the steroids which were administered as therapy in this particular patient but not in other adrenalectomized individuals similarly treated. Rather if this finding of 13 ug. aldosterone/gm. of creatinine (per day) is a valid one it may be that its source was regenerated adrenal tissue. The presence of regenerated adrenal tissue in patients some time following 'total' adrenalectomy for Cushing's syndrome is not too rare (100).

In studies recently published by Jones et al (288) H^3-aldosterone was injected into both non-pregnant women and those in the last trimester of gestation. From the specific activity of the urinary aldosterone released by acid hydrolysis and the value of the radioactive dose the secretion rate of aldosterone could be calculated. This technique showed that there is an increased rate of secretion of aldosterone in some pregnant women. But these investigators have reported also that the finding of increased urinary aldosterone during pregnancy is in some women due in large

part and, in others, apparently entirely to an alteration in the route of metabolism of this particular hormone. The amounts of free aldosterone extractable at neutral pH, are not increased in pregnancy urine but the quantities of aldosterone extractable following pH 1 acid hydrolysis are markedly elevated in the last trimester. This acid hydrolysis apparently releases aldosterone from a loose combination with an unknown substance (sulphate?). Since the aldosterone which is thus liberated retains its biological activity this route of conjugation which is apparently selectively increased in human pregnancy does not involve a prior reduction of the Δ4-3-ketone grouping in ring A as would conjugation with glucuronic acid. The latter pathway of metabolism of aldosterone was reported to be considerably decreased during pregnancy but the overall rate of metabolism of aldosterone does not, however, appear to be significantly altered in pregnant women.

It is of interest that those subjects studied by Jones et al (288) who did secrete increased amounts of aldosterone during gestation did not at the same time show clinical signs of hyperaldosteronism, e.g. oedema, hypertension, as do patients with diseases associated with similarly high aldosterone excretion. It would appear as if the state of pregnancy offered a protection and according to Jones et al and Landau and Lugibihl (183) it may be the greatly increased production of progesterone, a Na-excreting substance, which is responsible for this beneficial effect in pregnant women.

Moreover, as observed in the present study on adrenalectomized

pregnant women and in those of other investigators (30) an increased production of aldosterone is not a prerequisite for a successful gestation and delivery.

C. The production of 17-hydroxycorticosteroids in human pregnancy

A number of aspects of the production and metabolism of 17-hydroxycorticosteroids in human pregnancy have similarly been elucidated by investigations carried out within the past few years.

In the present study 2 totally adrenalectomized patients receiving corticosteroid replacement therapy and one normal woman showed in the last trimester of pregnancy values for total urinary 17-hydroxycorticosteroids which were within the range for normal non-pregnant women. Moreover, the pattern of urinary corticosteroids with respect to both the Δ 4-3-ketone and alpha-ketolic steroid components was in the normal pregnant subject qualitatively similar to those obtained for non-pregnant normals. The adrenalectomized women also showed corticosteroid excretion patterns which with respect to the $C_{21}O_5$ alpha-ketolic steroids were qualitatively similar to those observed for normal subjects. It would be reasonable to assume that the steroids present in the urine of these patients were derived, at least predominantly, from the hormone therapy administered. One of these cases, however, also showed relatively large quantities of less polar alpha-ketolic steroids in a urine specimen collected in the 9th month of pregnancy when she was receiving both hydrocortisone and

9-α-fluoro-hydrocortisone as replacement therapy. It is not likely that these unidentified compounds were derived from the administered hydrocortisone since the metabolism of the latter steroid is similar to that of cortisone (43) and the administration of cortisone to a non-pregnant adrenalectomized woman in the present study did not appear to result in a urinary excretion of comparable amounts of these less polar alpha-ketolic steroids. In pregnancy, however, the metabolism of administered hydrocortisone may be altered, perhaps due to the presence of the placenta and/or foetal adrenals. It is not likely either that these blue tetrazolium reactive steroids were derived from the 9-α-fluoro-hydrocortisone since they did not appear in comparable amounts in the urine of another similar patient who received still greater quantities of the fluorinated hydrocortisone. The possibility must also be considered that these less polar alpha-ketolic steroids may have been secreted by regenerated adrenal cortical tissue especially since this patient excreted as well appreciable amounts of Na-retaining hormone at the same time. Moreover, the latter individual continued to excrete aldosterone after termination of her pregnancy.

In the present study 3 normal subjects excreted quantities of cortisone plus hydrocortisone which ranged from 1/8th to 1/12th of those of compounds THF plus THE while a normal pregnant subject excreted relatively more of the free steroids, the amount being 1/4th of those of the reduced metabolites. Even greater proportions of the free 17-hydroxycorticosteroids relative to those of their ring A reduced metabolites appeared in the urine of 2

totally adrenalectomized pregnant patients. But this further change from the normal non-pregnancy pattern may have not been due entirely to the condition of pregnancy but in part, also, to the character of therapy administered. The present urinary studies tended, nevertheless, to suggest that a reduced rate of reduction of the Δ 4-3-ketone grouping in ring A of the 17-hydroxycorticosteroids occurs during pregnancy. One of the pregnant adrenalectomized women, moreover, continued to show this pattern of corticosteroid metabolism 4 days after delivery. A similar finding has recently been reported by Christy et al (289). Migeon et al (89) were, however, the first to describe this alteration in 17-hydroxycorticosteroid metabolism during pregnancy. They administered C-14-hydrocortisone to both non-pregnant women and those in the last trimester of gestation and observed a reduced clearance of radioactive steroid from the plasma in the latter subjects. This delayed clearance in pregnancy was associated with a urinary excretion of increased amounts of radioactive steroid in the free fraction but decreased quantities in the conjugated fraction in comparison with the pattern of corticosteroid excretion observed in the non-pregnant individuals. Migeon et al, therefore, concluded that the appearance of increased quantities of 17-hydroxycorticosteroids in the blood and urine of pregnant women is due at least in part to a decreased metabolism of these steroids during gestation. Others have subsequently reported similar observations (90)(50)(289). It is now apparent according to the findings of Taliaferro (93) and others (94) that this delayed metabolism of 17-hydroxycort-

icosteroids in pregnancy is related to the increased levels of oestrogens produced during gestation. A reduced turnover rate of administered hydrocortisone in newborn infants (91) has similarly been associated with increased levels of oestrogens. The manner in which the oestrogens exert this influence is not yet clear. It has been reported that these sex hormones increase the protein binding of corticosteroids (100) and thus presumably render them less available to destruction by the liver but Daughaday et al (290) could not observe this effect. According to Cohen et al(50) oestrogens may be implicated in the tendency for 17-hydroxycorticosteroids to be retained within the intravascular compartment during pregnancy. This latter observation would offer an explanation for the failure of pregnant women to show marked signs of Cushing's syndrome in the presence of much increased levels of gluco-corticoids in the circulation. Do the oestrogens, however, exert their influence on 17-hydroxycorticosteroid metabolism at the level of liver function? They may induce a decrease in the reduction of the $\triangle 4$-3-ketone grouping in ring A of the corticosteroid molecule. Glenn as reported by Cohen et al (50) found that oestrogens inhibited the degradation of hydrocortisone when both steroids were incubated with a liver microsome supernatant fraction. These findings with a purified fraction of rat liver tissue, 'in vitro', do not necessarily represent, however, what is happening in the human being. On the other hand it may be the step involving conjugation with glucuronic acid which is impaired in pregnancy since Christy et al (289) observed a delayed

clearance of compound THE when it was administered to pregnant women. Another hypothesis suggested by Jones et al (288) is that an interference of the reduction of 17-hydroxycorticosteroids by liver tissue may result during pregnancy because of a preferential metabolism of the greatly increased amounts of progesterone which are produced during gestation.

The most recent findings reported by Little et al (49) and others (47)(48)(50) on adrenal deficient pregnant women tend to indicate indirectly that the maternal adrenal glands must be the predominant if not the only source of corticosteroids in pregnancy. They exclude the possibility of any significant production of 17-hydroxycorticosteroids by placenta or foetal adrenal glands during gestation. The latter findings would be in agreement with the observations made in the present study with incubations of human placenta and thosereported by certain other investigators (286)(127). On the other hand, as previously mentioned, Troen (184) has reported a considerable production of Porter-Silber reactive, presumably hydrocortisone-like, steroids during prolonged perfusions of unstimulated placentae with Tyrodes solution. Since the placenta can apparently produce significant amounts (average of 8 mgm./placenta/12 hours) of corticosteroids under 'in vitro' conditions it might be expected that similarly large or even larger quantities could be elaborated 'in vivo'. But this appears not to be the case since attempts to remove corticosteroid replacement therapy in adrenal deficient women during pregnancy have generally precipitated signs of acute adrenal insufficiency (42)(49)(47).

Although some investigators have reported no increase in the secretion rate of 17-hydroxycorticosteroids during pregnancy (50) others including Christy et al (289) consider that the increased blood corticosteroid levels cannot be entirely accounted for by decreased metabolism during gestation. Jones et al (288), moreover, have pointed out that although the rate of corticosteroid metabolism is reduced in some conditions of cirrhosis the plasma 17-hydroxycorticosteroid levels are normal perhaps because adrenal secretion of these steroids is reduced in order to maintain this normal state. In pregnancy, on the other hand, plasma and urinary free 17-hydroxycorticosteroids are increased above normal levels in the face of a reduced clearance of these steroids from the circulation. It would appear, therefore, as if adrenal secretion of 17-hydroxycorticosteroids is maintained at a constant normal rate in pregnancy perhaps because the pituitary in this condition is less sensitive to increased levels of these steroids in the circulation and continues to secrete ACTH. Earlier histological studies (55) on adrenal glands from pregnant women and, more recently, studies by Whitely and Stoner (56) did not, however, show evidence for any changes in these glands which might suggest an increased activity or an abnormal stimulation by ACTH.

It may be as postulated by Jones et al (288) that an increased secretion rate of aldosterone, a decreased sensitivity of the kidney to the action of aldosterone, the reduced rate of metabolism of hydrocortisone and the accompanying stimulation of hydrocortisone secretion to maintain the raised blood con-

concentrations are all correlated, being all due to the increased progesterone output in human pregnancy. This idea is supported by their observation of a close relationship between the aldosterone secretion rate and pregnanediol excretion in pregnant women.

D. The production of progesterone in human pregnancy

Considerable evidence of indirect nature has accumulated which indicates that the production of progesterone is greatly increased in pregnant women. This evidence consists primarily of the finding of progressively increased quantities of pregnanediol, the major metabolite of progesterone, in the urine during gestation (105). Other derivatives of progesterone, pregnanolones, are also excreted in progressively greater amounts throughout the course of pregnancy (149). During the early part of human gestation progesterone is derived mainly from the secretion of the corpus luteum. This secretory function of the corpus luteum is maintained throughout early pregnancy and is under the influence of the chorionic gonadotrophin (HCG). By the 4th or 5th month of gestation there is regression of the corpus luteum and a decrease in the urinary excretion of HCG to low but constant levels. The quantities of pregnanediol in the urine continue, however, to increase progressively to term (149). The following observations suggest that the source of this progesterone produced in the latter half of pregnancy must be the placenta.

The possibility that maternal adrenal glands are a predominant source of progesterone during gestation can be excluded since in the present study and in those reported by others (40)

(45) adrenal deficient women (totally adrenalectomized or Addisonian) were found to excrete essentially normal amounts of pregnanediol during pregnancy. Three cases in which the corpus luteum was removed before the 4th month of pregnancy excreted somewhat subnormal amounts of pregnanediol but each pregnancy went to term (262). Appleby and Norymberski (51) found that pregnanediol excretion remained increased in 5 cases of intrauterine death. The latter observation suggests that the fetal adrenals are not an important source of progesterone during human gestation. Moreover, the finding that the concentration of progesterone in placental blood is about 4 times that in the maternal peripheral circulation also suggests a placental elaboration of this steroid (156).

According to Pearlman (159) who administered $4-C^{13}$-progesterone to women in the last trimester of gestation the endogenous production of progesterone as estimated by isotopic dilution studies approaches a rate of 250 mgm./day in late pregnancy. Extracts of placental tissue, however, yield surprisingly low quantities of progesterone. In earlier attempts (158) only from 0 to 1 mgm. of progesterone/kg. of full term placental tissue could be isolated. Haskins (157) found that placentae obtained by caesarian section contained on the average more than twice as much of progesterone as those obtained at term. A similar observation was apparent in the present study since 1.83 and 2.55 mgm. progesterone/kg. tissue were obtained by extraction of placentae removed at the time of caesarian section in comparison with 0.98 mgm./kg isolated by Berliner et al (177) from full term tissue.

These quantities of progesterone are very low when the daily excretion of metabolites of progesterone in late pregnancy is considered and suggest that the progesterone which is produced 'in vivo' must be rapidly secreted into the circulation and metabolized. Further evidence for this is afforded by the presence of only very small quantities of progesterone itself in the maternal circulation throughout gestation (154). The concentrations of pregnanediol in the peripheral circulation are somewhat increased in the last trimester (155).

In the present studies it was not possible to demonstrate a continued 'in vitro' production of progesterone during incubations of minced human placental tissue alone since the total amount of progesterone obtained from incubated tissue was not increased above the quantity which could be obtained merely by the extraction of the tissue. The addition of HCG, ACTH and prolactin did not appear to stimulate an increased production of progesterone by placental tissue whereas HCG stimulates a corpora luteal secretion of progesterone 'in vivo' (149). The possibility that in the present experiments the amounts of HCG added were too small to be effective has to be considered. Since placental tissue already contains an average of 20 U HCG/gm. according to reports of Diczfalusy (126) the addition of 7 and 10 U/gm. quantities might not be expected to have any influence. In an experiment when 32 U/gm. were added to the incubation medium of one portion of tissue there was still no increase above control levels in the amount of progesterone subsequently obtained from

the media. Endroczi et al (286) also could not obtain a stimulation of progesterone production following the addition of HCG or ACTH to incubations of human placental homogenates.

Prolactin is used clinically to induce lactation in some women following delivery (282). It is considered to be identical to luteotrophin which is necessary for the production of progesterone by the corpus luteum in hypophysectomized rats after they have been properly primed with FSH and LH hormones (100). Prolactin was therefore incubated with placental tissue in the present study over a 6 hour period. This did not result in an increase of progesterone concentration above the amount which was obtained from the control sample of placental tissue.

Thus, it would appear that placental tissue under the incubation systems used in the present study did not have an innate capacity to produce progesterone. Homogenates of placental tissue are also inactive in this respect (286). On the other hand, Hagopian et al (172) published preliminary findings which indicated that steroids with polarity of progesterone were elaborated in increased quantities following the addition of ACTH to perfusions of placenta. The extent of the increase was not given. These steroids subsequently were found to consist not of progesterone but of 6- ketoprogesterone and an unidentified compound neither of which showed progestational activity. The addition of radioactive progesterone to the perfusions resulted in the appearance of radioactivity in the other 2 steroids indicating that they were derived from progesterone. There was no net increase in the amounts of progesterone produced during perfusion but

rather a decrease due apparently to metabolic transformations. At a 1956 conference on gestation Dr. Pincus (127) described more fully similar perfusions of human placentae obtained at caesarian section with bovine blood; full term placentae were apparently non-functional. Although there was considerable variation from one placenta to the other average figures were given which showed that a total of 0.70 mgm. of progesterone-like material was produced per placenta during 4 hours of perfusion. This is a very low value when one considers the quantities which the placenta must be producing 'in vivo', approximately 250 mgm. /day (159), in late pregnancy. Moreover, in the present studies, 255 and 183 ug. of progesterone could be obtained merely by extracting 100 gm. of placental tissue. Since as much as 765 ug. of this steroid may be obtained from a normal sized, 300 gm., placenta by extraction alone it would appear, therefore, that the total production of 700 ug. of progesterone-like steroid during 4 hour perfusions is not very significant. In the latter perfusions the production of progesterone-fraction steroid was reported to be increased by the addition of both HCG and sheep pituitary gonadotrophin but the extent of the increase was not given.

It would appear that an 'in vitro' production of progesterone from endogenous precursors in placental tissue is not easily demonstrated as is the secretion of corticosteroids by adrenal cortical tissue under similar conditions.

In the present study the addition of Δ5-pregnene-3β-ol-20-one (pregnenolone) to an incubation of placental mince resulted

in the production of progesterone amounting to 357.79 ug./100 gm. tissue/hr. This conversion of pregnenolone to progesterone involves a simple oxidation of the 3β- hydroxyl group to the corresponding 3-ketone and is under the influence of a 3β- hydroxysteroid dehydrogenase. The double bond subsequently shifts from the Δ 5 to the Δ 4 position (74). This conversion has, however, previously been demonstrated with human placental tissue (163) and is not unique to placenta since other steroid producing glands, adrenals and testes (164) can also convert pregnenolone to progesterone under 'in vitro' conditions.

When cholesterol was incubated with placental tissue it was converted to progesterone at the rate of 31.33 ug./100 gm. of tissue/hour. This conversion was, however, much less than that observed with pregnenolone presumably because it involves more complex reactions. These would include the removal of 6 carbon atoms from the side chain at carbon 17 and an oxidation of the 20 carbon atom to form the Δ 5-pregnene-3β-α-20-one intermediate (283). Once the latter is produced the transformation to progesterone presumably occurs readily. In the incubation system used in the present studies it would appear as if conversion of cholesterol to pregnenolone occurred only slowly and may have been the rate- limiting step in the conversion of the 27 carbon steroid to progesterone. Solomon et al (168) previously described at a 1954 meeting a similar conversion of cholesterol to progesterone when the former steroid was added to the perfusion medium of human placentae.

E.. Adrenal function during pregnancy in the rat

Comparatively little is known regarding the influence of pregnancy on adrenal function in the rat and other species of laboratory animals. There are, however, no histological changes in the adrenals of rats during gestation which might indicate an alteration in adrenal function during pregnancy (265)(200)(291)(201). Although some workers have reported an increase in adrenal weight during pregnancy others could not find any change (292)(201). It is of interest though that adrenal glands of female rats are approximately twice as heavy as those of male rats, apparently due to the influence of oestrogens (235)(223).

In the series of rats examined in the present study the weights of the adrenals of pregnant rats were similar to those of non-pregnant female rats. In vivo and 'in vitro' experiments were carried out to determine whether changes in the secretion rates of the major gluco- and mineralocorticoid, corticosterone and aldosterone, respectively, could be demonstrated during pregnancy. These experiments included incubations under 'in vitro' conditions of adrenal glands from both pregnant and non-pregnant rats and also the collection of adrenal venous blood for over a period of 1 hour from both groups of rats under nembutal anaesthesia.

The number of experiments was limited because of the difficulty in obtaining at the same time a sufficient number of pregnant rats and non-pregnant ones of similar age. The small amount of adrenal tissue available in some of the incubations meant that the quantity of steroids secreted was measurable only within the lower limits of sensitivity of the chemical assays used.

There was usually a lapse of 1 to 2 months between incubation experiments before the appropriate number of animals became available. Extensive incubation studies with adrenal tissue of male rats carried out in this laboratory, however, have shown that the basal secretion rate of both aldosterone and corticosterone may vary over short periods of time and similar degrees of variation were observed in the present incubation studies with female rat adrenal tissue.

In 4 experiments when the adrenals were removed under ether anaesthesia the average secretion rate of corticosterone in the first period of incubation was considerably greater than that observed in the 2 succeeding periods. This was noted in both pregnant and non-pregnant groups. This high initial secretion rate of corticosterone is probably related to the finding of Sayer (284) that ether anaesthesia stimulates a secretion of ACTH by the anterior pituitary since it was not observed when nembutal was used for the adrenalectomy. The total average output of corticosterone during the 5 hour period of incubation per gram of adrenal tissue from pregnant and non-pregnant animals did not differ when the tissue was removed under ether anaesthesia but with use of nembutal it was 48.32% less in pregnant groups.

The secretion rates of aldosterone were measured in the same groups of rats. When ether was used the average secretion rate of aldosterone by adrenal tissue of pregnant rats was 47.6% greater in the first period of incubation but thereafter the differences between both groups of rats was negligible. The average total secretion of aldosterone for the 5 hour period of

incubation was 16.63% greater for the adrenals of the pregnant rats than for those of the non-pregnant animals. When nembutal was used as anaesthetic for adrenalectomy the aldosterone secretion rate of the adrenals from both groups was higher than that observed under ether. Adrenal glands of pregnant rats, furthermore, secreted amounts of aldosterone /gm./hr. which were increased by 28.5, 44.9 and 25.8% above those secreted by adrenal tissue of non-pregnant rats in the 3 succeeding periods of incubation; the average total secretion of aldosterone over a five hour period was 34.37% greater for adrenals of pregnant rats than for those of non-pregnant ones.

The average rate of secretion of aldosterone relative to that of corticosterone for adrenal tissue of pregnant rats was, therefore, only slightly greater than that observed for adrenals of non-pregnant groups when ether was used for adrenalectomy. This difference between both groups was, however, more marked with nembutal. A larger series of animals should be studied with nembutal before any conclusions could be drawn regarding differences in rates of secretion of corticosterone and aldosterone in adrenals from non-pregnant and pregnant rats. There was, however, a tendency for the secretion rates of corticosterone to decrease more rapidly with time but for those of aldosterone to be simultaneously increased in the adrenals of the pregnant rats.

In vitro findings, however, may not always reflect what is actually occurring in the intact animal since the excised tissue is removed from its blood supply which may contain substances that modify, that is, stimulate or depress, its function 'in vivo'.

For these reasons it was decided to carry out collections of adrenal venous blood.

These 'in vivo' studies required less animals and were completed within 6 weeks time. It must be pointed out, however, that this technique involves the collection of adrenal venous blood from animals which are in a state of acute stress (186). Moreover, the response to stress with respect to adrenal activity may very well differ in pregnant and non-pregnant rats. In either condition adrenal function during stress may not accurately reflect that activity in the normal animal at rest (96)(293). Singer and Stack-Dunne (186) were nevertheless still able to show a further response to ACTH when it was administered to male rats similarly treated. Also Das Gupta and Giroud (187) were able to demonstrate an increased 'in vivo' secretion of aldosterone in male rats with induced nephrosis in comparison with untreated male rats with this technique whereas others (294) could not show the same effect with incubations of adrenal glands from similar groups of rats under 'in vitro' conditions. It was hoped in the present study that if any definitive changes in adrenal function occurred during pregnancy in the rat they could similarly be demonstrated.

When 1 hour collections of adrenal venous blood from pregnant and non-pregnant rats under nembutal anaesthesia were subsequently carried out it was found that the concentration of aldosterone and corticosterone was less in the adrenal venous blood of pregnant rats than in that of non-pregnant ones. The differences in both aldosterone and corticosterone content between the 2 groups

were statistically significant when the steroid secretion rates were related to total body weight and to unit adrenal weight but were not significant when expressed simply per rat or per unit blood volume collected.

Both 'in vivo' and 'in vitro' experiments, therefore, showed a tendency for the rate of secretion of corticosterone to be decreased in pregnant rats. The aldosterone content in adrenal venous blood of pregnant rats was also decreased in comparison with that present in blood collected from non-pregnant rats. In vitro, however, there was a tendency for the average rate of secretion of aldosterone to be greater in incubations of adrenal glands from pregnant rats than in those not pregnant when the adrenal tissue was removed under membutal anaesthesia. In the latter experiments there appeared, therefore, a tendency for the average rate of secretion of aldosterone relative to that of corticosterone to be greater in the pregnant group of rats. This effect was not, however, observed in the 'in vivo' study perhaps because it may have been masked by the condition of stress in the latter experiments. This stress (despite the use of membutal anaesthesia) would induce increased secretion of ACTH. While this hormone shows some stimulation of aldosterone secretion in the rat both 'in vivo' and under 'in vitro' conditions (8) its capacity to stimulate the secretion of corticosterone is much more pronounced.

The present author knows of no similar investigations on adrenal function in the pregnant rat which may have been carried out by other workers. There is, however, a considerable amount

of literature regarding the effect of oestrogens on rat adrenal histology and function which may be pertinant to the results described above since oestrogens are known to influence adrenal function in human pregnancy.

The administration of oestrogens to male or female rats will result in adrenal hypertrophy only under certain conditions and the presence of the pituitary is necessary for this effect (265). Deanesly (229) noted that oestrogenization of male rats leads in early stages to cortical enlargement and in the latter stages to atrophic changes as a consequence of disorganization of pituitary function. Vogt (231) found that the administration of hexoestrol to rats led to a decreased secretion of corticosterone into adrenal venous blood of male rats. She postulated that this oestrogen effect was due to an inhibition of cholesterol synthesis. The latter would result in a decreased steroid synthesis and a consequent increase of stimulation to the pituitary to secrete ACTH. The final outcome would be hypertrophy and hyperplasia but diminished steroid secretion. It has subsequently been reported (293), however, that administration of oestrogen actually increases the basal secretion rate of certain corticosteroids including corticosterone when rats are kept free from any stress. This observation was made indirectly and was dependent upon the finding of increased stores of corticosteroids in adrenal tissue of unstressed rats following oestrogen administration. In conditions of stress, however, as during collections of adrenal venous blood from rats, Holzbauer (96) observed that oestrogens have an opposite effect, inducing a decreased secretion of cort-

icosterone. Whether oestrogens exert similar effects on aldosterone secretion rates was not determined.

McKerns could find no inhibition by oestradiol of ACTH stimulated rat adrenal tissue incubated under 'in vitro' conditions when oestrogens were added directly to the medium (232). When, however, oestradiol was injected into male rats for a certain period of time and the adrenals subsequently removed for incubation a decreased secretion of corticosterone was observed (233). Since ethinyl oestradiol and stilbestrol had inhibitory effects in both types of experiments the authors concluded that the effect of the natural oestrogen on adrenal function was indirect and mediated by the anterior pituitary. The latter view is supported by others (234)(235). According to Gompertz (236) the oestrogens act by influencing both hypophyseal secretion of ACTH and adrenocortical sensitivity to ACTH. But the influence which these sex hormones have on growth of the adrenal cortex in response to ACTH is apparently not associated with a compensatory effect upon secretory activity.

The tendency for the secretion rate of corticosterone to be reduced in pregnant rats in both 'in vivo' and 'in vitro' experiments carried out in the present study may be due at least in part to a similar influence of oestrogens produced in increased quantity during gestation. The observed differences in corticosteroid secretion rates between pregnant and non-pregnant female rats were, however, not pronounced and tend to suggest that there is no marked alteration in adrenal function during pregnancy in the rat. Moreover, it has been reported (205) that after the 14th

of 21 days of gestation in the rat the presence of the adrenals is not necessary for a normal delivery presumably due to a life-maintaining influence of a corpora luteal secretion which appears to be progesterone (213)(214)(215).

F. Studies with rat placenta

In the present studies rat placental tissue did not secrete aldosterone, corticosterone or any other Δ 4-3-ketonic steroids with similar polarity in measurable quantities, at least, when incubated under 'in vitro' conditions. This observation, with respect to aldosterone at any rate, would seem to agree with the report of Greep (247) and Hoffmann et al (248) that in the pregnant rat the products of conception are not a source of any substance which could influence water metabolism during gestation.

It was found also in the present study that the addition of rat placental tissue to incubations of adrenal glands obtained from male and female rats resulted in a decrease in the quantities of Δ4-3-ketonic steroids subsequently isolated from the incubation medium. This effect may have been due to a non-selective inhibition of normal respiratory processes of the adrenal tissue in the presence of relatively large amounts of foreign tissue. Another possibility to be considered was whether or not the corticosteroids secreted into the medium were being metabolized by placental tissue so that estimations of Δ4-3-ketonic steroids showed a decrease. A third factor might be a possible concentration or adsorption of corticosteroids by placental tissue involving little or no metabolism of these steroids. The

last 2 possibilities were investigated in experiments in which placental tissue was incubated with corticosterone. The latter studies suggested that if any metabolism of added corticosterone did occur its extent per unit weight of placental tissue was slight in comparison with the activity of liver tissue under similar conditions. The observed inhibiting effect of placenta on corticosteroid levels during incubation with adrenal tissue appeared to have been partly due to an adsorption or concentration of the steroids by the placental tissue. This adsorption appeared to be increased as a result of incubation. The latter observations are of interest in the light of certain findings of Curry and Beaton (250). They reported that cortisone overdosage leads to certain degenerative biochemical changes in non-pregnant rats but not to the same extent in pregnant ones. Although the placenta became decreased in weight its presence was necessary for this protective influence in pregnant rats. The authors postulated that the rat placenta may have a capacity to concentrate or metabolize adrenal hormones injected into the maternal circulation.

The failure to obtain a stimulation of adrenal cortical function in the presence of rat placental tissue in the present study agrees with the finding of Greer (243) that no ACTH is produced by the rat placenta. It is not likely that the inhibitory effect was due to the oestrogen content in this tissue since according to Selye and McKeown (245) the rat placenta is not a source of oestrogens during gestation. If progesterone, a precursor of adrenal cortical hormones, were secreted by the

placental tissue into the incubation medium one would expect to obtain larger amounts of corticosteroids during incubations with adrenal tissue rather than less. But McKeown and Zuckerman (246) have reported that the corpus luteum is the predominant source of progesterone throughout gestation in the rat whereas in the human the placenta takes over this function in the latter half of pregnancy.

G. Studies on metabolism of corticosteroids in rat pregnancy

Liver tissue from pregnant and non-pregnant female rats was incubated under 'in vitro' conditions with steroids in the present study to determine whether any difference in the rate of corticosteroid metabolism in pregnant rats could be demonstrated directly at the level of liver function. When corticosterone was used as the substrate the liver of pregnant rats was only about 10% more active in the 'in vitro' reduction of both the Δ4-3-ketone and alpha-ketol groups than was liver of non-pregnant rats. These differences although slight were of statistical significance. Pregnancy liver tissue was also 50 and 28% more active in metabolism of the Δ4-3-ketone and alpha-ketol groups, respectively, of aldosterone-21-monoacetate than was liver tissue of non-pregnant rats. Liver of both groups metabolized corticosterone to a greater extent than the aldosterone derivative when extent of reduction of either the Δ4-3-ketone or alpha-ketol groups is considered. Other investigators (72) have also found that various corticosteroid substrates are metabolized by liver tissue at different rates under 'in vitro' conditions. Unfortunately, aldosterone itself was not available in the re-

quired amounts for the present studies.

If a similarly increased activity per unit weight of liver occurs 'in vivo' in pregnant rats it might be of greater importance when the 30% increase in liver weight observed during gestation is taken into consideration. The latter increase was statistically significant and has also been observed by others (250). Berliner et al (287) have reported that loose connective tissue in the rat is capable of metabolizing corticosteroids and it may well be that this small increase in liver activity of pregnant rats, if it occurs 'in vivo' as well may be insignificant.

Yates et al (259) have reported that liver tissue from female rats when incubated 'in vitro' is from 2 to 10 times more active than male liver tissue in the reduction of ring A of a variety of corticosteroid substrates. When oestrogens were injected into male or female rats it was observed during subsequent incubations of their liver tissue that the capacity to reduce corticosteroids became increased. Administration of testosterone to either sex of rats had the opposite effect. The authors concluded that oestrogens induced formation of the hepatic enzymes which catalyse the reactions involved in the metabolism of the ring A in steroids. It may be that an increased elaboration of oestrogens in pregnancy was similarly implicated in the slight increase in extent of corticosteroid metabolism by incubated liver tissue of pregnant rats in comparison with that of non-pregnant female rats which was observed in the present study. At variance with the above hypothesis is the observation

of Glenn as reported by Cohen et al (50) that oestrogens inhibited the degradation of hydrocortisone when both steroids were incubated with a rat liver microsome supernatant fraction. The conflicting results may have been due to the different liver preparations used for incubation but are probably better explained in the light of the following observations. When thyroid hormone is administered to rats the reducing activity of liver tissue becomes subsequently increased when incubated 'in vitro' with corticosteroids (260). This effect of thyroxin is presumably due to a stimulation of synthesis of hepatic enzymes which regulate the reduction of the Δ4-3-ketone grouping of steroids. According to Badawi and Soliman (295) oestrogens when administered to male castrated rats induce an increased secretion of thyrotropin which in turn stimulates the production of thyroxin. It may be, therefore, that the oestrogen effect observed by Yates et al (259) was an indirect one mediated by thyroid hormone.

If this is so it might explain why opposite results were obtained (50) when oestrogens were added directly to the incubation medium. Moreover, since thyroid activity is apparently increased in pregnancy (296) this may account for the increased reducing capacity observed in the present study with excised liver tissue from pregnant rats. Pregnancy liver tissue may contain greater levels of thyroid hormone than those present in the liver of non-pregnant rats.

SUMMARY AND CONCLUSIONS

A. Incubations of Human Placentae

Human placentae obtained at caesarian section were minced and incubated in Krebs-Ringer solution with the aim of demonstrating a production of aldosterone and other corticosteroids under 'in vitro' conditions.

Seven UV light absorbing compounds were isolated from the incubation media. These included cortisone, hydrocortisone, progesterone and 4 unidentified compounds. No detectable amounts of aldosterone, however, could be obtained from the quantities of tissue used.

The trophic hormones chorionic gonadotrophin, adrenocorticotrophin and prolactin did not appear to stimulate the production of progesterone or of the measured corticosteroids since their separate addition to the incubation medium of placental tissue did not increase the concentration of these steroids above control levels.

The quantity of all steroids isolated from the incubation media decreased with time of incubation. In the experimental system used, progesterone was not elaborated from any steroid precursors present in the placentae at the time of caesarian section since the total quantity of progesterone was not increased during the incubation of placental tissue above the amount which could be obtained merely by extraction. When, however, known precursors of adrenal cortical hormones, cholesterol and $\Delta 5$-pregnene-3β-ol-20-one, were added to the incubations the pro-

gesterone content was increased above control levels., these increases corresponded to an 'in vitro' production of progesterone at the rate of 31.33 and 357.79 ug./100 gm. tissue/hr., respectively.

The more polar steroids, cortisone and a compound with chromatographic mobility similar to that of aldosterone X_1, were also not increased in quantity during incubation of placental tissue alone above the amount which could be obtained simply by extraction of this tissue without prior incubation. Unlike progesterone, however, there was still no increase above control levels upon the addition of cholesterol and Δ 5-pregnene-3βol-20-one to the incubation medium. This means that there was no production of cortisone or of compound X_1 under any of the 'in vitro' experimental conditions employed. While the quantities of compound X_1 were unchanged during a 3 hour incubation period some of the placental cortisone was apparently converted to hydrocortisone; the extent of this conversion appeared to depend, in part, on the initial concentration of cortisone. In one experiment there appeared also to be a metabolism of cortisone to more polar alpha-ketolic steroids during a prolonged incubation of placental tissue.

The reaction involving conversion of cortisone to hydrocortisone could, however, be reversed since upon the addition of relatively large quantities of hydrocortisone to the incubation media of 2 placentae a significant conversion of this steroid to cortisone was observed. If the placenta is not elaborating corticosteroids 'in vivo' the presence of larger quantities of cort-

isone than of hydrocortisone in this organ could be due to a similar conversion of hydrocortisone, the predominant 17-hydroxycorticosteroid in the maternal circulation in late pregnancy, to cortisone, 'in vivo'.

B. The Excretion of Corticosteroids and Metabolites of Progesterone in Normal and Adrenalectomized Pregnant and Non-Pregnant Women.

Two totally adrenalectomized patients receiving corticosteroid replacement therapy and one normal woman were studied in the last trimester of gestation. Each case showed total urinary 17-hydroxycorticosteroid levels within the range for normal non-pregnant women. Moreover, the pattern of urinary corticosteroids with respect to both the Δ 4-3-ketonic and alpha-ketolic steroid components was in the normal pregnant subject qualitatively similar to those obtained for non-pregnant normals. The adrenalectomized women also showed corticosteroid excretion patterns which with respect to the $C_{21}O_5$ alpha-ketolic steroids were qualitatively similar to those observed for normal subjects. It would be reasonable to assume that the steroids present in the urine of these patients were derived from the hormone therapy administered. One case, however, also excreted relatively large amounts of less polar alpha-ketolic steroids in the 9th month of pregnancy. The latter compounds may have been derived from regenerated adrenal tissue rather than from the exogenous hormone therapy since they did not appear in comparable quantities in the urine of other similarly treated adrenalectomized patients.

Three normal subjects excreted quantities of cortisone plus hydrocortisone which ranged from 1/8th to 1/12 of those of compounds THF plus THE while a normal pregnant woman excreted relatively more of these free steroids, the amount being 1/4th of those of the reduced metabolites. Even greater proportions of the free 17-hydroxycorticosteroids relative to those of their ring A reduced derivatives appeared in the urine of the adrenalectomized patients; this further change from the normal non-pregnancy pattern, however, may have not been due entirely to the state of pregnancy, but in part, also, to the character of steroid therapy administered. The studies tended to suggest that a reduced rate of reduction of the Δ 4-3-ketone grouping in ring A of 17-hydroxycorticosteroids occurs during pregnancy. One of the adrenalectomized women continued to show a similar pattern of corticosteroid metabolism 4 days after delivery.

There appeared to be no extra-adrenal source of aldosterone in one adrenalectomized pregnant woman studied in the 6th, 8th and 9th months of gestation. Four days post-partum she continued to excrete only less than 1.5 ug. of Na-retaining hormone per gm. of creatinine. A similarly negligible quantity was excreted by a non-pregnant adrenalectomized patient. But a determination on another adrenalectomized woman in her 9th month of pregnancy showed the presence of 13 ug. of Na-retaining hormone per gm. creatinine, in the urine. This value although well below the amount observed in normal pregnancy urine in the last trimester is still appreciable being more than the quantity excreted by non-pregnant normal individuals. This excretion of aldosterone as

well as the urinary corticosteroid pattern in this particular patient suggest the presence of regenerated adrenal tissue.

Pregnanediol determinations in the 2 adrenalectomized pregnant patients were within the range observed for normal pregnancy. The sharp decrease in pregnanediol excretion observed in one of these cases 4 days post-partum is also seen after termination of normal gestation.

There was considerable variation in the excretion of pregnane-3α, 17α, 20α- triol in the latter patients during pregnancy but this metabolite could not be detected in the urine 4 days after delivery in one case.

C. Adrenal Function During Late Pregnancy in the Rat

Experiments were carried out to determine whether changes in the secretion rate of corticosteroids could be demonstrated during late gestation in the rat. The studies included the incubation of adrenal glands from pregnant and non-pregnant female rats under 'in vitro' conditions; in 4 experiments adrenals were removed under ether anaesthesia and, in 2 others, under nembutal. In vivo investigations involving the collection of adrenal venous blood for a period of 1 hour from pregnant and non-pregnant female rats under anaesthesia were subsequently carried out. Both types of experiments were limited in number due to the difficulty of obtaining a sufficient number of pregnant rats and non-pregnant ones of similar age; it was, therefore, not established

conclusively whether or not adrenal function is altered during pregnancy in the rat. Nevertheless, a decrease in the secretion rate of corticosterone in pregnant rats was observed in the 'in vivo' study and also under 'in vitro' conditions when the adrenal tissue had been removed under nembutal anaesthesia. This latter difference between pregnant and non-pregnant rats was, however, slight when ether anaesthesia was used for adrenalectomy. Under ether anaesthesia the rate of secretion of corticosterone in adrenals of both groups of rats was considerably higher in the 1st period of incubation than in the 2 succeeding ones. This high initial secretion of corticosterone may have been due to the stimulation of adrenal tissue by ACTH which is secreted in increased amounts during ether anaesthesia.

The aldosterone content in adrenal venous blood of the pregnant animals was also decreased in comparison with that present in blood collected from non-pregnant rats. The adrenal glands of pregnant rats under 'in vitro' conditions showed, however, a tendency to secrete higher amounts of aldosterone than those from non-pregnant rats when the adrenals were removed under nembutal anaesthesia. This effect was less pronounced with ether anaesthesia. Under 'in vitro' conditions there appeared, therefore, a tendency for the average rate of secretion of aldosterone relative to that of corticosterone to be greater in the pregnant group of rats than in the non-pregnant ones. This latter effect was not, however, observed in the in vivo study, perhaps because of the condition of acute stress under which adrenal venous blood is collected. This stress would result in increased secretion of

ACTH which in turn would stimulate the secretion of corticosterone to a greater extent than that of aldosterone.

The observed differences in corticosteroid secretion rates between pregnant and non-pregnant female rats were, however, not pronounced and tend to suggest that there is no marked alteration in adrenal function during late pregnancy in the rat.

Adrenal weights of pregnant rats were similar to those observed for non-pregnant female rats. Adrenal glands of the females were, however, approximately twice as heavy as those of male rats in the same colony.

D. Incubations of Liver Tissue from Pregnant and Non-Pregnant Female Rats with Corticosteroids

The purpose of these experiments was to determine whether a difference in the rate of corticosteroid metabolism could be demonstrated in pregnancy at the level of liver activity.

When corticosterone was used as substrate in the incubations the liver of pregnant rats was 10% more active in the 'in vitro' reduction of both the Δ4-3-ketone and alpha-ketol groups than was liver tissue of non-pregnant rats. These differences although very slight were of statistical significance. Liver of both groups of rats metabolized the Δ4-3-ketone grouping of corticosterone to a greater extent than the alpha-ketol configuration.

Pregnancy liver tissue was also 50 and 28% more active in the reduction of the Δ4-3-ketonic and alpha-ketolic groups, respectively, of aldosterone-21-monoacetate than was similarly incubated liver tissue from non-pregnant female rats.

Liver tissue from both groups of rats metabolized corticosterone to a greater extent than the aldosterone derivative when the amount of reduction of either the Δ 4-3-ketone or alpha-ketol groups is considered.

Pregnancy liver tissue was, therefore, more active in the metabolism of corticosteroids under 'in vitro' conditions than was that of non-pregnant female rats. Although the observed differences in activity per unit weight of liver tissue were small they were statistically significant and may be more important when an average 30% increase in liver weight found in pregnant rats is taken into consideration.

E. Studies with Rat Placenta under In Vitro Conditions

Rat placental tissue did not secrete aldosterone, corticosterone or any other Δ 4-3-ketonic steroid of similar polarity in measurable quantity during incubations under the 'in vitro' conditions employed.

The addition of rat placental tissue to incubations of adrenal glands obtained from male or female rats resulted in a decrease in the quantities of Δ4-3-ketonic steroids isolated from the incubation media. This effect was greater when larger quantities of placental tissue were added.

Subsequent incubations of placental tissue with corticosterone suggest that the above effect of placental tissue may have been partly due to an adsorption or concentration of corticosteroids by this tissue. This adsorption appeared to be increased as a result of incubation. Any metabolism of corticosterone per

unit weight of placental tissue which may have occurred was slight in comparison with that observed with rat liver tissue under similar incubation conditions.

CLAIMS OF ORIGINALITY

The demonstration of an interconversion of cortisone and hydrocortisone during 'in vitro' incubations of human placentae obtained at caesarian section is an original observation. The trophic hormones ACTH, prolactin and chorionic gonadotrophin have not been previously incubated with placental mince.

The presented studies on corticosteroid production and metabolism in pregnant rats are also original. These include incubations under 'in vitro' conditions of placenta and liver tissue removed from pregnant rats. Investigations of adrenal cortical activity 'in vivo' and 'in vitro' in pregnant rats have not been previously reported.

BIBLIOGRAPHY

1. Giroud, C.J.P., J. Stachenko and E.H. Venning
   Proc. Soc. Exper. Biol. & Med.
   92:154, 1956.

2. Giroud, C.J.P., M. Saffran, A.V. Schally and E.H. Venning
   Proc.Soc. Exper.Biol. & Med. 92:855,1956.

3. Farrell, G.L., E.W. Rauschkolb and P.C. Royce.
   Am. J. Physiol. 182:269, 1955.

4. Singer, B. and M.P. Stack-Dunne.
   Nature 174:790, 1954.

5. Singer, B. and M.P. Stack-Dunne.
   J. Endocrinol. 12:130, 1955.

6. Venning, E.H., I. Dyrenfurth and J.C. Beck.
   J. Clin. Endocrinol. 16:1541, 1956.

7. Venning, E.H., I. Dyrenfurth, C.J.P. Giroud and J.C. Beck.
   Canad. M. A. J. 77:773, 1957.

8. Venning, E.H., O.J. Lucis, I. Dyrenfurth and J.C. Beck
   Proc. 40th mtg. Endocrine Soc. 1958,p.58.

9. Luetscher, J.A. Jr.
   Recent Progr. Hormone Res. 12: 175, 1956.

10. Beck, J.C., I. Dyrenfurth, C.J.P. Giroud and E.H.Venning.
    A.M.A. Arch. Int.Med. 96:463, 1955.

11. Leaf, A., F.C. Bartter, R.F. Santos and O. Wrong.
    J.Clin.Invest. 32:868, 1953.

12. Liddle,G.W., F.C. Bartter, L.E. Duncan, Jr., J.K. Barber and C. Delea. J. Clin. Invest. 34:949, 1955.

13. Ayres, P.J., O. Garrod, S.A.S. Tait and J.F. Tait.
    An International Symposium on Aldosterone Edited by A.F. Muller and C.M. O'Connor, J & A. Churchill,Ltd., London, 1958, p.143.

14. Rauschkolb, E.W. and G.L. Farrell.
    Endocrinology 59:526, 1956.

15. Swann, H.G. Physiol. Rev. 20:493, 1940.

16. Mason, H.I. M.H. Power, E.H. Rynearson, L.C. Ciaramelli, Choh Hao Li and H.M. Evans.
J. Clin. Endocrinol. 8:1, 1948.

17. Knobil, E., A. Morse, F.G. Hofmann and R.O. Greep.
Acta endocrinol. 17:229, 1954.

18. Dempsey,R.W. and G.B. Wislocki.
Endocrinology 35:409, 1944.

19. Wislocki, G.B. and H.S. Bennett.
Am. J. Anat. 73:335, 1943.

20. Venning, E.H. Endocrinology 39:203, 1946.

21. Tobian,L.Jr. J. clin. Endocrinol. 9:319, 1949.

22. Gemzell, C.A. J. Clin. Endocrinol. 13:898, 1953.

23. Garst, J.B. and N.S. Assali.
Proc. Soc. Exper. Biol. & Med. 92:815, 1956.

24. Christy, N.P., E.Z. Wallace and J.W. Jailer.
J.Clin. Invest. 34:899, 1955.

25. Venning, E.H. and I. Dyrendfurth.
J. Clin. Endocrinol. 16:426, 1956.

26. Venning, E.H., T. Primrose, L.C.S. Caligaris and I. Dyrenfurth. J. Clin. Endocrinol. 17:473, 1957.

27. Genest, J., W. Nowaczynski, E.,Koiw and B. Vitye.
Clin. Res. Proc. 5:15, 1957.

28. Martin,J.D. and I.H. Mills. Brit. M.J. 2:571, 1956.

29. Rinsler,M.G. and B. Rigby. Brit. M.J. 2:966, 1957.

30. Laidlaw,J.C., M. Cohen and A.G. Gornall.
J. Clin. Endocrinol. 18:222, 1958.

31. Hench,P.S. Proc. Staff Meet. Mayo Clinic. 13:161, 1938.

32. Hench,P.S., E.C. Kendall, C.H.Slocumb and H.F.Polley.
Proc. Staff Meet. Mayo Clinic 24:181, 1949.

33. Robinson,H.J., W.G.Bernhard, H.Grubin, H.Wanner, G.W. Sewekow and R.H. Silber. J. Clin. Endocrinol. 15:317, 1955.

34. French,H. Lancet 1:1398, 1908.

35. Fitzpatrick,G. Surg. Gynec. Obstet. 35:72, 1922.

36. Perkins,P.A. J.Amer. Med. Ass. 99:,1500 1932.

37. Sheldon,D.E. Am. J. Obstet. & Gynec. 42:269, 1945.

38. Samuels,L.T., G.T.Evans and J.L.McKelvey.
Endocrinology 32:422, 1943.

39. Cohen,M. Arch. Int. Med. 81:879, 1948.

40. Jailer,J.W. and A.I.Knowlton.
J. Clin. Invest. 29:1430, 1950.

41. Hunt,A.B. and W.M.McConakey.
Am. J. Obst. & Gynec. 66:970, 1953.

42. Hills,A.G., E.H.Venning, F.C.Dohan, G.D.Webster and E.M.Richardson. J. Clin. Invest. 33:1466, 1954.

43. Gray,C.H. and J.B.Lunnon. J. Endocrinol 13::XIX, 1956.

44. Halberg,E. and I.H.Kaiser. Acta endocrinol. 16:227, 1954.

45. Knowlton, A.I., G.H.Mudge and J.W.Jailer.
J. Clin. Endocrinol. 9:514, 1949.

46. Migeon,C.J., H.Prystowsky, M.M.Grumbach and M.C.Byron.
J. Clin. Invest. 35:488, 1956.

47. Beaulieu,E.E., H. Bricaire and M.F. Jayle.
J. Clin. Endocrinol. 16:690, 1956.

48. Beaulieu,E.E., M. deVigan, H.Bricaire and M.F.Jayle.
J. Clin. Endocrinol. 17:1478, 1957.

49. Little,B., O.W.Smith, A.G.Jessiman, H.A.Selenkow, W. van't Hoff, J.M.Eglin and F.D.Moore.
J. Clin. Endocrinol. 18:425, 1958.

50. Cohen,M., M.Stiefel, W.J.Reddy and J.C.Laidlaw.
J. Clin. Endocrinol. 18:1076, 1958.

51. Appleby,J.I. and J.K.Norymberski.
J. Endocrinol. 15:310, 1957.

52. Christy,N.P., W.E.L.Gordon, D.Longson, E.Z.Wallace and J.W.Jailer. Proc. 40th Mtg. Endocrine Soc. 1958 p.14.

53. Gemzell,C.A. Acta endocrinol. 17:100, 1954.

54. McKay,E., N.S.Assali and M.Henley.
Proc. Soc. Exper. Biol. & Med. 95:653, 1957.

55. Andersen,D.H. and H.S.Kennedy. J. Physiol. 77:159, 1933.

56. Whitely,H.J. and H.B.Stoner. J. Endocrinol. 14:325, 1957.

57. Jailer,J.W. Obst. & Gynec. Surv. 12:14, 1957.

58. Sandberg,A.A., K.Eik-Nes, C.J.Migeon and L.T.Samuels. J. Clin. Endocrinol. 16:1001, 1956.

59. Peterson,R.E., J.B.Wyngaarden, L.G.Serafin, B.B.Brodie and J.J.Bunim. J. Clin. Invest. 34:1779, 1955.

60. Burstein,S., K.Savard and R.I.Dorfman Endocrinology 53:88, 1953.

61. Burstein,S., K.Savard and R.I.Dorfman Endocrinology 52:448, 1953.

62. Burton,R.B., E.H.Kentmann and C.Waterhouse. J. Clin. Endocrinol. 13:48, 1953.

63. Mason,H.L. J. Biol. Chem. 172:783, 1948.

64. Engel,L.L., P.Carter and L.D.Fielding. J. Biol. Chem. 213:99, 1955.

65. Engel,L.L., P.Carter and M.J.Springer. Fed. Proc. 13:204, 1954.

66. Block,E., K.Benirschke and E.Rosemberg. Endocrinology 58:626, 1956.

67. Touchstone,J.C., H.Bulaschenko, E.M.Richardson and F.C.Dohan. J. Clin. Endocrinol. 17:250, 1957.

68. Richardson,E.M., J.C.Touchstone and F.C.Dohan. J. Clin. Invest. 34:285, 1955.

69. Eberlein,W.R. and A.M.Bongiovanni. J. Clin. Endocrinol. 15:1531, 1955.

70. Ulick,S. and S.Lieberman. J. Amer. Chem. Soc. 79:6567,1957.

71. Gomez Mont,F. and D.Berliner. Gaceta Med. Mexico 83:413, 1953.

72. Reaven,G.M. Endocrinology 57:580, 1955.

73. Gordon,E.S. J. Lab. & Clin. Med. 44:803, 1954.

74. Talalay,P. Physiol. Rev. 37:362, 1957.

75. Tomkins,G.M. and K.J.Isselbacher.
J. Amer. Chem. Soc. 76:3100, 1954.

76. Tomkins,G.M. Recent Progr. Hormone Res. 12:125, 1956.

77. Tomkins,G.M. J. Biol. Chem. 225:13, 1957.

78. Isselbacher,K.J. Recent Progr. Hormone Res. 12:134, 1956.

79. Schneider,J.J., M.L.Lewbart, P.Levitan and S.Lieberman.
J. Amer. Chem. Soc. 77 4184, 1955.

80. Gregory,J.D. and Y.Nose. Fed. Proc. 16:189, 1957.

81. Schneider,J.J. and M.L.Lewbart. J. Biol. Chem. 222:787, 1956.

82. Savard,K. and S.H.Goldfaden. Fed. Proc. 13:288, 1954.

83. Ungar,F., J.W.Davis, H.Rosenkrantz and R.I.Dorfman.
J. Biol. Chem. 207:375, 1954.

84. Glenn,E.M. and R.O.Recknagel.
Proc. Soc. Exper. Biol. & Med. 89:153, 1955.

85. Hubener,H.J., D.K.Fukushima and F.T.Gallagher.
J. Biol. Chem. 220:499, 1956.

86. DeCourcy,C. and J.J.Schneider.
J. Biol. Chem. 223:865, 1956.

87. Sandberg,A.A., E.Chang and W.R.Slaunwhite,Jr.
J. Clin. Endocrinol. 17:437, 1957.

88. Rosselet,J.P., J.W.Jailer and S.Lieberman
J. Biol. Chem. 225:977, 1957.

89. Migeon,C.J., J.Bertrand and P.E.Wall.
J. Clin. Invest. 36:1350, 1957.

90. Mills,I.H. Proc. 39th Mtg. Endocrine Soc. 1957, p.27.

91. Bongiovanni,A.M., W.R.Eberlein, M.Westphal and T.Boggs.
J. Clin. Endocrinol. 18:1127, 1958.

92. Diczfalusy,E., K.G.Tillinger and A. Westman.
Acta endocrinol. 26:303, 1957.

93. Taliaferro,I., F.Cobey and L.Leone.
Proc. Soc. Exper. Biol. & Med. 92:742, 1956.

94. Wallace,E.Z., H.I.Silverberg and A.C.Carter.
Proc. Soc. Exper. Biol. & Med. 95:805, 1957.

95. Uotila,U.U. Anat. Rec. 76:183, 1940.

96. Holzbauer, M., J. Physiol. 139:306, 1957

97. Keene, M.S.L. and E.E. Hewer. J. Anat. 61:324, 1927

98. Staemler,H.J. Arch. Gynak. 182:521, 1953.

99. Forbes,I.R. Endocrinology 56:699, 1955.

100. Personal communication.

101. Ulstrom,R.A., E.Colle and J.Reynolds.
Proc. 40th Mtg. Endocrine Soc. 1958, p.120.

102. Nichols,J., O.L.Lescure and C.J.Migeon.
J.Clin. Endocrinol. 18:444, 1958.

103. Lanman,J.T. and L.M.Silverman
Endocrinology 60:433, 1957.

104. Plotz,E.J. and M.E. Davis.
Proc. Soc. Exper. Biol. & Med. 195:92, 1957.

105. Venning,E.H. Brit. M. Bull. 11:40, 1955.

106. Davis,M.E. and E.J.Plotz. Acta endocrinol. 21:245, 1956.

107. Birke,G., C.A.Gemzell, L.O. Plantin and H.Robbe
Acta endocrinol. 27:389, 1958.

108. Kaiser,I.H. J. Clin. Endocrinol. 16:1251, 1956.

109. Kinnunen,O. Acta endocrinol. 17:226, 1954.

110. Migeon,C.J., A.R.Keller and E.Holmstron.
Bull. Johns Hopkins Hosp. 97:415, 1955.

111. Gersh,I. and A.Grollman, Am. J. Physiol. 126:368, 1939.

112. Carnes,W.H. Proc. Exper. Biol. & Med. 45:502, 1940.

113. Wilkins,L., W. Fleischmann and J.E. Howard
Endocrinology 26:385, 1940

114. Benirschke,K., E.Block and A.T.Hertig.
Endocrinology 58:598, 1956.

115. Block,E., K.Benirschke and R.I. Dorman.
J. Clin. Endocrinol. 15:379, 1955.

116. Klein,R., P.Taylor,Z.Laron, C.Papadotos, J.Fortunato and C.Byers. Proc. 39th Mtg. Endocrine Soc. 1957, p.61.

117. Gardner,L.I. and R.L.Walton.
Helvet. paediat. Acta. 9:311, 1954.

118. Wislocki,G.B. and E.W.Dempsey.
Anat. Rec. 123:133, 1955.

119. Wislocki,G.B. and E.W.Dempsey. Am.J. Anat. 83:1, 1948.

120. Wislocki,G.B., E.W.Dempsey and D.W.Fawcett.
Obst. & Gynec. Surv. 5:604, 1948.

121. Lanman,J.T. Endocrinology 61:684, 1957.

122. Borell,U. Acta endocrinol. 17:13, 1954.

123. Gey,G.O., G.E.Seegar Jones and L.M.Hellman.
Science 88:306, 1938.

124. Lanman,J.T. and J.Dinerstein.
Proc. 40th Mtg. Endocrine Soc. 1958,p.29.

125. Stewart,H.L., M.D.Machteld, E.Sano and T.L.Montgomery.
J. Clin. Endocrinol. 8:175, 1948.

126. Diczfalusy,E. Acta endocrinol. Supplementum XII 1953.

127. Pincus,G. Transactions, 3rd conference, The Josiah Macy, Jr. Foundation, p.91, 1956.

128. Clayton,B.E. and J.E.Hammant.
J. Endocrinol. 15:255, 1957.

129. Rosenfeld,G. and W.D.Bascon. Endocrinology 59:497, 1956.

130. Troen,P. and E.E.Grodon. J. Clin. Invest. 37:1516, 1958.

131. Badinand,A., R.Mallein and J.Cotte.
C.R. Soc. Biol. (Paris) 147:323, 1953.

132. Assali,N.S. and J.Hamermesz. Endocrinology 55:561, 1954.

133. Sulman,F.G. and F.Bergmann.
J. Obst. & Gynaec. Brit.Emp. 60:123, 1953.

134. Fraenkel,E. Arch. Gynak. 68:438, 1903.

135. Loeb,L. Zentralbl. Physiol. 22:498, 1908.

136. Loeb,L. Zentralbl. Physiol. 23:73, 1909.

137. Corner,G.W. and W.M.Allen. Am. J. Physiol. 88:340, 1929.

138. Butenandt,A., V.Westphal and W.Hohlweg.
Ztschr. physiol. chem. 227:84, 1934.

139. Hartmann,M. and A.Wettstein.
Helvet. chem. acta. 17:878, 1365, 1934.

140. Wintersteiner,O. and W.M.Allen. J. Biol. Chem. 107:321, 1934.

141. Marrian,G.F. Biochem. J. 23:1090, 1929.

142. Butenandt,A. Ber. dtsch. chem. Ges. 63:659, 1930.

143. Butenandt,A. Ber. dtsch. chem. Ges. 64:2529, 1931.

144. Venning,E.H. and J.S.L. Browne.
Proc. Soc. Exper. Biol. & Med. 34:792, 1936.

145. Venning,E.H. and J.S.L. Browne.
Endocrinology 21:711, 1937.
" 27:707, 1940.

146. Klopper,A., J. Obst. & Gynaec. Brit. Emp. 64:504, 1957.

147. Klopper,A., J.A. Strong and L.R. Cook.
J. Endocrinol. 15:180, 1957.

148. Bergstrand, C.G. and C.A.Gemzell.
J. Clin. Endocrinol. 17:870, 1957.

149. Venning,E.H. Transactions, 3rd conference, The Josiah Macy, Jr. Foundation, p.71, 1956.

150. Plotz,E.J. and M.E.Davis. Acta endocrinol. 21:259, 1956.

151. Hooker,C.W. and T.R.Forbes. Endocrinology 41:158, 1947.

152. Forbes,T.R. Endocrinology 49:218, 1951.

153. Fujii,K., K. Hoshino, I. Aoki and J. Yao.
Bull. Tokyo med. dent. Univ. 3:225, 1956.

154. Sommerville,I.F., J. Clin. Endocrinol. 17:317, 1957.

155. Sommerville,I.F. and G.N.Deshpande.
J. Clin. Endocrinol. 18:1223, 1958.

156. Pearlman,W.H. and M.Thomas. Endocrinology 52:590, 1953.

157. Haskins,A.L., Am. J. Obst. & Gynec. 67:330, 1954.

158. Pearlman,W.H. and E. Cerceo. J. Biol. chem. 198:79, 1952.

159. Pearlman,W.H. Biochem. J. 67:1, 1957.

160. Salhanick,H.J., J.E.Jones and D.Berliner.
Fed. Proc. Abstract. no. 1356, 1956.

161. Short,R.V. J. Endocrinol. 15:i, 1957. abstract.

162. Zander,J., T.R.Forbes,A.M. Von Munstermann and R.Neher.
J. Clin. Endocrinol. 18:337, 1958.

163. Samuels,L.T., M.L.Helmreich, M.B.Lasater and H. Reich.
Science 113:490, 1950.

164. Nissim,J.A. and J.M. Robson. J. Endocrinol. 8:329, 1952.

165. Pearlman,W.H., E. Cerceo and M.Thomas.
J. Biol. Chem. 208:231, 1954.

166. Mamoli,L. Ber. dtsch. chem. Ges. 71:2701, 1938.

167. Perlman,D. Science 115:529, 1952.

168. Solomon,S., A.L.Levy, R.Vande Wiele and S.Lieberman.
Proc. 126th Mtg. Amer. Chem. Soc, 1954
abstract 29C.

169. Berliner,D.L. and H.A.Salhanick.
J. Clin. Endocrinol. 16:903, 1956.

170. Herrmann,W. and L.Silverman. J. Clin. Endocrinol.
17:1482, 1957.

171. Herrmann,W. Proc. 39th Mtg. Endocrine Soc., 1957, p.26.

172. Hagopian,M., G.Pincus, J.Carlo and E.B. Romanoff.
Endocrinology 58:387, 1956.

173: DeCourcy,C., C. Gray and J.B.Lunnon.
Nature 170:494, 1952.

174. Johnson,R.H. and W.J.Haines. Science 116:456, 1952.

175. Little,B. and E.Rossi. Endocrinology 61:109, 1957.

176. Majnarich,J.J. and R.N.Dillon.
Arch. Biochem. and Biophysics. 49:247, 1954.

177. Berliner,D.L., J.E.Jones and H.A.Salhanick.
J. Biol. Chem. 223:1043, 1956.

178. Salhanick,H.A., L.M.Neal and J.P.Mahoney.
J. Clin. Endocrinol. 16:1120, 1956.

179. Villee,C.A. and J.M.Loring. Proc. 39th Mtg. Endocrine Soc., 1957, p.94.

180. Levitz,M., G.P.Condon and J.Dancis.
Endocrinology 58:377, 1956.

181. Ryan,K.J. Proc. 40th Mtg. Endocrine Soc., 1958.

182. Brown,H. and C.Migeon. J. Clin. Endocrinol. 16:1227, 1956.

183. Landau,R.L. and K.Lugibihl. J. Clin. Endocrinol. 18:1237, 1958.

184. Troen,P. Proc. 50th. Mtg. Amer. Soc. Clin. Invest., 1958.

185. Bush,I.E., J. Endocrinol. 9:95, 1953.

186. Singer,B. and M.P.Stack-Dunne. J. Endocrinol. 12:130,1955.

187. Das Gupta,D. and C.J.P. Giroud.
Proc. Soc. Exper. Biol. & Med. 98:334, 1958.

188. Rosenman,R.H., S.St.George,C.Freed and M.K. Smith.
J. Clin. Invest. 34:1726, 1955.

189. Reif, A.E. and B.B. Longwell. Endocrinology 62:573,1958.

190. Masson,G.M.C., S.B.Koritz and F.G.Peron.
Endocrinology 62:229, 1958.

191. Saffran,M. and M.J. Bayliss. Endocrinology 52:140, 1953.

192. Saffran,M. and A.V.Schally. Endocrinology 56:523, 1955.

193. Elliott, I.H. and A.V. Schally.
Canad. J. Biochem. & Physiol. 33:174, 1955.

194. Schonbaum,E., M.K. Birmingham, and M. Saffran.
Canad. J. Biochem. & Physiol. 34:527, 1956.

195. Birmingham,M.K., K.McKenns and E.Kurlents.
Proc. 39th Mtg. Endocrine Soc., 1957, p.56.

196. Guieysse,A. C.R. Soc. Biol. (Paris) 51:898, 1899.

197. Watrin,J. C.R. Soc. Biol. (Paris) 82:1405, 1919.

198. Tamura,Y. Brit. J. exp. Biol. 4:81, 1926.

199. Donaldson,J.C. Proc. Soc. Exper. Biol. & Med. 21:157, 1923.

200. Blumenfeld,C.M. Endocrinology 18:367, 1934.

201. Christianson,M. and I.C.Jones. J. Endocrinol. 15:17, 1957.

202. Rogoff,J.M. and G.N. Stewart. Am. J. Physiol. 79:508, 1927.

203. Carr,J.L. Proc. Soc. Exper. Biol. & Med. 29:128, 1931.

204. Corey,E.L. Physiol. Zool 1:147, 1928.

205. Davis,M.E. and E.J. Plotz. Endocrinology 54:384, 1954.

206. Lewis,J.T. Am. J. Physiol. 64:503, 1923.

207. Ingle, D.J. and G.T. Fisher. Proc. Soc. Exper. Biol. & Med. 39:149, 1938.

208. Wyman,L.C. Am. J. Physiol. 86:528, 1928.

209. McKeown,T. and W.R.Spurrell. J. Physiol 98:255, 1940.

210. Swingle,W.W., W.M.Parker, A.R.Taylor, H.W.Hays and J.A. Morrell. Am. J. Physiol. 119:675, 1937.

211. Collings,W.D. Endocrinology 28:75, 1941.

212. Emery,F.E. and E.L.Schwabe. Endocrinology 20:550, 1936.

213. Wells,J.A. and R.R. Greene. Endocrinology 25:183, 1939.

214. Schwabe,E.L. and F.E. Emery. Proc. Soc. Exper. Biol. & Med. 40:383, 1939.

215. Greene,R.R. Proc. Soc. Exper. Biol. & Med. 40:83, 1939.

216. Corey,E.L. Am. J. Physiol. 132:446, 1941.

217. Winter,C.A. and F.E. Emery. Anat. Rec. 66:401, 1936.

218. Hatai,S., J. Exper. Zool. 18:1, 1915.

219. Blumenfeld,C.M. Endocrinology 24:723, 1939.

220. Smith,D.E. Am. J. Physiol. 146:133, 1946.

221. Zalesky,M. Anat. Rec. 65:467, 1936.

222. Deanesley,R. Proc. Roy. Soc. Brit. 103:523, 1928.

223. Andersen,D.H. J. Physiol. 83:15, 1934.

224. Ellison,E.T. and J.C. Burch. Endocrinology 20:764, 1936.

225. Leiby,G.M. Proc. Soc. Exper. Biol. & Med. 31:15, 1933.

226. Heskett, B.F. and J.W.Hoffman.
Quart. Bull. Northwestern Univ. M.School
17:203, 1943.

227. Vogt,M., J.Physiol. 104:60, 1945.

228. Smith,O.W. Endocrinology 40:116, 1947.

229. Deanesley,R., J. Endocrinol. 1:36, 1939.

230. Allen,B.M. and H.Bern. Endocrinology 31:586, 1942.

231. Vogt,M. J. Physiol. 130:601, 1955.

232. McKerns,K.W. Endocrinology 60:130, 1957.

233. McKerns,K. and E. Kaleita.
Proc. 40th Mtg. Endocrine Soc., 1958, p.32.

234. Selye,H., J.B. Collip and D.L. Thomson.
Proc. Soc. exp. Biol., N.Y., 32:1377, 1935.

235. Carter,S.B., J. Endocrinol. 13:161, 1956.

236. Gompertz,D., J. Endocrinol. 17:107, 1958.

237. Josimovich,J.B., A.J. Ladman and H.W. Deane.
Endocrinology 54:627, 1954.

238. Cohen,A., C.R. Soc. Biol. (Paris) 148:321, 1954.

239. Blackman,S.S. Bull. Johns Hopkins Hosp. 78:180, 1946.

240. Tobin, C.E. Am. J. Anat. 65:151, 1939.

241. Wislocki, G.B. and E.W. Dempsey.
Anat. Rec. 123:33, 1955.

242. Astwood,E.B. and R.O. Greep.
Proc. Soc. Exper. Biol. & Med. 38:713,1938.

243. Greer, M.A. Endocrinology 45:178, 1949.

244. Selye,H. J.B. Collip and D.L. Thomson.
Endocrinology 19:151, 1935.

245. Selye, H. and T. McKeown.
Proc. Roy. Soc., London 119B:31, 1935.

246. McKeown,T. and S. Zuckerman.
Proc. Roy. Soc. 124 B:464, 1938.

247. Greep, R.O. Transactions, 3rd conference, The Josia Macy, Jr. Foundation, P.229, 1956.

248. Hofmann, F.G., E. Knobil and W.L. Caton.
Endocrinology 53: 198, 1953.

249. Hisaw, F.L. and J.T. Velando. Endocrinology 49:732, 1951.

250. Curry, D.M. and G.H. Beaton. Endocrinology 63:155, 1958.

251. Brown, J.H.U. and A. Anason. Endocrinology 62:103, 1958.

252. Chart, J.J., E.S.Gordon, P.Helmer and M. LeSher.
J. Clin. Invest. 35:254, 1956.

253. Miller, L.L. and L.R. Axelrod. Metabolism 3:438, 1954.

254. Eisenstein, A.B., J. Lab. & Clin. Med. 40:796, 1952.

255. Eisenstein, A.B., J. Biol. Chem. 215:585, 1955.

256. Forchielli, E., H. Rosenkrantz and R.I. Dorfman.
J. Biol. Chem. 215:713, 1955.

257. Schneider, J.J. and P.M. Horstmann.
J. Biol. Chem. 191:327, 1951.

258. Schneider, J.J. and P.M. Horstmann.
J. Biol. Chem. 196:629, 1952.

259. Yates, F.E., A.L. Herbst and J. Urguhart.
Proc. 40th Mtg. Endocrine Soc., 1958, p.95.

260. McGuire, J.S. and G.M. Tomkins.
Proc. 40th Mtg. Endocrine Soc., 1958, p.24.

261. Schneider, J.J., J. Biol. Chem. 199:235, 1952.

262. Henry, J.S., E.H. Venning and J.S.L. Browne. New International Clinics, vol. IV, series 48. Copyright, 1938, by J.B. Lippincott Company.

263. Venning, E.H. and M.P. Ripstein. Rev. canad. de. biol. 7:200, 1948.

264. Goldmann, E.E. Tubingen Teil. 2. Beitrag Z. Klin. Chir., 78:1, 1912.

265. Parkes, A.S. Physiol. Rev. 25:203, 1945.

266. Krebs, H.A. and K. Henseleit. Z. Physiol. chemie. 210:33, 1932.

267. Krebs, H.A. and L.V. Eggleston. Biochem. J. 34:442, 1940.

268. An investigation of the urinary corticosteroid pattern in adrenal cortical disease by the technique of paper chromatography. M.Sc. Thesis - S. Sybulski.

269. Savard, K., J. Biol. Chem. 202:457 and 717, 1953.

270. Schindler, O. and T. Reichstein. Helv. Chim. Acta 34:108, 1951.

271. Bush, I.E. Biochem. J. 50:370, 1952.

272. Burton, R.B., A. Zaffaroni and E.H. Keutmann. J. Biol. Chem. 193:769, 1951.

273. Weichselbaum, T.E. and H.W. Margraf. J. Clin. Endocrinol. 17:959, 1957.

274. Umberger, E.J. Analyt. Chem. 27:768, 1955.

275. Nowaczynski, W., M. Goldner and J. Genest. J. Lab. & Clin. Med. 45:818, 1955.

276. Neher, R. and A. Wettstein. J. Clin. Invest. 35:800, 1956.

277. Dyrenfurth, I., S. Sybulski, V. Notchev, J.C. Beck and E.H. Venning. 18:391, 1958.

278. Bernstein, S. and R.H. Lenhard. J. Org. Chem. 18:1146, 1953.

279. Eberlein, W.R. and A.M. Bongiovanni. Proc. 39th Mtg. Endocrine Soc., 1957, p.81

280. Silber, R.H. and C.C. Porter. J. Biol. chem. 210:923, 1954.

281. Simpson, S.A. and J.F. Tait. Proc. Soc. Exper. Biol. & Med. 50:150, 1952.

282. Kurzrok, R., R.W. Bates, O. Riddle, E.G. Miller, Jr. Endocrinology 18:18, 1934.

283. Heard, R.D.H., E.G. Bligh, M.C. Cann, P.H. Jellinck, V.J. O'Donnell, B.G. Rao and J.L. Webb Recent Progr. Hormone Res. 12:45, 1956.

284. Sayers, G. Ciba Foundation Colloq. Endocrinol. 7:254, 1953.

285. Nowaczynski, W.J., P.R. Steyermark, E. Koiw, J. Genest and R.N. Jones Canad. J. Biochem. & Physiol. 34:1023, 1956.

286. Endroczi, E., G. Telegdy and J. Martin. Acta Physiologica (Hungaricae) 14:311, 1958.

287. Berliner, D.L. and T.F. Dougherty. Proc. Soc. Exper. Biol. & Med. 98:3, 1958.

288. Jones, K.M., R. Lloyd-Jones, A. Riondel, J.F. Tait, S.A.S. Tait, R.D. Bulbrook and F.C. Greenwood. Acta endocrinol. 30:321, 1959.

289. Christy, N.P., E.Z. Wallace, W.E.L. Gordon and J.W. Jailer. J. Clin. Invest. 38:299, 1959.

290. Daughaday, W.H. Arch. intern. Med. 101:286, 1958.

291. Donaldson, J.C. Anat. Record. 38:239, 1928.

292. Donaldson, J.C. Am. J. Physiol. 68:517, 1924.

293. Holzbauer, M. J. Physiol. 139:294, 1957.

294. Fiegelson, E.B., J.W. Drake and L. Recant. J. Lab. & Clin. Med. 50:437, 1957.

295. Badawi, H.M. and F.H. Soliman. Experentia. 12:412, 1957.

296. Toxaemias of Pregnancy. A Ciba Foundation Symposium. London J. & A. Churchill, Ltd., 1950.

297. Greene, R.R., J.A. Wells and A.C. Ivy. Proc. Soc. Exper. Biol. & Med. 40:83, 1939.