ABSTRACT

Radioactive squalene, lanosterol and cholesterol were isolated in small amounts from tissue extracts after incubation of sheep and pig placental and fetal liver tissue slices with acetate-1-^{14}C.

In comparing the capacity of sheep and pig fetal and maternal adrenal tissue preparations removed at different stages of gestation to metabolize pregnenolone-7α-^{3}H and progesterone-7α-^{3}H, it was demonstrated that both substrates were metabolized extensively by maternal adrenals of both species. The principal metabolites isolated were cortisol, corticosterone, 11-deoxycortisol and deoxycorticosterone. In addition, 17α-hydroxyprogesterone, 20α-dihydroprogesterone, cortisone and androstenedione were formed from both substrates, whereas 17α-hydroxypregnenolone was formed only from pregnenolone. Pig maternal adrenal glands also formed 11β-hydroxyprogesterone from both substrates. Dehydroepiandrosterone was formed only after incubation of sheep adrenals with pregnenolone. Although the same metabolites were isolated after incubation of fetal adrenal glands, the proportion of corticosteroids formed increased with the stage of gestation at which the adrenals were removed. The most interesting aspect of this study was the observation that fetal adrenal 11β-hydroxylase activity appeared to increase with gestational age in both species.

The significance of these results are discussed in relation to feto-placental steroid biosynthesis and metabolism

and the role of the fetal adrenal in the initiation of parturition in both species.

Résumé

ETUDES SUR LA BIOSYNTHESE ET LE METABOLISME DES STEROIDES HORMONAUX PAR LES TISSUS PLACENTAIRES ET FOETAUX CHEZ LA BREBIS ET LA TRUIE.

De petites quantités de squalène, de lanostérol et de cholestérol radioactifs furent isolées à partir d'extraits de tissus après l'incubation de tranches de tissu foetal hépatique et de tranches de tissu placentaire avec de l'acétate-1-^{14}C.

En comparant la capacité de préparations de tissu surrénal maternel et foetal enlevé à différents stades de la gestation à métaboliser la prégnénolone-7α-^{3}H et la progestérone-7α-^{3}H, il fut démontré que ces deux substrats furent activement métabolisés par les glandes surrénales des deux espèces. Les principaux métabolites isolés furent le cortisol, le corticostérone, le 11-deoxycortisol et le deoxycorticostérone. En outre, le 17α-hydroxyprogestérone, le 20α-dihydroprogestérone, le cortisone et l'androsténédione furent formés à partir des deux substrats tandis que le 17α-hydroxyprégnénolone fut formé uniquement à partir de prégnénolone. Les glandes surrénales maternelles de la truie formèrent aussi le 11β-hydroxyprogestérone à partir des deux substrats. Le déhydroépiandrostérone ne fut formé qu'après l'incubation des surrénales de la brebis avec du prégnénolone. Quoique les mêmes métabolites furent isolés après l'incubation des glandes surrénales foetales, la proportion des corticostéroides formés augmenta avec le stade

de gestation auquel les surrénales furent enlevées. L'observation que l'activité du 11β-hydroxylase foetal surrénal sembla augmenter avec l'âge gestationel chez les deux espèces constitua l'aspect le plus intéressant de cette étude.

L'importance de ces résultats est discutée relativement à la biosynthèse et au métabolisme des stéroides foeto-placentaires, ainsi qu'au rôle des glandes surrénales foetales dans l'initiation de la parturition chez les deux espèces.

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GENERAL INTRODUCTION

It is generally accepted that both progesterone* and estrogens are involved in reproductive endocrinology in all higher forms of animal life and especially in viviparous mammals where progesterone with or without estrogen plays a role in pregnancy maintenance via effects upon the implantation site and myometrium (Deanesly, 1966). It has also been clearly demonstrated that in those species where pregnancy is prolonged beyond the normal luteal phase of the ovarian cycle, new pregnancy specific endocrine mechanisms are invoked to continue the supply of these hormones, which would be lost if the corpus luteum were to regress as in the normal nonpregnant cycling female. These pregnancy specific mechanisms include prolongation of the life span of the corpus luteum, usually through a placental gonadotropin, and in some instances, the acquisition of a steroid-producing capacity by the placenta.

Perhaps the most striking feature of the endocrine mechanisms during pregnancy has been the feto-placental interaction in hormone production. The concept of a feto-placental endocrine unit has been introduced by Diczfalusy and his colleagues through studies, mainly in vivo, on the mid-gestation human fetus and placenta. According to this

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* See Appendix I for glossary of trivial and systematic names of steroids used in this thesis.

concept the bulk of steroid hormones elaborated during pregnancy are formed by the feto-placental unit from circulating sterol and steroid precursors, rather than from acetate. The concept also states that both the fetus and the placenta represent incomplete steroidogenic systems because of the lack, or non-functioning of certain essential enzyme systems. However, the enzymes absent from the placenta are present in the fetus, and those lacking in the fetus are present in the placenta. In this way, the feto-placental unit is capable of elaborating C_{21}, C_{19}, and C_{18} steroids. Thus progesterone is formed by the placenta mainly from maternal blood cholesterol (Hellig et al., 1969, 1970), while the precursors for placental production of estrogens are blood borne androgens derived almost entirely from the fetus (Siiteri and MacDonald, 1966).

In comparative studies with over 13 diverse species of animals, those placentae that synthesized estrogens could do so only from preformed androgens, demonstrating a similarity to the human situation (Ryan, 1969). In addition, the capacity of fetal adrenal enzymes to synthesize androgens, which are in turn converted to estrogen by the placenta, has been demonstrated not only for the human but also for the sheep and macaque (Davies et al., 1970). Placental synthesis of progesterone from pregnenolone proceeds effectively in a large number of mammalian species (Ryan, 1969; Townsend and Ryan, 1970; Matsumoto et al., 1969), but literally nothing

is known of either cholesterol or acetate conversion to progesterone in any species other than man.

Further insight into the endocrine control of pregnancy and particularly in the control of parturition has been stimulated by the studies of Liggins et al. (1967) and Drost and Holm (1968). These authors indicated the importance of a functional fetal pituitary-adrenal axis for the occurrence of natural parturition in sheep since fetal adenohypophysial aplasia or intra-uterine fetal hypophysectomy or adrenalectomy was shown to prolong pregnancy. Furthermore, Van Rensburg (1967), Halliday and Buttle (1968) and Liggins (1968, 1969b) have demonstrated that in the sheep, premature parturition could be induced by infusion of synthetic corticotropin (Synacthen, Ciba) or the glucocorticoids cortisol and dexamethasone into the fetal lamb in utero. Subsequently, several groups in England, Australia and New Zealand have shown that parturition in the sheep is the result of an orderly sequence of changes in maternal or fetal blood concentration of progesterone, cortisol, estrogen, oxytocin and prostaglandin F2α (Bedford et al., 1972a; Nathanielsz et al., 1972; Thorburn et al., 1972; Liggins et al., 1972; Chard, 1972). However, the trigger which initiates this sequence of events leading to parturition in the sheep, and which, if indeed any, of these factors is the prime determinant of labor, remains to be elucidated.

As part of a program designed to provide information on the feto-placental interrelationships of steroids during

pregnancy in domestic animals, the studies to be reported in this thesis will be concerned with a) the capacity of placental and fetal liver preparations of the sheep and pig to transform labeled acetate to cholesterol in vitro and b) the biosynthesis of various steroids, particularly corticosteroids, from labeled pregnenolone and progesterone by both fetal and maternal adrenal glands removed from sheep and pigs at different stages of gestation.

CHAPTER I

REVIEW OF THE LITERATURE

A. INTRODUCTION

In considering the role of steroid hormones in comparative reproduction, it is important to bear in mind that the evolution of reproduction in general, and of viviparity in particular, has involved predominantly endocrine mechanisms. Within these endocrinological adaptations the steroid hormones have appeared to be the principal mediating agents in the control of pregnancy. Hence, the generality has been offered that progestins and estrogens are essential for reproduction in all higher forms of animal life. However, the roles that these two types of hormones play once gestation has been established have not been completely defined, nor has their essentiality in an absolute or relative sense been incontrovertibly established for all species of mammals. Until fairly recently the biochemistry and physiology of steroid hormones during pregnancy in species other than the human female havebeen largely ignored. However, with the advent of new and more refined techniques for the separation and estimation of steroid hormones it is now becoming possible to approach this problem in a more systematic and meaningful manner. As will be evident in the following sections, the biochemistry and physiological role of steroid hormones during the course of pregnancy in mammals still requires

extensive study. This review of the literature will be confined to the comparative aspects of the biochemistry and role of steroid hormones during pregnancy in mammals and reference to the human will be cited only for comparative purposes.

B. THE ROLE OF THE CORPUS LUTEUM DURING PREGNANCY

In the presence of fertilized and implanting ova, changes occur in the ovary which, in general, define its character for the remainder of the gestation period. Corpora lutea which would normally regress at the end of a cycle are established as corpora lutea of pregnancy. Follicles which would have been expected to enlarge and to ovulate with the onset of another cycle are inhibited as is ovulation itself. Existing information on the role of the ovary during pregnancy justifies the view that it is capable of secreting progesterone and/or estrogens which are essential for continued growth of the blastocyst and for full differentiation of the uterine endometrium (Deanesly, 1966). However, in some mammalian species the ovaries can be removed after a certain stage of gestation without resorption or abortion of the fetus. It is now becoming evident that in those species in which oophorectomy does not result in termination of pregnancy, the placenta is capable of providing the estrogen and progesterone necessary for the continued maintenance of pregnancy.

The endocrine role of the corpus luteum during pregnancy in mammals has been determined largely by studying the effect of its removal on the subsequent course of pregnancy. As shown in Tables 1 and 2, mammalian species can be conveniently classified into those that require the ovary and/or pituitary throughout pregnancy (oophorectomy intolerant) and those that do not (oophorectomy tolerant). They can be further classified as to gestational length and total or partial replacement of ovarian function by the placenta (Amoroso and Finn, 1962; Ryan and Ainsworth, 1967; Ryan, 1969). If mammals are classified in this manner it can be seen that there is a good correlation between the requirements for a corpus luteum and/or pituitary and the presence or absence of progesterone and estrogens in the placenta.

Class I. Consists mainly of species with gestation periods of 60 days or less, which require the ovary and occasionally the pituitary for pregnancy maintenance. In general, these species have not had progesterone or estrogen isolated from the placenta, the ovaries do not regress before term and aromatization by placental tissue has not been demonstrated (Table 1). However, the conversion of pregnenolone to progesterone by rat and rabbit placental tissue preparations has recently been demonstrated (Townsend and Ryan, 1970; Matsumoto et al., 1969).

Class 2. Consists of species with gestational periods of over 100 days that still require the ovary for pregnancy maintenance. These species are capable of placental aromatization, but progesterone has not been isolated from the

TABLE 1*

Correlation between the requirement for a corpus luteum and placental hormone synthesis of progesterone and estrogens during pregnancy in oophorectomy intolerant mammals

Species	Gestation Length (Days)(a)	Regression of Corpus Luteum Before Term (b)	Presence of Progesterone Demonstrated in Placenta (c)	Presence of Estrogen Demonstrated in Placenta (d)	Aromatization By Placental Tissue (e)	Comments (f)
			CLASS I - SHORT GESTATION			
Opossum	12.5	NO	—	—	—	No placental endocrine function
Hamster	16-19	NO	—	—	—	
Mouse	19-20	NO	—	NO	NO	Tolerates hypophysectomy-placental gonadotropin
Rat	22	NO	YES	NO	NO	Tolerates hypophysectomy-placental gonadotropin
Rabbit	30-32	NO	NO	NO	NO	Intolerant of hypophysectomy
Dog	58-63	YES	NO	NO	NO	Intolerant of hypophysectomy
			CLASS II - LONG GESTATION			
Sow	113-115	NO	NO	YES	YES	Intolerant of hypophysectomy
Goat	146-151	NO	NO	YES	YES	Intolerant of hypophysectomy

* Table reproduced with modifications from Ryan (1969)

a) Compiled from Zarrow (1961)

b) Compiled from Amoroso and Finn (1962)

c) Compiled from Short (1961); Rat: Wiest et al. (1968)

d) Mouse: Harkness et al., (1964); Rat, Rabbit & Dog: Compiled from Newton (1938); Sow and Goat: Compiled from Velle (1963)

e) Mouse: Rembiesa et al., (1971); Rat: Townsend and Ryan (1970); Rabbit and Sow: Ainsworth and Ryan (1966); Goat: Ainsworth and Ryan (1970); Dog: unpublished observation cited by Ryan (1969).

f) Compiled from Amoroso and Porter (1966).

TABLE 2 *

Correlation between the requirement for a corpus luteum and placental hormone synthesis of progesterone and estrogens during pregnancy in oophorectomy tolerant mammals

Species	Gestation Length (Days) (a)	Gestational Age for Oophorectomy in which Abortion Not Inevitable (Days) (b)	Regression of Corpus Luteum Before Term (c)	Presence of Progesterone Demonstrated in Placenta (d)	Presence of Estrogen Demonstrated in Placenta (e)	Placental Pregnenolone-Progesterone conversion (f)	Aromatization By Placental Tissue (g)	Comments (h)
				CLASS 3 - SHORT GESTATION				
Cat	63	49	YES	—	—	—	—	Intolerant of hypophysectomy
Guinea-pig	67-68	20-27	NO	YES	—	—	NO	Tolerates hypophysectomy
				CLASS 4 - LONG GESTATION				
Sheep	144-152	55	YES	YES	YES	YES	YES	Tolerates hypophysectomy
Macaque	168	25	—	YES	YES	YES	YES	Tolerates hypophysectomy
Human	280	30-73	YES	YES	YES	YES	YES	Tolerates hypophysectomy
Cow	277-290	207	NO	YES	YES	YES	YES	
Horse	330	200	YES	YES	YES	YES	YES	
Armadillo	150	40	NO	—	—	—	YES	

* Table reproduced with modification from Ryan (1969)

a) Compiled from Zarrow (1961)

b) Compiled from Short (1961) and Deanesly (1966)

c) Compiled from Amoroso and Finn (1962)

d) Guinea pig: Heap and Deanesly (1966); Sheep, macaque, human and horse compiled from Short (1961) and Cow (Velle, 1963)

e) Compiled from Newton (1938)

f) Sheep and cow: Ainsworth and Ryan (1967); Macaque and horse: Ainsworth and Ryan (1969); Human: Pion et al. (1965, 1966)

g) Guinea-pig, sheep, cow and horse: Ainsworth and Ryan (1966); Macaque: Ainsworth et al. (1969); Human: Ryan (1959); Armadillo: Brinck-Johnson et al. (1967) and Brinck-Johnsen (1970)

h) Compiled from Amoroso and Porter (1966).

placenta (Table I).

Class 3. Consists of species with short gestational periods that do not require the ovary. However, their endocrine requirements are such that they do not appear to offer an endocrine model which can be related to other species (Table 2).

Class 4. Consists of species with long gestational periods of over 150 days, which generally do not require the ovary for pregnancy maintenance and have the capacity for placental progesterone formation and aromatization (Table 2). All the species listed in Table 2 with prolonged gestation periods have developed placental aromatization and thus the basis for feto-placental interaction. In addition, when the ovaries are not required for pregnancy maintenance, the placenta or feto-placental unit assumes an active endocrine role. Hence, it is from these ovariectomy tolerant mammals, which includes man, that experimental models for the study of feto-placental endocrine interaction have been derived.

In those species listed in Tables 1 and 2 where the ovary was required for the maintenance of pregnancy, it has been established in several instances that the progesterone and/or estrogen was of ovarian origin. In the rat, the ovarian concentrations of progesterone remain extremely high during pregnancy and correlate roughly with the peripheral plasma level patterns (Wiest et al., 1968; Wiest,

1970). Furthermore, in rats ovariectomized on the 13-14th day of pregnancy, the peripheral plasma progesterone levels at day 16 had dropped dramatically when compared with control animals at the same stage of pregnancy (Csapo and Wiest, 1969). Yoshinaga et al. (1969) have shown ovarian venous plasma estrogen values to increase significantly just prior to parturition in the rat. In addition, Townsend and Ryan (1970) were unable to demonstrate estrogen formation after incubation of rat placental tissue preparations with 4-androstene-3, 17-dione-4-^{14}C, strongly indicating that the estrogen was of ovarian origin.

Marked differences in endocrine functions during pregnancy in domestic species (Tables 1 and 2) are also apparent. Ovariectomy and hypophysectomy at any stage of pregnancy in both the pig and goat causes abortion, although pregnancy can be maintained in both species after ovariectomy by suitable therapy with progesterone (du Mesnil du Buisson and Dauzier, 1957; Meites et al., 1951). Thus it was concluded that in both these species the integrity of the pituitary and corpus luteum was essential for maintenance of pregnancy and that the placenta did not produce progesterone in sufficient amounts to prevent abortion. This conclusion received support from the findings of Linzell and Heap (1968) who reported no net production of progesterone by the feto-placental unit of anaesthetized goats. More recently, Thorburn and Schneider (1972) showed that bilateral ovariec-

tomy of pregnant goats resulted in a dramatic fall in peripheral plasma progesterone concentrations followed by abortion 36-48 hours later. In studies with the pregnant sow, the total luteal content of progesterone remained high throughout pregnancy, at a level comparable to that observed in the luteal phase of the estrus cycle (Duncan et al., 1960; Rombauts et al., 1965; Masuda et al., 1967). The maintenance of this high level of progesterone is in accordance with the fact that the ovaries are indispensable throughout pregnancy in this species.

In an attempt to evaluate corpus luteum function during pregnancy in the cow, Erb et al. (1968a) measured progestin levels (progesterone and a closely related metabolite 20β-hydroxy-preg-4-en-3-one) in corpora lutea and progesterone levels in ovarian venous plasma and jugular venous plasma throughout pregnancy. They concluded that the corpus luteum in the bovine was functional throughout pregnancy and that the extra-ovarian sources of progesterone may be considerable after 200 days of pregnancy, as indicated by the constant elevated levels of progesterone in jugular venous plasma after the 3rd month, and the lower levels of progesterone in ovarian venous plasma from 199-237 days of pregnancy. However, in the last month of pregnancy Estergreen et al. (1967) reported ovarian venous levels of progesterone again increased and this they felt to be of significance for normal parturition.

In contrast to the other domestic species referred to above, the ovary of the sheep can be removed after the 50th day of pregnancy without effect on the subsequent course of gestation (Casida and Warwick, 1945; Denamur and Martiner, 1955). In the pregnant ewe, Edgar and Ronaldson (1958) found that the maximum level of progesterone in the ovarian venous blood reached during the estrus cycle was maintained when pregnancy supervened and remained fairly constant until the last month of pregnancy. Thereafter, the concentration fell and no progesterone could be detected at 15 days prepartum. Moore et al. (1972) using a more sensitive technique confirmed these latter findings and also demonstrated a gradually increasing level of progesterone in both uterine venous plasma and fetal venous plasma as pregnancy progressed, beginning at about the 80th day. Although the levels of progesterone in the fetal vein rose, at no stage did they reach the levels recorded in the uterine vein. Similar differences have been reported by Linzell and Heap (1968) and it appears that little progesterone passes from the maternal to the fetal circulation. Thus it is evident that although the corpus luteum appears to remain functional for most of the gestation period extra-ovarian sources of progesterone are capable of maintaining pregnancy in this species.

C. STEROID ENDOCRINE ROLE OF THE MAMMALIAN PLACENTA

1. Isolation of steroid hormones from placental tissue.

It has been postulated that in those mammalian species in which oophorectomy does not result in the termination of pregnancy an extra-ovarian source, the fetal placenta, has assumed a major role in providing the estrogens and progestins required for the maintenance of pregnancy. As demonstrated in Tables 3, 4 and 5, progesterone, steroids related to progesterone, and estrogens, have been isolated from the placenta of several species. The possibility that placental production is adequate to maintain pregnancy has been suggested by the fact that in many of these species abortion does not occur following oophorectomy. However, in species in which progesterone has not been isolated from the placenta, abortion generally occurs after removal of the ovaries. In addition, either or both 20α- and 20β-hydroxyprogesterone has been isolated from placental tissue of the human, sheep, rat and mare (Table 4). Although possessing progestational activity, these compounds appear to be unable to maintain pregnancy, at least in the rat (Wiest, 1968) and mouse (Wiest and Forbes, 1964). In addition, the importance of their relative amounts in relationship to progesterone has not been defined, although recent work has suggested that 20α-dihydroprogesterone may be important for the onset of parturition in the rat (Wiest, 1970; Kuhn and Briley, 1970).

TABLE 3

Species in which progesterone has been isolated from placental tissue

Species	Placental level[a] µg/kg
Human	500-10,000
Chimpanzee	comparable to human
Rhesus monkey	35-72
Horse	60-370
Cow[b]	4.4
Sheep	4-9
Common seal	46
Indian rhinoceros	41
Giraffe	10-12
Guinea-pig[c]	17

[a] Compiled from Short (1961).

[b] Detected in only one of six placenta analyzed (Bowerman and Melampy, 1962).

[c] Heap and Deanesly (1966).

TABLE 4

Species in which steroids related to progesterone have been isolated from placental tissue

Species	Steroid isolated	Placental level (µg/g)	Reference
Human	20α and 20β-dihydroprogesterone	1.82	Zander et al., 1958
Sheep	20α-dihydro-progesterone	0.01	Short and Moore, 1959
Rat	20α-dihydro-progesterone	0.27	Wiest, 1970
Mare	20β-dihydro-progesterone	-	Short, 1957

TABLE 5

Species in which estrogens have been isolated from placental tissue

Species	Steroid isolated	Placental level (µg/kg)	Reference
Human	estrone	41	Schmidt-Elmendorff, 1961
	estradiol-17β	101	
	estriol	180	
	16-epiestriol	2-3	Diczfalusy and Halla, 1958
	16-ketoestradiol-17β	6	Diczfalusy and Munstermann, 1959
Cow	estrone	35	Veenhuizen et al., 1960
	estradiol-17β	36	
	estradiol-17α	37	
Sheep	estradiol-17α	15-24	Velle, 1958c
Pig	estrone	1.3-120	Rombauts, 1964
Goat	estradiol-17α	-	Velle, 1963
Mouse	estrone	0.08-0.7	Harkness et al., 1964
	estradiol	0.14-0.76	
	estriol	0.25-2.8	
Guinea pig	estriol	3.9	Church and Eleftheriou, 1966
	estrone	48.5	
	estradiol-17β	2.2	
	estradiol-17α	6.7	
	16-ketoestradiol-17β	17.6	
	16-epiestriol	16.5	
Armadillo	estriol	0.5	Brink-Johnsen, 1970
	estrone	0.4	
	estradiol-17β	0.9	

Evidence that the placenta produces estrogens has been assumed in animals that do not abort after oophorectomy. The isolation of estrogenic substances from the placenta of a number of species has been reported (Table 5). It will be noted that species differences exist, particularly with regard to the isolation of estradiol-17α, which has been isolated in significant amounts only from bovine and sheep placental tissue. As with progesterone, there appears to be an inverse correlation between the presence of estrogens in the placenta and the need for the corpus luteum in the maintenance of pregnancy.

2. Biosynthesis and metabolism of progesterone and related compounds.

In general, progesterone is formed in the corpus luteum of pregnancy for all or part of gestation and/or in the placenta for varying periods up to the time of parturition, when the corpus luteum regresses. In the human, progesterone is formed by the placenta primarily from cholesterol, which is probably of maternal liver origin (Bloch, 1945; Davis et al., 1956; Frandsen and Stakemann, 1964), as well as from pregnenolone and pregnenolone sulfate (see Table 6). A small part of the cholesterol may be formed by the placenta by de novo synthesis from acetate (Van Leusden and Villee, 1965; Zelewski and Villee, 1966; Villee and Tsai, 1969). In the mammalian corpus luteum, biosynthesis of progesterone proceeds predominantly via complete de novo synthesis from ace-

TABLE 6

Biosynthesis of progesterone by the mammalian placenta

Species	Substrate	Placental preparation	Reference
Human	cholesterol	perfusion mitochondria	Solomon, 1960 Jaffe et al., 1965 Morrison et al., 1965.
	pregnenolone	mince	Nissin and Robson, 1952.
		homogenate	Sobrevilla et al., 1964.
		perfusion, homogenate	Pion et al., 1965, 1966.
		organ culture	Meeker et al., 1971a.
	pregnenolone sulfate	homogenate	Pearlman et al., 1954.
		perfusion	Palmer et al., 1966a.
Cow, sheep	pregnenolone	homogenate	Ainsworth and Ryan, 1967.
Mare, rhesus monkey, baboon Macaque cynomologus, orangutan	pregnenolone	homogenate	Ainsworth and Ryan, 1969; Ainsworth et al., 1969.
Rabbit	pregnenolone	slice	Matsumoto et al., 1969.
Rat	pregnenolone	homogenate	Townsend and Ryan, 1970.
Guinea pig	pregnenolone	slice	Bedwani and Marley, 1971.
Mouse	pregnenolone	quarters	Rembiesa et al., 1971b.

tate (Savard et al., 1965). After fetal demise in human pregnancy, progesterone production remains essentially the same (Frandsen and Stakemann, 1964), which suggests that the fetal contributions of cholesterol, pregnenolone or pregnenolone sulfate are not necessary for normal progesterone production and that a considerable portion of human progesterone formation is basically a maternal-placental function.

Progesterone is not extensively metabolized by the human placenta (see Table 7). The reduction of the 20-oxo groups to a 20α-hydroxy group is one of the best characterized transformations of progesterone and related steroids (Little et al., 1959). In vivo, the 20α-hydroxysteroid: NAD (P) oxidoreductase system functions to oxidize 20α-hydroxy steroids coming from the fetus to the corresponding 20-oxo compounds, rather than the reverse (Palmer et al., 1966b). The principal metabolic transformation of progesterone by the human placenta is through 6β-hydroxylation resulting in the formation of 6β-hydroxyprogesterone (Berliner and Salhanick, 1956; Palmer et al., 1966b; Kitchin et al., 1967).

Although it has been demonstrated that a number of mammalian placental preparations are capable of converting pregnenolone to progesterone (Table 6), literally nothing is known of either acetate or cholesterol conversion to progesterone by the placenta or fetus of other mammalian species. Recently, Nitchuk and Ainsworth (1972) have shown that both sheep and

TABLE 7

Metabolism of progesterone by the mammalian placenta

Species	Placental preparation	Metabolites isolated	Reference
Human	*in vitro* perfusion	6-ketoprogesterone	Hagopian et al., 1956
	mince	6β-hydroxyprogesterone	Berliner and Salhanick, 1956
	homogenate	20α-dihydroprogesterone	Little et al., 1959
	homogenate	androstenedione	Warren and Cheatum, 1964
	in situ perfusion	20α-dihydroprogesterone 6β-hydroxyprogesterone	Palmer et al., 1966b
	in situ perfusion	20α-dihydroprogesterone 5β-pregnane-3, 20-dione	Kitchin et al., 1967
	homogenate	6β-hydroxyprogesterone androstenedione 17α-hydroxyprogesterone 16α-hydroxyprogesterone testosterone	Jungmann and Schweppe, 1967
Cow, sheep	homogenate	20α-dihydroprogesterone 20β-dihydroprogesterone 5β-pregnane-3α, 20α-diol 5β-pregnane-3α, 20α-diol	Ainsworth and Ryan, 1967
Sheep	*in vitro*	20α-dihydroprogesterone 20β-dihydroprogesterone 5β-pregnane-3α, 20α-diol 5β-pregnane-3α, 20α-diol 5β-pregnane-3α, 20-dione 5β-pregnane-3α-ol-20-one 6β-hydroxyprogesterone	Nancarrow and Seamark, 1972a
Mare	homogenate	20α-dihydroprogesterone 5α-pregnane-3, 20-dione 5α-pregnane-3β-ol-20-one 5α-pregnane-20α-ol-3-one 5α-pregnane-3β, 20α-diol 5α-pregnane-3β, 17α, 20α-triol	Ainsworth and Ryan, 1969a

Baboon, rhesus monkey	homogenate	20α-dihydroprogesterone 6β-hydroxyprogesterone 5α-pregnane-3, 20-dione 5α-pregnane-3β-ol-20-one 5α-pregnane-3β, 20α-diol	Ainsworth et al., 1969
Rhesus monkey	in situ perfusion	5α-pregnane-3, 20-dione 5α-pregnane-3β-ol-20-one	Leung and Solomon, 1972
Squirrel monkey	homogenate	6β-hydroxyprogesterone 5α-pregnane-3β-ol-20-one	Ainsworth et al., 1969
Orangutan	homogenate	20α-hydroxyprogesterone 6β-hydroxyprogesterone 5α-pregnane-3β-ol-20-one	Ainsworth and Ryan, 1969b
Rat	homogenate	5α-pregnane-3α-ol-20-one 5α-pregnane-3α, 20β-diol	Townsend and Ryan, 1970
Mouse	homogenate	20α-dihydroprogesterone 16-ketoprogesterone	Vinson and Chester Jones, 1964
	quarters	20α-dihydroprogesterone 21-hydroxyprogesterone 5α-pregnane-3, 20-dione 5α-pregnane-3β-ol-20-one 5α-pregnane-3β, 20α-diol androstenedione 5α-androstane-3α-ol-17-one	Rembiesa et al., 1971b

pig placenta tissue were capable of synthesizing sterols and cholesterol from acetate in vitro. However, the contribution of this potential source of cholesterol to the overall availability of cholesterol in the feto-placental endocrine system of each of these species has yet to be determined.

In contrast to the limited metabolism of progesterone by the human placenta both in vivo and in vitro, it appears that progesterone is actively metabolized by the placenta of a number of mammalian species (Table 7). In addition to the formation of either or both 20α and 20β reduced metabolites of progesterone, an active Δ^4-reductase has been demonstrated in the placenta of all these species, which results in the formation of a series of metabolites in which the orientation of the A ring is either 5α or 5β, depending on the species. In those species in which data are available, it has been suggested (Ainsworth and Ryan, 1969) that the insignificant rise in blood progesterone levels observed during pregnancy in several of these species could be as a consequence of placental endocrine activity. If this is the case then pregnane rather than pregnene derivatives would be present in uterine venous blood.

Table 8 summarizes the enzyme activities which have been demonstrated in vitro in the placenta of different mammalian species and compares the distribution of the enzyme activities with those which have been demonstrated in the human placenta. This Table emphasizes very clearly the

TABLE 8

Distribution of enzyme activities in the placenta of different mammalian species, as suggested by *in vitro* experiments

Species	17 -20(C_{21}) Desmolases	Hydroxysteroid dehydrogenase						Δ^4 - Δ^5 isomerase	Δ^4 - 5α reductase	Δ^4 - 5β reductase	Steroid hydroxylases			Aromatizing system
		3α	3β	17α	17β	20α	20β				6β	17α	16α	
Human	±	-	+	-	+	+	-	+	-	+	+	±	±	+
Cow	-	+	+	+	+	+	+	+	-	+	-	+	-	+
Sheep	-	+	+	+	+	+	+	+	-	+	+	+	-	+
Horse	-	-	+	+	+	+	+	+	+	-	+	+	-	+
Macaque cynomologous	-	-	+	-	+	+	-	+	+	-	-	-	-	+
Squirrel monkey	-	-	+	-	+	-	-	+	+	-	+	-	-	+
Baboon	-	-	+	-	+	+	-	+	+	-	+	-	-	+
Orangutan	-		+	-	+	+	-	+	+	-	+	-	-	
Rhesus monkey	-		+	-	+	+	-	+	+	-	+	-	-	+
Mouse	+		+	-	-	+	-	+	+	-	-	-	-	-
Rabbit			+	-	-			+	+	-		-	-	-
Rat	-		+	-	-	-	+	+			-	-	-	-
Guinea pig			+	-	-			+				-		-
Marmoset monkey				-	-							-	-	+
Sow				-	+							-	-	+
Armadillo				-	+						-	+	+	+
Goat				+	+							+	-	+

important differences which exist between species in the nature of the metabolites which may be formed from the same precursor by the same endocrine tissue. It should also be noted (see Table 7) that mouse placental tissue appears to be capable of 21-hydroxylation as evidenced by the isolation of deoxycorticosterone after incubation of placental tissue with radioactive pregnenolone or progesterone. In addition, androgens were isolated from the same incubations indicating that mouse placental tissue is also capable of side-chain cleavage.

3. Biosynthesis of estrogens by mammalian placental tissue.

In species which do not abort after oophorectomy or in those where only progesterone is required to maintain pregnancy after such surgery, it is assumed that the placenta is the source of estrogen. As shown in Table 9, placental preparations from a number of mammalian species are capable of forming estrogens, particularly from C_{19} androgen precursors. In general, it can be stated that in the mammalian placentae studied it appears that C_{21} steroids make little, if any, contribution to estrogen formation due to an apparent lack of a 17, 20 (C_{21})-desmolase enzyme system. Moreover, in the studies with human placenta it has been established that the major precursors of estriol are 16-hydroxylated androgens, which are of fetal origin (Diczfalusy, 1969). However, it will be noted that in some instances

TABLE 9

Biosynthesis of estrogens by the mammalian placenta

Species	Substrate	Placental preparation	Estrogen formed	Reference
Human	androstenedione	homogenate, microsomes	estrone	Ryan, 1959
	16α-hydroxy-dehydroepi-androsterone	supernatant fractions 9000xg	estriol	Magendantz and Ryan, 1964
	estrone estradiol-17β testosterone	homogenate	estriol	Slaunwhite et al., 1965
	androstenedione testosterone	homogenate	estrone estradiol-17β	Jungmann et al., 1966; Slaunwhite et al., 1965.
	androstenedione testosterone	perfusion in vitro	estrone estradiol-17β	Cédard et al., 1966
	androstenedione testosterone dehydroepi-androsterone	perfusion in vitro	estrone estradiol-17β	Nakayama et al., 1967a
	androst-5-en-3β, 16α, 17β-triol	perfusion in situ	estriol	Dell' Acqua et al., 1967a
	16α-hydroxy-dehydroepi-androsterone 16α-hydroxy-androstenedione 16α-hydroxy-testosterone	perfusion in situ	estriol	Dell' Acqua et al., 1967b
	dehydroepi-androsterone dehydroepi-androsterone sulfate	perfusion in situ	estrone estradiol-17β	Lamb et al., 1967
	estradiol	microsomes	2-hydroxyestrone	Fishman and Dixon, 1967

dehydroepi-androsterone	homogenate	estrone estradiol-17β estriol	Wu et al., 1968; 1970
dehydroepi-androsterone androstenedione	perfusion *in situ*	estrone estradiol-17β estriol	Nakayama et al., 1968
Δ^7-testosterone Δ^7-androsteno-lone $\Delta^{4,7}$-androsta-dien-19-ol-3, 17-dione	microsomal fraction	dihydroequilin-17β	Givner et al., 1968
3β, 17β-dihy-droxyandrost-5 en-16-one	perfusion *in situ*	16-ketoestradiol 16-epiestriol	Reynolds et al., 1968
17α-hydroxy-progesterone	perfusion *in vitro*	estrone estradiol-17β	De Miquel and Troen, 1968
15α-hydroxy-androstenedione	10,000 xg supernatant	15α-hydroxyestradiol	Stern et al., 1968
dehydroepi-androsterone dehydroepi-androsterone sulfate, testos-terone	perfusion *in situ*	estrone estradiol-17β	Bolté et al., 1969
estradiol-17β	homogenate	estrone 2-hydroxyestrone	Smith and Axelrod, 1969a
dehydroepi-androsterone	homogenate	estrone estradiol-17β 2-hydroxyestrone	Smith and Axelrod, 1969b
epitestosterone	homogenate	estradiol-17β	Higuchi and Villee, 1970

	15α-hydroxy-androstenedione	perfusion in situ	15α-hydroxyestrone 15α-hydroxyestradiol	Meeker et al., 1971b
Rhesus monkey	androstenedione dehydroepi-androsterone	homogenate	estrone estradiol-17β	Ainsworth et al., 1969
Macaque cynomologus Squirrel monkey	androstenedione	homogenate	estrone estradiol-17β	Ainsworth et al., 1969
Baboon	androstenedione	homogenate	estrone estradiol-17β	Ainsworth et al., 1969
	androstenedione dehydroepi-androsterone	microsomal fraction	estrone estradiol-17β	
	testosterone	microsomal fraction	estrone estradiol-17 2-hydroxyestrone 2-hydroxyestradiol 4-androstene-3β, 17β-diol 19-aldoandrostenedione 2β-hydroxytestosterone 19-aldotestosterone 19-hydroxytestosterone 19-hydroxyandrostenedione androstenedione	Milewich and Axelrod, 1971, 1972a, b
Marmoset monkey	androstenedione	homogenate	estrone	Ryan et al., 1961
Orangutan	androstenedione	homogenate	estrone estradiol-17β	Ainsworth and Ryan, 1969b

Goat	androstenedione	homogenate microsomal fraction	estrone estradiol-17β estradiol-17α	Ainsworth and Ryan, 1970
Sheep	dehydroepi-androsterone androstenedione	homogenate	estrone estradiol-17β estradiol-17α	Ainsworth and Ryan, 1966
	androstenedione	microsomal fraction	estrone estradiol-17β estradiol-17α	
	androstenedione epitestosterone	slice	estrone estrone sulfate estradiol-17β estradiol-17α	Harvey et al., 1972
	androstenedione testosterone epitestosterone	mince	estrone estradiol-17β estradiol-17α	Pierrepoint et al., 1970
	androstenedione epitestosterone	mince	estrone sulfate estradiol-17β sulfate estradiol-17α sulfate	Pierrepoint et al., 1971a
Cow	dehydroepi-androsterone androstenedione	homogenate microsomal fraction	estrone estradiol-17β estradiol-17α	Ainsworth and Ryan, 1966
	androstenedione	microsomal fraction	estrone estradiol-17β	
	androstenedione testosterone	mince	estrone estradiol-17β	Pierrepoint et al., 1969
Sow	dehydroepi-androsterone androstenedione	homogenate	estrone estradiol-17β	Ainsworth and Ryan, 1966
	dehydroepi-androsterone androstenedione	microsomal fraction	estrone	

Mare	dehydroepi-androsterone	homogenate	estrone	Ainsworth and Ryan, 1966
	androstenedione	microsomal fraction	estradiol-17β	
Mouse	testosterone	slices	-	Rembiesa et al., 1971a
Guinea pig, rabbit	dehydroepi-androsterone	homogenate	-	Ainsworth and Ryan, 1966
	androstenedione	microsomal fraction		
Rat	testosterone	homogenate	-	Sybulski, 1969
	androstenedione	homogenate	-	Townsend and Ryan, 1970
Armadillo	16α-hydroxy-dehydroepi-androsterone	homogenate	estriol	Brinck-Johnsen et al., 1967
	testosterone androstenedione	homogenate	estrone estradiol-17β	Brinck-Johnsen, 1970

estriol has been identified as a metabolite in in vitro studies, after incubation of placental tissue with either androgen or estrogen precursors which do not contain a hydroxyl group at C-16. In those species in which the nature of urinary and plasma estrogens have been investigated (see Section D) the estrogens identified in placental tissue correspond to those found to be quantitatively important in either urine or plasma, thereby giving support to the belief that in species that do not require the ovary for pregnancy maintenance the estrogen is of placental origin. In contrast, in those species in which it has been established that estrogens are of ovarian origin, viz rat, rabbit and mouse, androgen precursors when incubated with placental tissue from these species do not give rise to estrogen. The only exception to date is the guinea pig. Placental preparations from this species appear to be unable to form estrogen from either dehydroepiandrosterone or androstenedione (Ainsworth and Ryan, 1966), yet it has been established that pregnancy can be maintained after removal of the ovaries. The source of estrogen in the guinea pig during pregnancy has not yet been determined.

The mechanism by which estrogen is formed from androgens by the human placenta has been the subject of considerable interest. The evidence up to 1962 has been given by Dorfman and Ungar (1965) who discussed various alternate mechanisms. Further evidence was given by Ryan (1963) and Wilcox and

Engel (1965a, b). As shown in Fig. 1 the mechanism by which estrogen is formed from C_{19}-4-en-3-one compounds is via 19-hydroxylation and subsequent elimination of the C_{19} carbon and formation of the aromatic ring system. The mechanism by which the C_{19} carbon is eliminated has not been fully explained. Axelrod and Goldzicher (1962) proposed that the mechanism of aromatization consists of oxygenation of the C-19 group to a primary alcohol or more probably an aldehyde, followed by diaxial transelimination of the 19β- carbon group and the 1α- hydrogen with the formation of an unstable $\Delta^{1(10),4}$-3-ketone. This in turn undergoes spontaneous aromatization to the stable pi-bonded aromatic phenol, which derives the hydrogen of the phenolic hydroxyl group from the 2β-axial hydrogen. A more recent view is that the 19-oxo compound when formed is next hydroxylated in the 1β-position and this hydroxyl group together with the aldehyde group at C_{19} are eliminated together (Townsley and Brodie, 1968). It is interesting to note (Table 9) that Milewich and Axelrod (1971, 1972a, b) have succeeded in isolating both 19-hydroxy and 19-oxo derivatives of androstenedione and testosterone from incubations of baboon placental tissue, with the latter as precursors, thereby indicating that the mechanism of aromatization is probably similar to that described for human placental tissue viz. through formation of 19-hydroxylated intermediates. Of further interest to this discussion is the observation of Preumont et al. (1969) concerning the

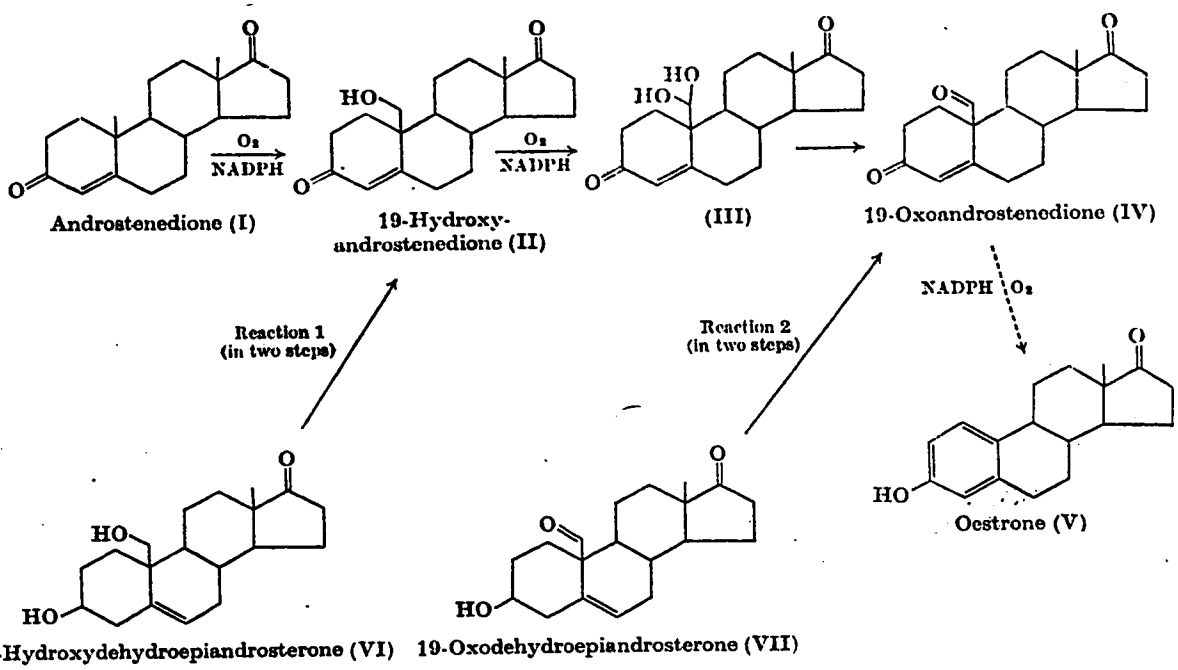


Fig. 1. Mechanism for the production of estrone formed from C_{19}-4-en-3-one compounds. (Figure derived from Akhtar and Skinner, 1968).

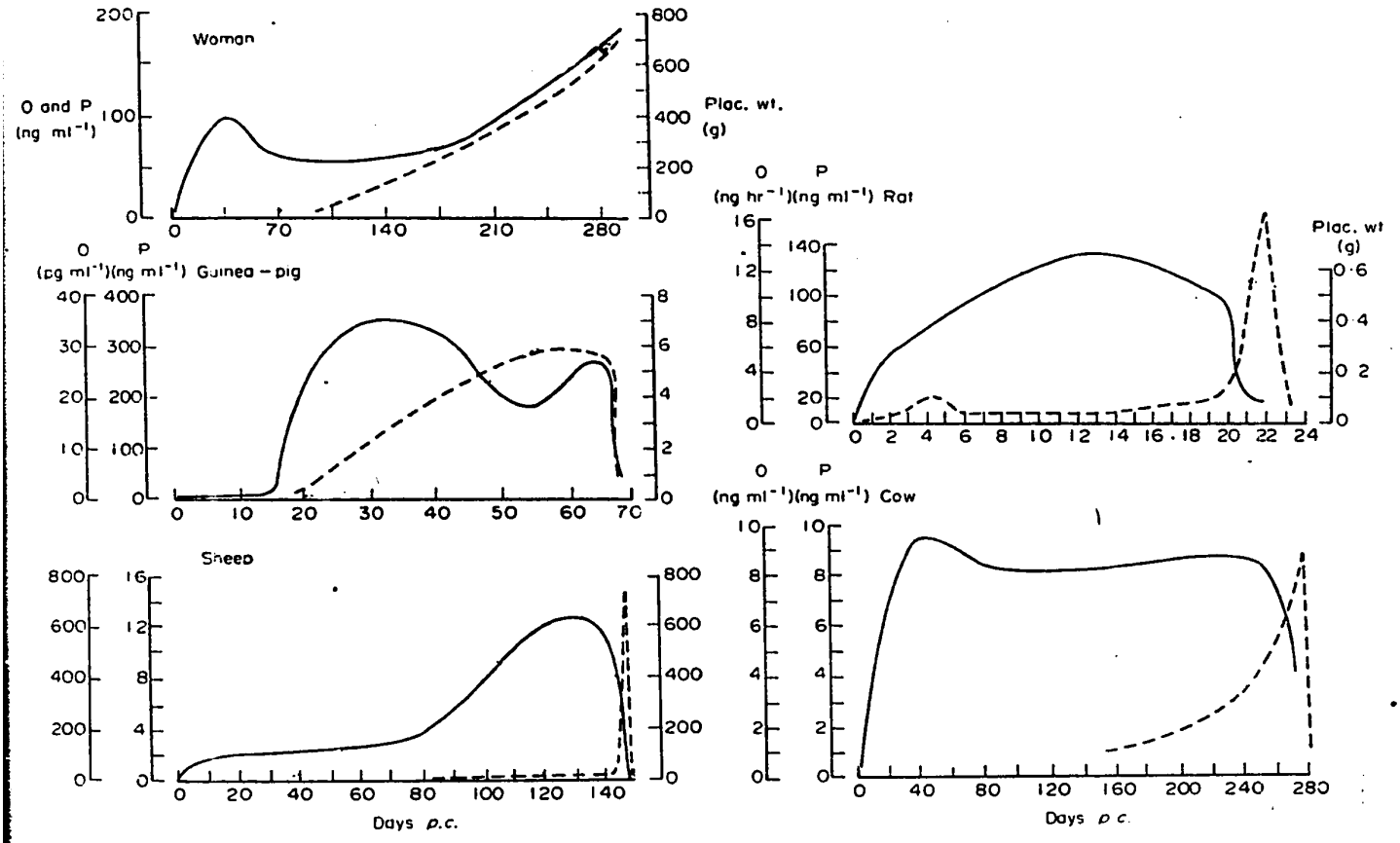
specificity of aromatization of 16-hydroxylated androgens by human placental and corpus luteum tissue. They observed that a number of 16-hydroxylated androgens were unable to undergo aromatization by corpus luteum tissue but could be readily aromatized by placental tissue. In contrast, it was observed that both androstenedione and dehydroepiandrosterone were readily aromatized by corpus luteum tissue. As a result of these observations, these authors suggested that a substrate specificity exists for the aromatizing system of the corpus luteum under the conditions employed, and, furthermore, that ovarian and placental aromatizing systems are different. A further possibility is that a specific enzyme exists in the human placenta for aromatization of 16-hydroxylated C_{19} precursors.

D. STEROID HORMONES IN BODY FLUIDS OF MAMMALS DURING PREGNANCY

Until recently a comparison of the plasma levels of progesterone and estrogens between different species during pregnancy has been complicated by the variety of analytical and sampling techniques that have been employed. However, with the development of protein binding and radioimmunoassay techniques for the quantitation of steroid hormones in plasma, it is now becoming possible to approach this question in a more meaningful way. As will be evident in the subsequent discussion, the information that is now available on the levels of steroid hormones in plasma emphases the complexity of the endocrine regulation of the maintenance of pregnancy in different species.

1. Changes in plasma progesterone and estrogen levels during pregnancy in mammals.

Text fig. 2 shows the variaton in levels of progesterone and estrogens during gestation for five species of mammals. These examples have been chosen to illustrate and compare species where the source of progesterone in pregnancy is predominantly placental and others where it is almost entirely of ovarian origin. In women it appears that the origin of progesterone is placental after the ninth to eleventh week of pregnancy (Holmdahl et al., 1971), in the guinea pig and sheep progesterone originating from the pla-



(Figure derived from Bedford et al., 1972a)

Fig. 2. Circulating hormone levels during pregnancy in five species. Solid line, progesterone; dashed line, estrogens. References: woman: progesterone (Short, 1961; Yannone et al., 1969; Yoshimi et al., 1969), estrogens (Roy and Mackay 1962); guinea pig: progesterone and estrogens (Challis et al., 1971); sheep: progesterone (Bassett et al., 1969), estrogens (Challis, 1971); rat: progesterone (Csapo and Wiest, 1969), estrogens (Yoshinaga et al., 1969); cow: progesterone (Donaldson et al., 1970; Stabenfelt et al., 1970), estrogens (Robinson et al., 1970).

centa is capable of supporting pregnancy but from a later stage of gestation than in women; in the cow pregnancy maintenance is almost wholly dependent on progesterone of ovarian origin; whereas in the rat, progesterone is derived almost entirely but not exclusively from the ovaries.

Some interesting comparisons can be drawn from these examples. In women and guinea pigs, the plasma levels of progesterone increase during pregnancy and reach levels greater than 100 ng/ml, these levels being attained at the time of parturition. In comparison, the plasma levels of progesterone in the sheep and cow are approximately one-tenth those found in women and guinea pigs, and the levels tend to decline shortly before parturition. Although it has been established that this decline in sheep applies in most instances, in some individual animals the fall is not as marked (Bassett et al., 1969; Donaldson et al., 1970; Fylling, 1970). The rat appears to occupy an intermediate position in that the plasma levels are high (about 100 ng/ml) and there is a rapid decrease over the last two days of pregnancy. In the rat, however, the decrease in plasma progesterone is coincident with an increase in the level of 20α-hydroxy-progesterone, which is formed by the corpus luteum due to an increase in activity of 20α-hydroxysteroid dehydrogenase (Wiest, 1968).

The change in plasma estrogen levels during pregnancy show distinct differences in each of the species. In women

and guinea pigs estrogen can be detected in plasma during the first trimester and the levels continue to increase steadily until parturition. However, although estrone and estradiol-17β contribute to the increased plasma levels of estrogen observed in the human, the principal increase is due to the increase in plasma estriol (Tulchinsky et al., 1972). In contrast, the plasma estrogen level in the sheep rises very sharply over the last 24 hours prior to parturition, whereas in the rat the increase, although sharp, takes place more gradually. More comprehensive data than that depicted graphically in fig. 2 has been obtained by Henricks et al. (1972) for the changes in plasma estrogen levels prior to parturition in the cow. These authors showed that the maximum level of estrogen at 14 days prepartum in 10 cows was 510 pg/ml. From this time until parturition the concentration rose rather consistently to a level of about 2600 pg/ml. In each case, however, the level is highest at the time of (woman, sheep, rat), or just before (guinea pig, cow), the onset of parturition.

Changes in the levels of plasma progesterone and estrogens have also been followed during pregnancy in other mammalian species. The species in which changes in plasma progesterone levels have been determined during pregnancy are listed in Table 10. For each species, the highest level obtained during pregnancy and whether or not there is a decline in progesterone levels prior to parturition is listed.

TABLE 10

Circulating maternal peripheral plasma progesterone levels during pregnancy

Species	Gestation (days)	Maximum (ng/ml)	Decline before parturition	Reference
Rhesus monkey	168	3-7	Yes	Hodgen et al., 1972
Bonnet monkey	170	10-20	No	Stabenfeldt and Hendrickx, 1972
Goat	148	6-10	Yes	Irving et al., 1972
Golden hamster	16	18-42	Yes	Lukaszewska and Greenwald, 1970
Rabbit*	31	410-2360	Yes	Mikhail et al., 1961

* value of progesterone in the ovarian venous blood

The data obtained for the bonnet and rhesus monkey show some similarities in that an initial peak occurred in plasma progesterone levels in most cases at about 30 days post-conception. Thereafter, the levels of plasma progesterone in the rhesus monkey remained essentially constant at about 3-8 ng/ml until the time of parturition, whereas the plasma levels in the bonnet monkey showed a definite upward trend beginning at about day 150 of pregnancy and continued through the last blood sample obtained within 12 hours of delivery on about day 170. From a comparative viewpoint, the changes in plasma progesterone in these two species during pregnancy are opposite to those found in the cow and ewe (see fig. 2) in which progesterone levels decline over the last two weeks of gestation. The plasma levels in the goat are similar to those found in the sheep with a prepartum decline occurring over the last 10-14 days of gestation. The changes in plasma progesterone levels in the hamster and rabbit are similar to those observed in the rat, in that the levels begin to decline at about mid-gestation. This becomes more evident if the concentrations of progesterone in the corpus luteum or ovarian vein plasma levels are compared at different stages of gestation (rat: Hashimoto et al., 1968; rabbit: Hilliard et al., 1968; hamster: Lukaszewska and Greenwald, 1970).

In addition to the species listed in Fig. 2, the changes in plasma estrogen levels have also been determined

during pregnancy in the goat and rhesus monkey. The pattern of plasma estrogens in the goat is similar to that observed in the sheep although the levels tend to be higher and the increase associated with parturition is not as dramatic (Challis and Linzell, 1971; Thorburn et al., 1972). In the rhesus monkey the plasma concentration of estradiol-17β shows a slow rise until a pleateau is reached at about day 100. Thereafter, these levels are maintained until term at a level of about 500-600 pg/ml (Hodgen et al., 1972). The sustained increase observed in women during the third trimester or the sharp rise observed just before or at the time of parturition in other species in which plasma estrogen levels have been measured during pregnancy was not seen in the latter part of pregnancy in the rhesus monkey.

2. <u>The nature of naturally occurring progesterone metabolites in the urine of pregnant mammals</u>.

During pregnancy in the human female, progesterone is excreted in the urine in amounts corresponding to a few micrograms per litre (Ismail and Harkness, 1967). It is now well established that progesterone is excreted in the urine principally as 5β-pregnane-3α, 20α-diol (pregnanediol) which is conjugated to glucuronic acid and which in late pregnancy may be excreted in amounts up to 50 mg/day (Klopper and Billewicz, 1963). In addition, many other C_{21} pregnane derivatives related to pregnanediol have been isolated from human pregnancy urine and have been listed by Jeffery and

Klopper (1970). In contrast to human pregnancy urine, the nature and quantities of progesterone metabolites in pregnancy urine from other mammalian species has received much less attention. Table 11 lists the naturally occurring C_{21} pregnane derivatives which have been isolated from the urine of a number of pregnant mammals. Although pregnanediol was isolated from the urine of all the species studied, in instances where estimates were made of pregnanediol concentration in urine collected during late pregnancy it was observed that the levels were considerably lower than those found in human pregnancy urine. In addition, a few studies on the isolation of radioactive metabolites from urine after injection of radioactive progesterone into mature mammals have shown that progesterone is metabolized to pregnanediol in addition to several other pegnanediol metabolites (Table 12). Studies on the metabolism of progesterone-4-^{14}C and 17α-hydroxyprogesterone-6, 7-^{3}H by immature chimpanzees have indicated that the overall metabolism of these two substrates, based on the isolation of urinary radioactive metabolites, is similar to that observed in the human, except for the high proportion of metabolites which are excreted in the unconjugated form (Romanoff et al., 1963). From these studies it is evident that progesterone can be metabolized to urinary pregnanediol by species other than the human female. It is also probable that the amount of isomeric pregnane derivatives excreted is in direct proportion to the

TABLE 11

Isolation of naturally occurring C_{21} steroids from the urine of pregnant mammals

Species	Metabolites isolated	Reference
Pig	5β-pregnane-3α, 20α-diol 5β-pregnane-3α-ol-20-one 5β-pregnane-3α, 6α-diol-20-one 5α-pregnane-3β-ol-20-one	Edgerton and Erb, 1971
Sheep	5β-pregnane-3α, 20α-diol	Robertson and Coulson, 1958
Mare	5β-pregnane-3α, 20α-diol 5α-pregnane-3α, 20α- diol 5α-pregnane-3β, 20α-diol	Marker and Rohrmann, 1939
	5β-pregnane-3α, 20β-diol	Brooks et al., 1952
Cow	5β-pregnane-3α, 20α-diol 5α-pregnane-3α, 20α-diol 5α-pregnane-3β, 20α-diol	Marker, 1938
	5β-pregnane-3α, 20α-diol	Klyne and Wright, 1959
Goat	5β-pregnane-3α, 20α-diol	Klyne and Wright, 1957
Rabbit	5β-pregnane-3α, 20α-diol	Verley et al., 1950
Chimpanzee	5β-pregnane-3α, 20α-diol	Fish et al., 1942. McArthur and Fitzgerald, 1970.
Rhesus monkey	5β-pregnane-3α, 20α-diol	Chamberlain et al., 1964. Liskowski and Wolf, 1972.
Baboon	5β-pregnane-3α, 20α-diol 5β-pregnane-3α, 20β-diol	De la Pena and Goldzieher, 1967
	5β-pregnane-3α, 20α-diol	Merkatz and Beling, 1969

TABLE 12

Radioactive metabolites isolated from the urine of mature mammals after administration of radioactive progesterone

Species	Substrate	Radioactive metabolites isolated	Reference
Pig	progesterone-4-^{14}C	5β-pregnane-3α, 20α-diol 5β-pregnane-3α-ol-20-one 5α-pregnane-3β-ol-20-one	Schomberg et al., 1966
Sheep	progesterone-4-^{14}C	5β-pregnane-3α, 20α-diol 5α-pregnane-3β, 20α-diol 5β-pregnane-3α-ol-20-one 5α-pregnane-3α-ol-20-one 5α-pregnane-3α, 20α-diol 5α-pregnane-3β, 20β-diol 5β-pregnane-3β, 20α-diol 5α-pregnane-3β-ol-20-one 5β-pregnane-3α, 20β-diol 5β-pregnane-3β, 20β-diol	Stupnicki and Williams, 1968
Baboon	progesterone-4-^{14}C	5β-pregnane-3α, 20α-diol 5α-pregnane-3α, 20α-diol 5β-pregnane-3α-ol-20-one	Goldzieher and Axelrod, 1969

plasma progesterone levels which in most animal species are considerably lower than those found in the human.

In contrast to many mammalian species, the feces appear to be an important excretory route for steroids in the sheep and the cow. Hunt et al. (1961) studied the distribution of radioactivity in the feces and urine of a luteal phase heifer injected with estrone-16-^{14}C and found that the ratio of ^{14}C metabolites in the urine and feces in two experiments was between 1:2 and 1:3. Mellin and Erb (1966) reported a similar distribution of radioactivity in the urine and feces of a luteal phase heifer after injection of estradiol-17β-^{14}C. In the ewe, Terqui et al. (1968) and Terqui (1972) have shown that after injection of labeled estrone and estradiol-17β into pregnant and non-pregnant animals the major part of the radioactivity was excreted via the feces. However, in similar studies with sows, Terqui et al. (1968) recovered 88-99% of the injected radioactivity in the urine. Although differences exist between the ruminants and the sow in the importance of the fecal route of excretion of steroid metabolites, the significance of this route of excretion in terms of the quantities and spectrum of steroid metabolites present during pregnancy has yet to be determined.

3. Quantitation and identification of naturally occurring estrogen metabolites in the urine of pregnant mammals.

Jeffery and Klopper (1970) have listed the numerous phenolic steroids which have been isolated from human preg-

nancy urine. In addition to estrone, estradiol-17β and the four isomers of estriol which are quantitatively the most significant phenolic steroids excreted in urine during human pregnancy, various other phenolic steroids with oxygen functions at carbon atoms 2, 6, 11 or 15 and double bonds at carbon atoms 11 or 16 have been isolated in varying amounts. In contrast to the array of phenolic steroids which have been isolated from human pregnancy urine, the estrogens isolated from pregnancy urine of other mammalian species have been limited in most instances to estrone and the isomeric estradiols (Table 13).

Estriol is the most abundant urinary estrogen produced during human pregnancy and at term the levels reached 30 mg/day (Heikkila, 1971). In contrast, most other gravid mammals excrete little or no estriol in the urine. Short and Eckstein (1961) were unable to detect estriol in the urine from pregnant rhesus monkeys, though Laumas (1965) detected small amounts of a compound which he believed to be estriol. Subsequently, more definitive evidence for the presence of estriol in the urine of pregnant rhesus monkeys was provided by Hopper and Tullner (1967). Estriol or estriol like compounds have also been described in the urine of the baboon (Merkatz and Beling, 1969), chimpanzee (McArthur and Fitzgerald, 1970), gorilla (Hopper et al., 1968), sheep (Fèvre and Rombauts, 1966) and cow (Mellin et al., 1966), but only in the gorilla has estriol been shown

TABLE 13

Isolation of naturally occurring estrogens from the urine of pregnant mammals.

Species	Metabolites isolated	Reference
Pig	estrone	Velle, 1958b; Rombauts, 1962; Rommel, 1964; Edgerton and Erb, 1969, 1971.
	estradiol-17β	Velle, 1958b; Edgerton and Erb, 1969, 1971.
	estriol	Velle, 1958b.
Sheep	estrone estradiol-17β estriol	Fèvre and Rombauts, 1966.
Mare	estrone	De Jongh et al., 1931; Savard, 1961; Rommel, 1964.
	estradiol-17β	Wintersteiner et al., 1935; Rommel, 1964.
	estradiol-17α	Hirschmann and Wintersteiner, 1937-38.
	equilin	Girard et al., 1932a, b, c.; Savard, 1961.
	equilenin dihydroequilin-17α dihydroequilenin-17α dihydroequilenin-17β	Hirschmann and Wintersteiner, 1937-38; Glen et al., 1956; Gaudry and Glen, 1958.
	3-desoxyequilenin	Prelog and Fuhrer, 1945.
	$\Delta^{5,7,9}$-estratienol-3-one-17	Heard and Hoffman, 1940; Glen et al., 1958.
Cow	estrone	Pope et al., 1957; Velle, 1958; Klyne and Wright, 1959; Mellin et al., 1966; Rommel 1964.
	estradiol-17β	Velle, 1958a; Mellin et al., 1966.
	estradiol-17α	Klyne and Wright, 1959; Mellin et al., 1966.
	estriol	Mellin et al., 1966.

Goat	estrone estradiol-17β	Klyne and Wright, 1957.
Chimpanzee	estrone estradiol-17β estriol	McArthur and Fitzgerald, 1970.
Rhesus monkey	estrone estradiol-17β estriol	Hopper and Tullner, 1967; Liskowski et al., 1970. Hopper and Tullner, 1967. Hopper and Tullner, 1967; Liskowski et al., 1970.
Gorilla	estrone estradiol-17β estriol	Hopper et al., 1968.
Baboon	estrone estradiol-17β estriol	Merkatz and Beling, 1969.

to be the major urinary estrogen excreted, the level being estimated at approximately 3 mg/day in late pregnancy urine (Hopper et al., 1968).

More definitive evidence for the presence of estriol in the urine of pregnant mammals has been obtained after intravenous injection of a radioactive estrogen or estrogen precursor followed by analysis of urine for the presence of radioactive estrogen metabolites. Using this technique radioactive estriol has been isolated from pregnancy urine of several mammalian species, as shown in Table 14. In addition, estrogens with hydroxyl groups in positions other than at C-16 and isomers of estriol have been identified in the urine of certain species (Table 14).

The ring B unsaturated estrogens, equilin and equilenin and their corresponding dihydro derivatives are excreted exclusively by the pregnant mare (Table 15). Although equilin and equilenin were first isolated from the urine of pregnant mares by Girard et al. (1932a, b) the biosynthetic pathway for formation of these compounds has yet to be elucidated. Gaudry and Glen (1958) have shown that ring-B unsaturated estrogens begin to appear in the urine of pregnant mares about the fourth month of pregnancy. Thereafter, the amounts increase until parturition, at which time these compounds represent about 65% of the total estrogens. These results suggest that the feto-placental endocrine system may be implicated in their synthesis. It can be seen from Table 15 that

TABLE 14

Radioactive estrogens isolated from the urine of pregnant mammals after administration of estrogens or estrogen precursors

Species	Substrate	Metabolite isolated	Reference
Pig	estrone-4-^{14}C estradiol-17β-6, 7-^{3}H	estrone estradiol	Terqui et al., 1968
	estrone-4-^{14}C estradiol-17β-6, 7-^{3}H	estrone 2-methoxyestrone 16α-hydroxyestrone 16-ketoestradiol-17β estradiol-17β estradiol-17α estriol 16-epiestriol 17-epiestriol 16, 17-epiestriol	Terqui, 1971
Sheep	estrone-4-^{14}C estradiol-17β-6, 7-^{3}H	estrone 2-methyoxyestrone 16α-hydroxyestrone estradiol-17α estriol 16-epiestriol 17-epiestriol	Terqui, 1972
Chimpanzee	estrone-16-^{14}C	2-methoxyestrone estradiol-17β estriol	Jirku and Layne, 1965
Baboon	estradiol-17β-^{14}C dehydroepiandrosterone-7-^{3}H dehydroepiandrosterone-7-^{3}H-sulfate	estrone estradiol-17β estriol	Townsley and Kling, 1970.

Rhesus monkey	dehydroepiandrosterone--4-^{14}C dehydroepiandrosterone--7-^{3}H-sulfate	estrone estradiol-17β estriol	Snyder et al., 1971
Baboon	estradiol-17β-4-^{14}C	estrone 2-methyoxyestrone estradiol-17β 15α-hydroxyestradiol-17β 16-ketoestradiol-17β estriol 16α-hydroxyestrone	Leung et al., 1972

TABLE 15

Biosynthesis of ring-B unsaturated estrogens by the pregnant mare

Steroid injected	Route of injection	Urinary estrogens isolated		Reference
		Radioactive	Non-radioactive	
acetate-1-^{14}C	jugular vein	estrone equilin equilenin		Heard et al., 1954.
cholesterol-4-^{14}C	jugular vein		estrone equilin equilenin	Heard and O'Donnel, 1954
testosterone-4-^{14}C	jugular vein	estrone		Heard et al., 1955
estrone-16-^{14}C	jugular vein		equilin equilenin	Heard et al., 1954
dihydroequilenin-17β-4-^{14}C	jugular vein	equilenin	estrone equilin	Savard et al., 1960
dehydroisoandrosterone-7-^{3}H-sulfate	jugular vein	estrone dehydroepiandrosterone	equilin equilenin $\Delta^{5,7,9}$-estratrienol-3-one-17	Bhavnani et al., 1968
Δ^{7}-dehydroisoandrosterone-4-^{14}C-sulfate	jugular vein	estrone	equilin equilenin $\Delta^{5,7,9}$-estratrienol-3-one-17	
estrone-4-^{14}C	jugular vein	estrone	equilin equilenin	
dehydroisoandrosterone-7-^{3}H and androstenedione-4-^{14}C	umbilical vein	estrone	equilenin equilin	

dehydroisoandrosterone-7-^{3}H and androstenedione-4-^{14}C	umbilical vein	estrone estradiol-17α estradiol-17β	equilin equilenin dihydroequilin-17α dihydroequilenin-17α	Bhavnani et al., 1969
Sodium acetate-1-^{14}C and cholesterol-7-^{3}H	umbilical circulation	estrone[a] estradiol-17α[a] equilin[b] equilenin[b] dihydroequilenin-17α[b]		Bhavnani et al., 1971

[a] contained both ^{3}H and ^{14}C

[b] contained only ^{14}C

the ring-B unsaturated estrogens can be isolated in radioactive form from urine after injection of ^{14}C-labeled acetate. The absence of radioactivity in these compounds after injection of labeled cholesterol or several other steroid precursors into either the maternal or fetal circulation suggests that these compounds are not synthesized via a pathway involving cholesterol as an obligatory intermediate. Evidence supporting this concept was obtained by Bhavnani et al. (1971) who showed that after injection of ^{14}C-acetate and ^{3}H-cholesterol into the fetal circulation of pregnant mares' only estrone and estradiol-17α contained both labels when subsequently isolated from the urine; the ring-B unsaturated estrogens contained only ^{14}C. These results demonstrated that the ring-B unsaturated estrogens were not formed via the classical pathway of steroid formation but via a series of reactions, distinct from those leading to the formation of estrone and estradiol, which utilize intermediates prior to the formation of cholesterol. These observations provide the first example of a biosynthetic pathway leading to steroid formation in which cholesterol is not an obligatory intermediate.

It is of interest to compare the changes in plasma estrogen levels with the changes in urinary estrogen levels during pregnancy in those species to which both parameters have been measured. In the cow, as with the human female, it has been shown that the changes in plasma estrogen levels

correlate closely with the urinary levels (Robertson, 1973; Hunter et al., 1970). However, in contrast to the human where it has been established that estriol is the predominant estrogen present in both plasma and urine (Fischer-Rasmussen, 1971), in the cow the principal estrogen present in plasma is estrone (Robertson, 1973) whereas estradiol-17α is the predominant urinary estrogen (Hunter et al., 1970).

Differences in the nature of the estrogens present in different body fluids and tissues has been demonstrated also for the baboon and ewe. In the baboon it has been shown that the principal estrogen present in placental tissue in vitro and in vivo is estradiol-17β (Ainsworth et al., 1969; Milewich and Axelrod, 1971; Solomon and Leung, 1972) whereas estrone is the major urinary estrogen (Merkatz and Beling, 1969; Leung et al., 1972; Townsley and Kling, 1972). In contrast, the principal circulating estrogen in the pregnant ewe is estrone (Robertson and Smeaton, 1973) and the principal urinary estrogen is estradiol-17α (Fèvre et al., 1965; Fèvre and Rombauts, 1966). These latter authors also showed that the urinary estrogen levels of estradiol-17α and estrone begin to rise rapidly between 90-100 days of pregnancy. The levels of estrone continue to rise steadily until about 140 days at which time there is a sharp increase which continue until parturition (levels vary between 20-80 μg/24 hrs). In contrast, the levels of estradiol-17α appear to plateau or decline between 120-140 days of pregnancy at levels of

about 30-50 µg/24 hrs and then rise sharply beginning at about 140 days until parturition to levels of 50-90 µg/24 hrs. This pattern of urinary estrogen excretion contrasts markedly with the observed changes in plasma estrogens, which can only be detected at basal levels until approximately 24-48 hrs before parturition when the levels rise and fall dramatically over a period of 48-72 hrs (Challis, 1971; Thorburn et al., 1972; Liggins et al., 1972; Robertson and Smeaton, 1972).

Changes in the plasma levels of estrogens in the sow are available only for late pregnancy (Shearer et al., 1972; Robertson and King, 1973). The data of Robertson and King (1973) shows that estrone is the principal plasma estrogen during late pregnancy. This observation corresponds with the urinary excretion of estrogens in which estrone is also the principal estrogen excreted by the sow during pregnancy (Rombauts, 1962; Lunaas, 1962; Raeside, 1963). It is also of interest to observe that the rise in plasma estrogens parallels very closely the rise in urinary estrogens during pregnancy in the sow. In late pregnancy the level of estrone in sow urine is of the order of 10-12 mg/24 hrs (Rombauts, 1962; Raeside, 1963) compared to the plasma level of estrone which is about 4 ng/ml (Robertson, 1973). It has also been demonstrated that estrone is the principal urinary end product after injection of estradiol-17β into the sow, thereby indicating that estradiol-17β may be transformed

periphally prior to excretion (Lunaas, 1963). Of further interest in relation to urinary estrogen excretion during pregnancy in the sow is the observation that the levels of urinary estrone do not differ significantly from those during the estrus cycle until the end of the third week after breeding, at which time the levels rise to a peak by the end of the fourth week. The levels then drop to levels comparable to those obtained at estrus until approximately 5 to 6 weeks before parturition, when the urinary estrone levels again increase and rise steadily but steeply until parturition (Rombauts, 1962; Lunaas, 1962; Raeside, 1963). It has been suggested that the early peak in estrone coincides with the time during which the fetal membranes become expanded and vascularized (Lunaas, 1962). It is also logical to assume that the estrogen produced at this time is of ovarian origin and more particularly is synthesized by the corpus luteum, since it has been demonstrated that the corpus luteum of the sow readily synthesizes estrogen from C_{19} precursors (Preumont et al., 1969). In contrast, it would appear that the placenta is the major source of estrogen synthesis in late pregnancy as the levels of urinary estrogen drop precipitously shortly after parturition (Rombauts, 1962; Raeside, 1963).

In the rhesus monkey it has been established that estrone is the major urinary estrogen and that its concentration increases moderately during the last two months of pregnancy

(Short and Eckstein, 1961; Laumus, 1965; Hopper and Tullner, 1967; Liskowski et al., 1970). Over the week before parturition estrone excretion increases markedly (Liskowski et al., 1970). This pronounced increase in late pregnancy was not observed by Laumas (1965) or Hopper and Tullner (1967). However, Hodgen et al. (1972) showed that although the urinary estrone levels rose significantly over the last week of pregnancy, the peak occurred approximately four days before parturition, after which time the estrone levels decreased markedly during the three days preceding delivery. These latter authors also showed that the plasma levels of estradiol-17β rose slightly just before parturition.

E. ENDOCRINE CHANGES ASSOCIATED WITH PARTURITION IN MAMMALS

The mechanism whereby the fetus is expelled from the uterus is regulated by a complex interaction of endocrine, neural and mechanical factors. The factors which regulate the development of myometrial contractility are vital for each species since they have to ensure the fetus is born at the optimum stage of maturity for extra-uterine existence. A feature which is characteristic of most, if not all, mammals is the essential role played by progesterone and/or estrogens in the maintenance of the pregnant state. The interruption of progesterone secretion results in the rapid termination of gestation and it has been proposed that the withdrawal of progesterone may constitute an important stimulus to the onset of parturition. Whether this statement can be applied to all species is still the subject of considerable debate. In recent years the hypothesis that progesterone blocks myometrial activity during pregnancy and that its withdrawal induces parturition, has received considerable attention (Csapo, 1961, 1969). Other theories have implicated a change in the progesterone-estrogen ratio , an increase in uterine volume, a rise in estrogen secretion, a release of pharmacology active substances (oxytocin, prostaglandins, acetecholamines etc.) and activation of neural mechanisms as contributory factors to the onset of delivery. These topics have been discussed in detail in several reviews and will not be reiterated here (Schofield,

1968; Csapo, 1969; Fuchs, 1971a, b; Jung, 1970). In light of present knowledge regarding the essential nature of progesterone in the maintenance of pregnancy and the recent evidence that implicates the fetus in the initiation of parturition, the changes in steroid hormone levels leading to parturition in several species will be discussed in so far as they reflect changes in the endocrine environment leading to stimulation of uterine activity, cervical relaxation and the onset of labor.

The changes in plasma progesterone and estrogen levels during pregnancy in a number of species have been reviewed in a previous section (D-1). The comparisons of changes in plasma progesterone levels have suggested that the circulating level of progesterone declines before parturition, in some, but not all species. In those species in which there is no apparent withdrawal of progesterone before the onset of parturition, it is necessary to examine more closely the evidence which is available. In the pregnant guinea pig it has been shown that although there is a 100-fold increase in plasma progesterone concentrations, this is accompanied by a decrease in the metabolic clearance rate of approximately 90% and an increase in production rate of only about 3-fold (Illingworth et al., 1970; Challis et al., 1971). This alteration in the kinetics of metabolism is related to the production during pregnancy of a progesterone binding protein, a plasma protein which has a high affinity for pro-

gesterone (Milgrom et al., 1970). This protein has the capacity to conserve a large pool of progesterone in circulation and buffers variations in endogenous secretion by protecting the steroid from rapid metabolism by hepatic and extrahepatic tissues. The coypu, another hystricomorph rodent, has been shown to have a similar progesterone conserving mechanism (Illingworth and Heap, 1971). A further interesting feature of the action of progesterone in the pregnant guinea pig has been described by Porter (1970), who demonstrated that this steroid had little effect on the spontaneous or oxytocin-induced activity of the myometrium. It has been suggested that myometrial activity in this species may be regulated by a factor other than progesterone, by changes in the local concentrations of readily available progesterone or the progesterone-estrogen ratio before the onset of increased uterine activity at the time of parturition (Challis et al., 1971). The hormone regulation of pregnancy in this species still remains a puzzle and cannot be related to theories on initiation of parturition which have been advanced for other species.

The pregnant woman is a further example where the progesterone withdrawal theory meets criticism,since plasma progesterone levels may reach their highest levels at the time of parturition. It is again possible, however, that changes in local concentration of progesterone or increased uterine metabolism may contribute to the removal of the pro-

gesterone block at term, but direct experimental evidence to verify these statements is lacking at the present time.

A further important aspect that needs to be considered in the context of factors that influence parturition in different species is the changes in the secretion of estrogens during pregnancy. Estrogens are known to have numerous effects on the mammalian uterus; they enhance rhythmic contractions in the non-pregnant animal, cause blood vessel dilation and increased permeability, and aid the electrical conductance of the myometrium and its response to oxytocin stimulation (Carsten, 1968). In the mammalian species which have been considered in Section D-1, the plasma concentration of estrogens reach their highest levels at the onset of parturition. Thus, a rapid rise in biologically active and readily available estrogen immediately prior to parturition could provide an important component of the physiological trigger of parturition, either by converting the uterine environment from that of a 'progesterone dominant' uterus to one of estrogen dominance and thereby overcoming the progesterone block, or by having a direct stimulatory effect on myometrial contratility.

If progesterone plays a major role in the maintenance of gestation and estrogen plays an integral part in the initiation of parturition, it might be expected that exogenous hormone treatment would affect the normal time of delivery. In general, such experiments have frequently resulted in

inconclusive data since the results are dependent upon the stage of pregnancy at which treatment is administered (Deanesly, 1966). In rabbits (Heckel and Allen, 1938, 1939), rat (Selye et al., 1935) and cats (Courrier, 1945) administration of estrogens may lead to prolongation of gestation although stimulation of the uterine contractions causes the fetuses to die. The results in rats obtained by Selye et al. (1935) are apparently contradictory to those of Csapo (1969) but the differences may be due to dosage since Selye et al. (1935) administered 500 µg of estrone per diem, whereas Csapo (1969) observed that 100 µg of estradiol propionate was sufficient to induce parturition. In a few species, progesterone prolongs gestation, as in rabbits and rats (see Deanesly, 1966), however, no effect has been demonstrated in the guinea pig (Ehrhardt and Kneip, 1942; Zarrow et al., 1963; Porter, 1969) or the cow (McDonald and Hays, 1958).

In the sheep, daily administration of 80 mg progesterone over the last week of pregnancy failed to delay the time of parturition, although in some cases labor was protracted (Bengtsson and Schofield, 1963). Furthermore, continuous intramuscular infusion of progesterone has failed to delay parturition induced prematurely by ACTH (Liggins, 1969b). Further observations by Liggins et al. (1972) has shown that 150 mg progesterone administered daily to pregnant ewes at the same time as dexamethasone was being infused into the fetus failed to delay the onset of labor, but despite con-

tinuous uterine activity similar to that observed in normal labor, the cervix failed to dilate over a period of 3 to 5 days and fetal death subsequently occurred due to amniotic infection. These results suggest that a fall in plasma progesterone concentration is unlikely to be a major factor controlling the time of onset of parturition in this species. This does not mean that progesterone withdrawal would not lead to parturition. Rather, it may be considered that the fall in progesterone concentration that normally occurs at term may contribute to the mechanism of parturition and may be important in relation to efficiency of uterine activity and of normal labor.

In spite of the fact that parturition is a frequent and recurrent phenomenom and that a vast amount of literature on the subject has accumulated over the past several decades, it is only recently that interest in this field has been rekindled. It should also be noted that Hipprocrates (460 B.C.) was the first to suggest that the fetus plays a major role in determining its own destiny. In the Hippocratic text it is stated "when the child is grown big ... he incontinently passes out into the outside world, free from any bonds". It is only within recent years that evidence implicating the fetus in the initiation of events leading to parturition has been verified experimentally, largely through the elegant studies of Liggins and his co-workers in the sheep (Liggins et al., 1972).

The studies of Holm (1966), Liggins et al. (1967) and Drost and Holm (1968), in particular, have indicated the importance of a functional fetal pituitary-adrenal axis for the occurrence of natural parturition in sheep since fetal adenohypophyseal aplasia or intrauterine fetal hypophysectomy or adrenalectomy indefinitely prolongs gestation. Furthermore, Liggins (1968, 1969b), Van Rensburg (1967) and Halliday and Buttle (1968) have demonstrated that in the sheep premature parturition can be induced after injection or infusion of synthetic corticotropin (Synacthen) or the glucocorticoids cortisol and dexamethasone into the fetus in utero. That the fetal adrenal cortex may be involved in the events leading to normal parturition in sheep has been further substantiated by Basset and Thorburn (1969) who showed that the levels of fetal plasma corticoids began to increase several days before parturition, the highest concentrations being reached at the time of birth. No comparable increase was demonstrated in maternal plasma levels. These observations were similar to those seen in acute studies (Alexander et al., 1968) and gave additional support to the view that activation of the fetal adrenal cortex is involved in the initiation of parturition in sheep. Prior to these studies on the sheep, much of the evidence for the existence of an autonomous fetal pituitary-adrenal axis had been derived from experiments with rats, mice and rabbits (Jost, 1966). Recently, evidence for an autonomous pituitary-adrenal axis in the rhesus monkey

was demonstrated by Kittinger et al. (1972) who showed that decapitation of fetuses at 150-156 days of pregnancy led to a progressive decline in the secretion rate of cortisol from the fetal adrenal. Injection of ACTH resulted in a stimulation of the secretion rate to levels comparable with those of intact fetuses stimulated by ACTH. In contrast, constant infusion of maximally stimulating quantities of ACTH into the mother did not increase the fetal adrenal secretion rate of cortisol, suggesting that ACTH does not cross the placental barrier and that rhesus monkey fetuses near term have an autonomous pituitary-adrenal function. Indirect evidence that a pituitary-adrenal axis functions also in human fetuses has been proposed from observations on anencephalic infants which are characterized by atrophy of the adrenal cortex and grossly impaired hypothalamic and pituitary development (Benirschke, 1956; Johannison, 1968).

Studies on the induction of parturition in sheep and goats by administration of a synthetic corticotrophin (Synacthen, Ciba) or glucocorticoids (cortisol or dexamethasone) to the fetus have been extended by Liggins et al. (1972) and Thorburn et al. (1972) to establish experimental models by which the endocrine events associated with parturition can be accurately followed. The validity of the conclusions drawn from observations on such models can be questioned but it is significant that the major endocrine changes recognized in the ewe and the fetus at the time of

normal parturition (Bedford et al., 1972a; Nathanielsz et al., 1972) also occur before premature parturition induced with corticotropin or glucocorticoids. In addition, to the changes in maternal plasma progesterone and estrogen and fetal plasma corticoids leading to parturition in the ewe, a further endocrine event has been described by Liggins and Grieves (1971), Liggins et al. (1972) and Thorburn et al. (1972). These authors have demonstrated the presence of prostaglandin F2α in uterine venous plasma during labor and have shown a several fold increase in its concentration in myometrium and maternal cytoledons with the onset of labor, but the concentration in fetal cotyledons showed no change. As a result of these investigations and those of Bedford et al. (1972a) and Nathanielsz et al. (1972) the sequence of observed changes in the concentration of fetal plasma corticoids, maternal progesterone and estrogen and uterine venous plasma prostaglandin F2α shortly before and at the time of parturition and the possible sequence of events leading to parturition in the pregnant ewe can be summarized as shown in Fig. 3 and Fig. 4. The main conclusions which can be drawn from these studies are that parturition can occur when the fetal adrenals are stimulated with ACTH or when dexamethasone is administered to the fetus in the absence of either a fall in plasma progesterone or arise in circulating estrogen. It is unlikely that a change in levels of either of these two steroids is the prime determinant

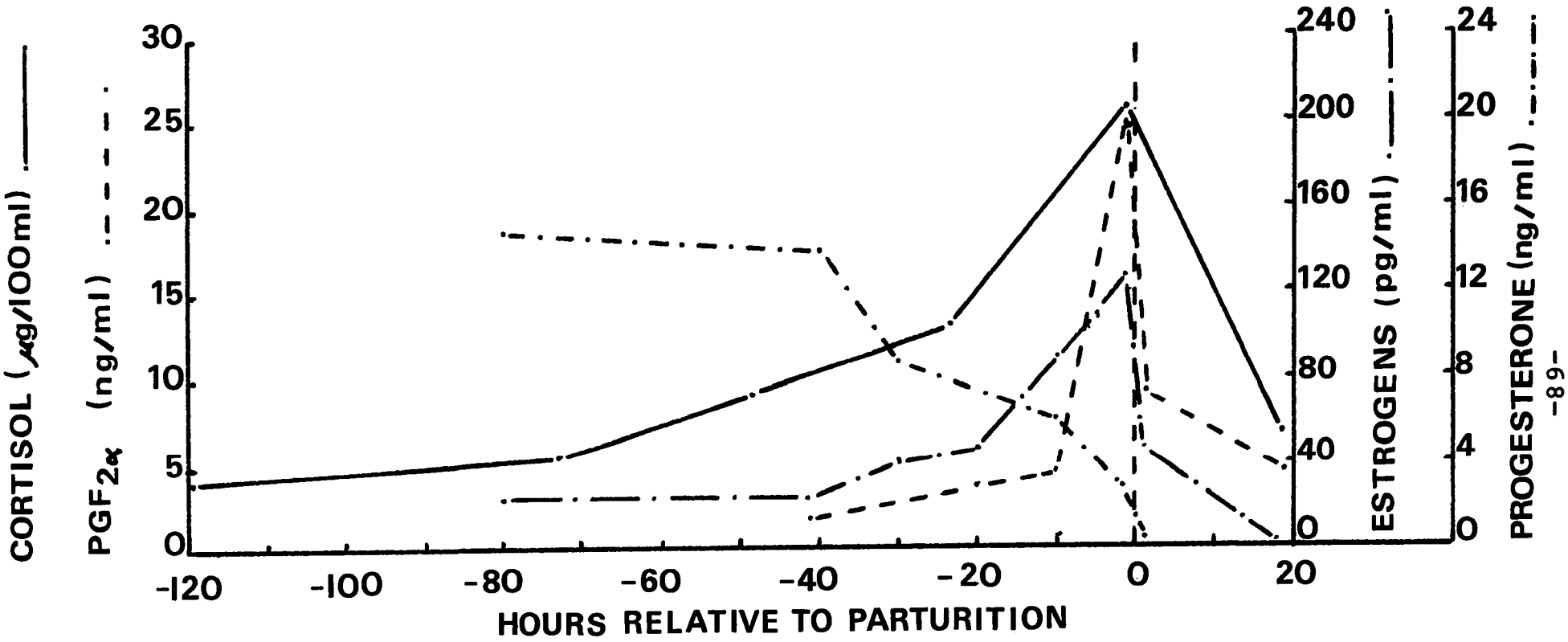


Fig. 3. Composite graph to illustrate changes in uterine venous blood concentration of progesterone, total unconjugated estrogens, prostaglandin F2α ($PGF_{2\alpha}$) (Challis et al., 1972)* and fetal plasma cortisol (Nathanielsz et al., 1972)** in sheep.

* values from one animal

** values mean of 10 animals

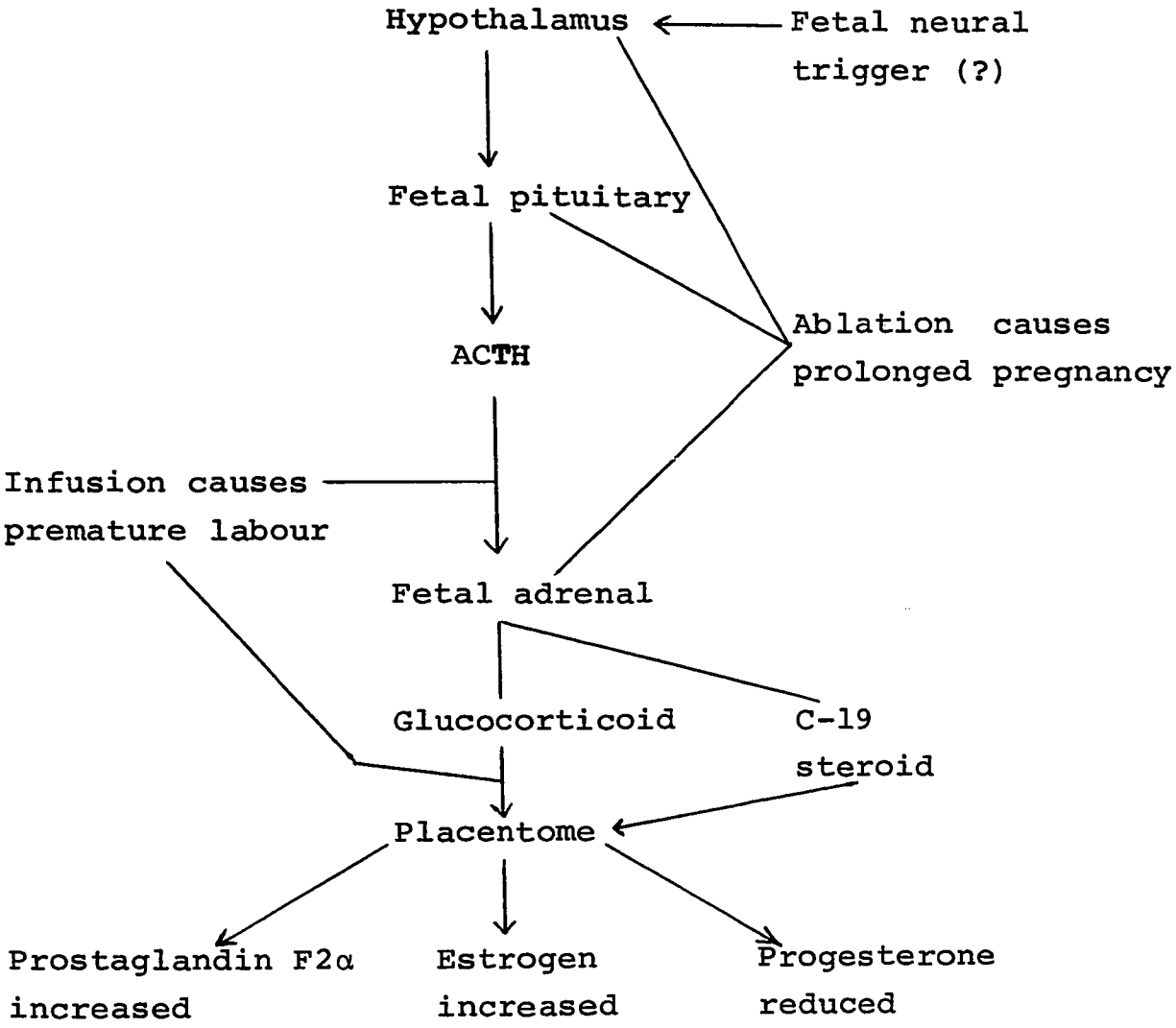


Fig. 4. Diagram of the possible fetal mechanisms controlling ovine parturition. (Derived from Liggins et al., 1972).

of parturition. It remains to be determined whether prostaglandin F2α can fulfill this role. Recent work by Challis et al. (1972) in which the concentration of unconjugated estrogens and prostaglandin F2α was measured in uterine venous blood of a sheep just before parturition has shown that the level of unconjugated estrogen began to rise approximately 30 hrs before parturition whereas the levels of prostaglandin F2α did not rise significantly until approximately 8 hrs before lambing. It may be that the sharp rise in estrogen secretion immediately before parturition stimulates the uterine secretion of prostaglandin F2 just before delivery. However, it seems probable at the present time that the normal mechanism(s) of parturition involves an interplay of these three hormones (Fig. 4). Liggins (1972) has suggested that ovine parturition may be the outcome of a cascade phenomenon whereby the initial stimulus of prostaglandin F2α synthesis is provided by the increase in fetal plasma corticoids. This is followed by an explosive release of free estrogens and prostaglandin F2α as each stimulates production of the other. However, the recent observations of Challis et al. (1972) relating to the temporal changes of estrogen and prostaglandin F2α secretion in uterine venous plasma would tend to negate this hypothesis. Furthermore, it is obvious that some of the earliest changes associated directly or indirectly with the onset of parturition are to be observed in the fetal lamb. Hopkins and Thorburn (1971)

have demonstrated a depression in fetal thyroxine levels 5 to 10 days before parturition and as a prerequisite to increased corticoid secretion by the fetal adrenal there must be an activation of the fetal hypothalamus to allow increase in fetal ACTH. The events that have been discussed constitute a later maternal component of the regulation of parturition. It has not yet been established whether estrogens are formed from an increased supply of maternal or fetal precursors, or whether they are derived from an alteration in feto-placental conjugation. However, it is of interest that unexpectedly high levels of estrogen sulfates have been demonstrated in sheep fetal plasma with little or no free estrogen being detected (Findlay and Cox, 1970). Both fetal and placental tissues have also been shown to possess both sulfokinase and sulfatase enzyme systems capable of synthesizing and cleaving estrogen and neutral steroid sulfates (Ainsworth, 1972; 1973). However, the physiological significance of these observations in relation to the availability and metabolism of both neutral and phenolic steroids in the feto-placental endocrine system of the sheep has not yet been established.

F. DYNAMICS OF STEROID PRODUCTION AND FETO-PLACENTAL TRANSFER OF STEROIDS DURING PREGNANCY IN MAMMALS.

The general principles, techniques and theoretical considerations involved in the application of isotope-tracer kinetic methods to studying in vivo the dynamics of steroid hormones have been the subject of several excellent reviews (Gurpide et al., 1963; Tait and Burstein, 1964; Mann and Gurpide, 1966; Baird et al., 1969; Gurpide, 1972). The two most widely used descriptive parameters in the study of steroid dynamics in various physiological states are the metabolic clearance rate (MCR), which is defined most generally as the volume of blood from which the steroid is completely and irreversibly cleared per unit time, and the production rate (PR), which is defined as the total amount of steroid irreversibly entering the blood per unit time. It is beyond the scope of this review to consider the practical and theoretical aspects involved in the determination of these two parameters and the reader is referred to the reviews quoted above for a full and in depth treatment of the subject.

By use of isotope-tracer kinetic methods, the production rates of several hormones have been determined in human females during pregnancy and non-pregnancy as shown in Table 16. It can be seen that in late pregnancy the PR of progesterone is increased about 100 times, that of estriol about 1000 times and that of aldosterone about 10 times above the levels obtained

TABLE 16[a]

Urinary production rates of cortisol, corticosterone, aldosterone, progesterone, estradiol and estriol in the human female during pregnancy

Species	Mean PR (mg/24 hr) (non-pregnant)	Gestation		Reference
		PR at 4-5 months (mg/24 hr)	PR at term (mg/24 hr)	
Cortisol	18-22	20-23	20-24	Cope and Black, 1959; Burstein et al., 1967.
Corticosterone	1.8-3	4-5	4-6	see Pasqualini, 1970.
Aldosterone	0.1-0.15	0.3-0.8	0.9-1.5	Jones et al., 1959; Vande Wiele et al., 1960; Watanabe et al., 1963.
Progesterone	3-4	-	200-350	Pearlman, 1957; Vande Wiele et al., 1960.
Estradiol	0.15-0.25	7-10	23-30	Gurpide et al., 1963; Siiteri and MacDonald, 1963.
Estriol	0.2-0.3	60-100	200-270	Gurpide et al., 1963

[a] Table derived from Pasqualini et al., 1970.

during the non-pregnant state. The PR of corticosterone shows only a slight rise whereas that of cortisol remains the same as in the non-pregnant state. Since the MCR for progesterone, aldosterone and estrogens remains essentially the same in both the pregnant and non-pregnant states, the increase in PR is due almost entirely to increased secretion of the steroid from either or both the maternal or fetal compartment, which is reflected in the increase in plasma levels and urinary metabolites.

Progesterone production during human pregnancy has been studied more extensively than the other hormones cited in Table 16, and estimates of production rates have been made by several different methods. Zander and von Munstermann (1956) calculated the placental production of progesterone after measurement of progesterone concentration in the uterine venous effluent and peripheral plasma and found values of 190-280 mg/day in the last trimester. Pearlman (1957) measured the specific activity of urinary pregnanediol following intravenous administration of progesterone and calculated the progesterone urinary PR to be 250 mg/day at 35-36 weeks of pregnancy. Progesterone production has also been estimated from the 24-hour excretion of pregnanediol (Shearman, 1959) and from the 15% urinary recovery rate of administered progesterone in the form of urinary pregnanediol (Klopper and Michie, 1956). The PR of progesterone calculated from these studies was 260 mg/day (range 90-420 mg/day). These values

agree very well with the plasma production rates obtained during late pregnancy by Little and Billiar (1969) and by Lin et al. (1972). These authors calculated the plasma PR of progesterone in the third trimester of pregnancy to be 210 ± 77.8 mg/day. Although the values obtained by these different methods are similar for pregnant women, in non-pregnant women and males the blood method should be used since Arcos et al. (1964) have shown that urinary PR estimations in these latter instances are unreliable because of the large contribution of precursors other than progesterone to the specific activity of pregnanediol.

The techniques of isotope tracer kinetics have also been used to study the kinetics of cortisol, progesterone and estrogen metabolism in the pregnant sheep. Paterson and Harrison (1967, 1968)have shown that the MCR of cortisol in ewes rose from a steady value of 0.60 - 0.66 l plasma/min (860-950 l/day) to 0.86 l/min (1240 l/day) just before lambing. In similar studies with ewes in late pregnancy Dixon et al. (1970) obtained values for the MCR between 1600-1900 l of plasma/day while Beitins et al. (1970) obtained a mean value of 1392 l/day in three ewes at about 120 days of gestation. Recently, several authors have calculated the MCR and PR of cortisol in the fetus of the pregnant ewe (Table 17). It will be noted that the MCR of cortisol in the fetus is approximately one tenth of that found in the mother. The differences in the MCR and PR observed

TABLE 17

Metabolic clearance rates and blood production rates of cortisol in the fetus of the pregnant ewe.

Gestational age	Number of animals	MCR (l/day)	PR (mg/24hr)	Reference
134-140	3	106	1.49	Dixon et al., 1970.
Approx. 120	3	92	2.14	Beitins et al., 1970.
130-140	6	58	0.94	Nathanielsz et al., 1972.

by the three groups of workers may be due in part to the difference in breeds used. Dixon et al. (1970) and Beitins et al. (1970) performed their experiments on Dorset ewes whereas Nathanielsz et al. (1972) used Welsh Mountain ewes. The fetus of the Dorset breed is approximately 1.5-2.0 times larger than that of the Welsh Mountain breed at comparable stages of pregnancy.

Using similar techniques Bedford et al. (1972a, b) have investigated the MCR and PR of estrogens and progesterone during pregnancy in sheep and in particular during the week preceding parturition. Similar studies on the MCR and PR of fetal cortisol have been made by Nathanielsz et al. (1972). A summary of the results is presented in Table 18. The MCR of progesterone in blood is high and very similar to that reported for pregnant women (Little and Billiar, 1969; Lin et al., 1972) even though the weight of a sheep is only about half that of the human adult. The MCR of progesterone rose slightly towards the end of pregnancy but this increase was not observed when the values were expressed in terms of l/kg. The PR of progesterone was closely reflected by changes in the blood concentrations. The PR was highest during the last two weeks of pregnancy, being about 5 times greater than that observed during the first 62 days. However, large variations in PR were apparent after about day 100 of pregnancy. The PR in ewes bearing lambs over 4 kg birth weight was significantly greater than those ewes with smaller lambs.

TABLE 18

Kinetics of estrogen, progesterone and cortisol production during pregnancy in sheep

	PROGESTERONE			
	Days of pregnancy			
	7-62	66-103	116-134	135-145
MCR_{Blood} (l/min)	3.2	3.2	3.2	4.0
Blood level (ng/ml)	2.0	3.8	6.0	7.5
PR (µg/ml)	7.0	15.0	22.0	35.0

	ESTROGEN[a]			
	Time before parturition			
	-16.5 days	-8.5 hrs	-20 min	+10 min
MCR_{Blood} (l/min)	3.87(2.85)			
Blood level (pg/ml)	5.5(3.4)	197.5(20.0)	559(108)	496(83)
PR (µg/min)	0.018(0.007)	0.66(0.05)	1.9(0.2)	1.7(0.2)
$SR_{ut.v.}$ (µg/min)			1.39(0.23)	

	CORTISOL (FETUS)[b]		
	Time before parturition		
	-4 days	-3 days	-2 days
MCR_{Blood} (l/min)	58(26.4)	64.8(24.9)	89(31.8)
Plasma level (µg/100 ml)	1.8	4.0	12.2
PR (ng/day)	0.94	2.59	9.17

[a] Figures represent the values obtained for estrone. The corresponding values for estradiol-17β are represented in parentheses.

[b] Figures in parentheses represent the MCR_{blood} in terms of l/kg.

The authors have suggested that this difference maybe related to stimulation of placental progesterone secretion due to rapid increase in uterine volume associated with fetal growth, which is most pronounced during the last two weeks of pregnancy (Barcroft, 1946). The conversion ratio of progesterone to 20α-dihydroprogesterone was also estimated and found to be 77%. The relative concentration of compounds under steady state conditions were similar to the endogenous levels reported by Short and Moore (1959), indicating that the 20α-dihydroprogesterone in blood is derived almost entirely from progesterone. It should also be noted that the radioactivity present in these two steroids represented only about 10% of the total radioactivity present in the blood during constant infusion of labeled progesterone (Bedford et al., 1972b). A further point observed by these authors is that there is approximately a 25% extraction of progesterone and a production (9.1%) of 20α-dihydroprogesterone by the head. Whether this is due to uptake and metabolism by the brain has not been established.

For the estrogens a significantly higher MCR of estradiol-17β was observed between day 141 of pregnancy and parturition (3.2 l/min) compared to that observed between days 100 and 140 (2.3 l/min). In Table 18 the average value is listed. Furthermore, the MCR of estrone was significantly greater than estradiol-17β. Table 18 also lists the changes in plasma levels and PR of estrone and estradiol-17β for one

sheep just before, during and just after parturition. The plasma levels and PR of both estrogens began to rise rapidly just before parturition. At the time of parturition the PR of estrone was approximately 10 times that of estradiol-17β. The authors (Bedford et al., 1972a) also established that immediately before parturition there is a good correlation between uterine secretion rates of estradiol-17β and estrone and their PR, thus supporting the view that the uterus is the principal source of estrogens at this time.

Table 18 also shows that the fetal MCR of cortisol remains relatively unchanged during the several days prior to parturition although there are indications that the MCR may increase during the last 48 hrs of intra-uterine life. There is also a marked and statistically significant increase in the PR of cortisol in the fetal compartment when the values found up to 4 days prepartum are compared to those during the last 48 hours of intra-uterine life. These results strongly suggest that the increase in circulating fetal plasma levels and PR of cortisol is causally related to parturition, since prevention of this rise by fetal hypophysectomy or adrenalectomy will prolong pregnancy (Liggins et al., 1967; Drost and Holm, 1968). The results also show that the amount of cortisol produced within the fetal compartment is sufficient to produce parturition when compared with amounts used by Liggins (1968) to precipitate premature parturition by infusion of glucocorticoids into the fetus.

The MCR of progesterone in the sheep can be compared with that of the guinea pig where placental progesterone production is also appreciable. In the non-pregnant guinea pig the MCR is high (about 0.125 l/min/kg). In pregnancy, however, the level is reduced markedly to about 0.01 l/min/kg (Illingsworth et al., 1970). This reduction in MCR is thought to be associated with the increased concentration of a high affinity binding protein for progesterone in the blood (Milgrom et al., 1970; Allouch et al., 1972). Similarly, in comparing the production rates of progesterone in pregnancy, the maximum PR in sheep averaged 56 mg/day (Bedford et al., 1972b), in women the maximum PR averaged 210 mg/day (Lin et al., 1972) and in guinea pigs the maximum PR averaged 1.2 mg/day (Challis et al., 1971).

An experimental design has been proposed by Mann and Gurpide (1966) and Gurpide and Vande Wiele (1969) which allows the measurement of the rates of secretion of steroid hormones into fetal and maternal circulations and feto-maternal transfer of hormones under physiological conditions. The technique requires the simultaneous administration of labeled hormones into the fetal and maternal systemic systems. Using this experimental approach Dixon et al. (1970) and Beitins et al. (1970) showed that the transfer of cortisol from mother to fetus was minimal and suggested that a placental barrier exists in the sheep during pregnancy with respect to transfer of cortisol. Similar results were obtained

by Bayard et al. (1972) using labeled dexamethasone. These results substantiate the observations of Liggins (1969b) and Fylling (1971) who have shown that the minimum effective dose of dexamethasone required to induce parturition in late pregnancy is 0.05-0.1 mg/24 hrs infused into the fetus and 6 mg/24 hr when infused into the ewe.

G. STEROID ENDOCRINE ROLE OF THE MAMMALIAN FETUS

The endocrine glands of the vertebrate embryo and fetus are increasingly recognized as exerting a profound influence on fetal differentiation and development (Jost, 1960; Deanesly, 1966). In eutherian mammals in which maternal and placental metabolism interact with fetal maturation, the secretion and metabolism of fetal hormones are important, and, frequently, limiting mechanisms regulating the total fetal hormonal milieu. The scope of this portion of the literature review will be limited to the role the fetal endocrine glands play in the elaboration of steroids which are presently known to be biologically important either as such or as precursors for placental steroid biosynthesis. In this context it is recognized that fetal adrenal corticoid secretion is essential for normal parturition in a number of species (see Section E) and certain aspects of fetal development (Bloch, 1968). The fetal adrenal is also important as a source of androgen precursors for placental synthesis of estrogens, at least in the human (Diczfalusy, 1969). Differentiation and development of the genital tract and external genitalia are known to be influenced by hormones presumably secreted by the gonads themselves (Jost, 1965; 1970). Hormonal factors may also be involved in gonadal organogenesis. The fetal liver, at least in the human, also performs a paraendocrine function during pregnancy in being largely responsible for the 16-hydroxylation of dehydroepi-

androsterone to provide the principal precursor for placental estriol synthesis (Diczfalusy, 1969).

1. Fetal adrenal steroid biosynthesis and metabolism.

Investigations of steroid hormone biosynthesis by mammalian fetal adrenal glands have followed the experimental approaches developed in studies on other endocrine glands; identification of steroids in adrenal tissue and adrenal venous blood, in adrenal tissue preparations after in vitro incubation studies with or without the addition of radioactive substrates, in fetal perfusion studies with radioactive substrates and the detection of steroid hydroxylating and dehydrogenase enzyme systems by histochemical techniques. In view of the difficulties encountered in methodology and collection of fetal adrenal vein blood, the rigorous identification of the hormones secreted by or isolated from fetal adrenal tissue has rarely been achieved. However, several reports claiming identification of steroids in extracts of fetal adrenal tissue or culture medium after culture of adrenal tissue and from fetal adrenal vein blood have been published and are summarized in Table 19. Identification in most instances was based on paper or gas chromatographic evidence.

The biosynthetic pathways involved in the formation of adrenal corticosteroids and C_{19} androgens by adult adrenal tissue of several mammalian species can be summarized in Fig. 5. It can be seen that two principal pathways may be

TABLE 19

Steroids identified in fetal adrenal tissue or fetal adrenal venous blood of mammals

Species	Steroids identified	Reference
Human	androstenedione	Bloch et al., 1955.
	androstenedione, dehydroepiandrosterone, 11β-hydroxyandrostenedione	Bloch et al., 1956.
	cortisol[a], cortisone[a], corticosterone[a]	Stark et al., 1965.
	dehydroepiandrosterone sulfate, 16α-hydroxydehydroepiandrosterone sulfate, pregnenolone sulfate, 3β, 20α-dihydroxy-5-pregnene sulfate, 3β, 17α-dihydroxy-5-androstene disulfate, 3β, 17β-dihydroxy-5-androstene disulfate	Huhtaniemi et al., 1970.
	cortisol, cortisone	Murphy and Diez d'Aux, 1972.
Rat	corticosterone	Milkovic and Milkovic, 1963.
	corticosterone, deoxycorticosterone, cortisol	Kalavsky, 1971.
Sheep	cortisol, corticosterone	Chester Jones et al., 1964.
	cortisol, corticosterone, aldosterone	Alexander et al., 1968.
	cortisol	Nathanielsz et al., 1972.
Pig	cortisol, corticosterone	Druzhinima, 1958.
Cow	corticosterone	Druzhinima, 1958.
Armadillo	cortisol, corticosterone	Bloch and Bernischke, 1965.

[a] identified in culture medium.

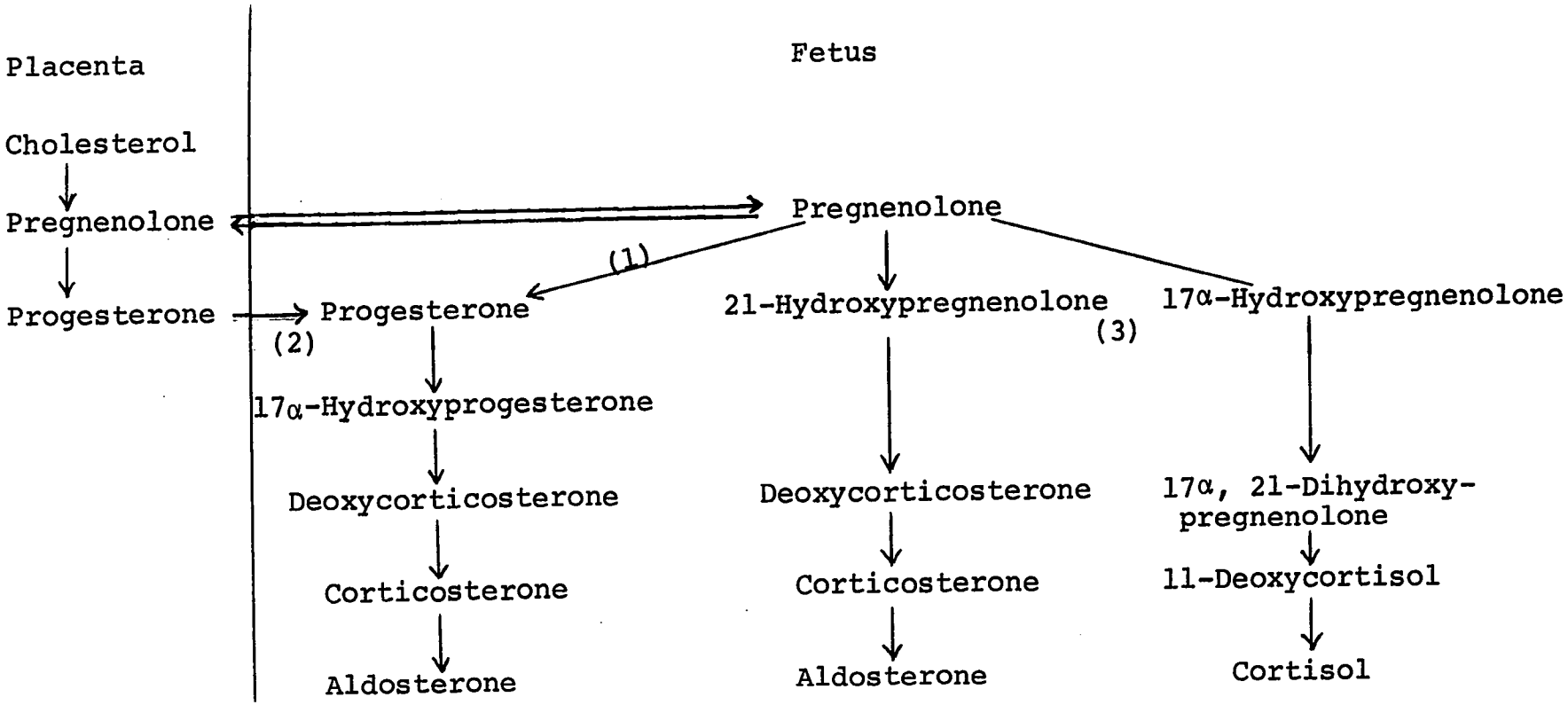


Fig. 5. Biosynthesis of corticoids by the human fetus *in vivo*. The pathways are based mainly on the perfusion studies reported by Pasqualini (1970).

(1) The conversion of pregnenolone to progesterone has not be demonstrated *in vivo*.

(2) Biosynthesis of corticosteroids from progesterone via the 'Δ^4-pathway'.

(3) Biosynthesis of corticosteroids from pregnenolone via the 'Δ^5-pathway'.

involved in the biosynthesis of cortisol; the first involves 17-hydroxylation of pregnenolone followed by 3β-hydroxysteroid dehydrogenation and Δ^5-Δ^4 isomerism to 17α-hydroxyprogesterone; the second requires the initial conversion of pregnenolone to progesterone with subsequent 17α-hydroxylation again giving rise to 17α-hydroxyprogesterone. This compound can then be hydroxylated at C-21 and at C-11 to yield cortisol. Although the biosynthetic roules illustrated in Fig. 5 appear to be the major pathways for corticosteroid formation in various species of mammals (Sandor, 1969), alternative biosynthetic pathways have been proposed, although these appear to be minor (Dorfman and Ungar, 1965; Vinson and Whitehouse, 1970). Aldosterone, the principal mineralocorticoid in many mammalian species is formed via 18-hydroxylation of corticosterone (Sandor, 1969; Vinson and Whitehouse, 1970).

Much of the information on the biosynthetic capacity of the mammalian fetal adrenal has been obtained by incubation of fetal adrenal tissue with radioactive substrates or by perfusion techniques. The perfusion technique has been carried out experimentally in two ways: injection of radioactive substrates into the umbilical veins of fetuses <u>in situ</u> followed by clamping of the cord and separation of the fetus from mother, or perfusion of the isolated fetus with blood-saline containing the radioactive substrate for 20 to 60 minutes. In both experimental approaches, fetal tissues,

including adrenals and blood are analyzed for radioactive steroids. In most instances these techniques have been carried out on the human fetus at mid-pregnancy. The information which is now available on the biosynthesis and metabolism of steroids by mammalian adrenal tissue is summarized in Tables 20 and 21. It will be observed that the information available on the steroid biosynthetic capacity of mammalian fetal adrenal tissue is minimal when compared to that for the human fetal adrenal. However, as a result of the extensive studies on the human fetus at mid-term and incubation studies with the isolated human fetal adrenal gland, several conclusions can be reached regarding the significance of fetal adrenal steroid biosynthesis and metabolism in the human. These conclusions are described in detail by Diczfalusy (1969) and by Pasqualini (1970) and will only be summarized here. In contrast to the repeated in vitro demonstration of the conversion of various 3β-hydroxy-Δ^5-steroids to the corresponding α,β-unsaturated 3-ketosteroids by fetal adrenal tissue (see Table 21) efforts to demonstrate the conversion of pregnenolone or dehydroepiandrosterone to Δ^4-3-ketosteroids in vivo have been unsuccessful (see Table 20). This apparent paradox between in vitro and in vivo results has not yet been resolved, although studies by Pasqualini and his group (Pasqualini, 1970) have shown that the 3β-hydroxysteroid dehydrogenase Δ^5-Δ^4 isomerase enzyme system exists in the fetal adrenal but that its activity is dependent on the

TABLE 20

Steroids identified in fetal adrenal tissue after perfusion of labeled substrates into the fetus

Species	Substrate	Metabolites identified	Reference
Human	acetate	cholesterol	Telegdy et al., 1969.
	acetate, cholesterol	pregnenolone dehydroepiandrosterone	Telegdy et al., 1969.
	cholesterol	pregnenolone 17α-hydroxypregnenolone	Coutts and MacNaughton, 1968, 1969.
	pregnenolone	dehydroepiandrosterone pregnenolone sulfate 17α-hydroxypregnenolone sulfate, dehydroepiandrosterone sulfate	Solomon, 1966.
	16α-hydroxypregnenolone	16α-hydroxyprogesterone	Reynolds et al., 1969.
	17α-hydroxypregnenolone	dehydroepiandrosterone sulfate, 17α-hydroxypregnenolone sulfate	Pion et al., 1967.
	17α-hydroxypregnenolone	17α-hydroxyprogesterone	Jackanicz et al., 1969.
	17α-hydroxypregnenolone	cortisol, dehydroepiandrosterone	Reynolds et al., 1969.
	21-hydroxypregnenolone	deoxycorticosterone corticosterone	Pasqualini et al., 1970.
	3β,17α,21-trihydroxypregnenolone	dehydroepiandrosterone cortisol	Pasqualini et al., 1968.
	progesterone	corticosterone sulfate deoxycorticosterone sulfate	Bird et al., 1965.

	progesterone	20α-hydroxyprogesterone 16α-hydroxyprogesterone 17α-hydroxyprogesterone cortisol	Solomon et al., 1967.
	17α-hydroxyprogesterone	17α-hydroxyprogesterone 17α-hydroxy-20α-dihydro-progesterone, androstene-dione, testosterone	Coutts et al., 1969.
	17α-hydroxyprogesterone	cortisol	Jackanicz et al., 1969.
	5β-pregnane-3α, 20α-diol	5β-pregnane-20α-ol-3α-yl sulfate	Cooke et al., 1967.
	androstenedione testosterone	testosterone sulfate	Mancuso et al., 1967.
	androstenedione testosterone	androstenedione 11β-hydroxyandrostene-dione	Benagiano et al., 1967.
	corticosterone	aldosterone	Pasqualini et al., 1966.
	corticosterone	21-hydroxypregn-4-ene-3, 11, 20-trione, 11β, 20β, 21-trihydroxy-pregn-4-en-3-one, 6β, 11β, 21-trihydroxypregn-4-ene-3, 20-dione, 11β-hydroxypregn-4-en-3, 20-dione-21yl sulfate	Pasqualini et al., 1969.
Rhesus monkey	pregnenolone pregnenolone sulfate	17α-hydryxopregnenolone 17α-hydroxyprogesterone dehydroepiandrosterone androstenedione 11β-hydroxyandrostene-dione, cortisol, progesterone, dehydro-epiandrosterone sulfate	Gorwill et al., 1971.
	progesterone	17α-hydroxyprogesterone androstenedione	Leung and Solomon, 1972.

TABLE 21

Steroids identified after incubation of fetal adrenal tissue preparations with radioactive substrates *in vitro*.

Species	Substrate	Tissue preparation	Metabolites identified	Reference
Human	acetate	slice	dehydroepiandrosterone androstenedione 11β-hydroxyandrostenedione pregnenolone, cortisol	Bloch and Benirschke, 1959.
	acetate	slice	cholesterol, dehydroepi-androsterone, cortisol	Bloch and Benirschke, 1960.
	acetate	slice	cholesterol	Givner and Jaffe, 1971.
	20α-hydroxy-cholesterol	slice	17α, 20α-dihydroxy-cholesterol, dehydroepiandrosterone cortisol, corticosterone	Shimizu et al., 1965.
	pregnenolone	homogenate	dehydroepiandrosterone	Villee et al., 1962.
	pregnenolone	slice mince	16α-hydroxypregnenolone androstenedione 11β-hydroxyandrostenedione	Villee and Driscoll, 1965.
	pregnenolone	homogenate	pregnenolone sulfate dehydroepiandrosterone sulfate, dehydroepiandrosterone androstenedione progesterone	Cooke et al., 1968.
	pregnenolone	homogenate	5α-pregnane-3, 20-dione	Charreau et al., 1968.
	pregnenolone	slice	21-hydroxypregnenolone	Pasqualini et al., 1970.
	pregnenolone	homogenate	pregnenolone sulfate 17α-hydroxypregnenolone sulfate dehydroepiandrosterone sulfate	Jaffe and Payne, 1971.
	pregnenolone	slice	dehydroepiandrosterone dehydroepiandrosterone sulfate androstenedione 11β-hydroxyandrostenedione cortisol	Milner, A.J. and Mills, I.H. 1970a.
	progesterone	slice	11-deoxycortisol cortisol 11-deoxycortisol androstenedione 11β-hydroxyandrostenedione	Milner, A.J. and Mills, I.H. 1970b.

	pregnenolone sulfate	homogenate	17α-hydroxypregnenolone sulfate dehydroepiandrosterone sulfate 16α-hydroxydehydroepi-androsterone sulfate	Perez-Palacios et al., 1968.
	21-hydroxy-pregnenolone	slice	deoxycorticosterone, corticosterone	Pasqualini et al., 1970.
	progesterone	homogenate	androstenedione, 17α-hydroxyprogesterone	Solomon et al., 1950.
	progesterone	slice homogenate	16α-hydroxyprogesterone 17α-hydroxyprogesterone deoxycorticosterone	Villee et al., 1961.
	progesterone	homogenate slice	dehydroepiandrosterone	Villee et al., 1962.
	progesterone	homogenate	16α-hydroxyhydrocortisone	Villee, 1965.
	progesterone	mince	11β-hydroxyandrostenedione 16α-hydroxyprogesterone cortisol	Villee and Driscoll, 1965.
	progesterone	organ culture	cortisol	Bloch et al., 1965.
	dehydroepi-androsterone	slice	16α-hydroxydehydroepi-androsterone	Nakayama et al., 1967b.
	dehydroepi-androsterone	homogenate	7α-hydroxydehydroepi-androsterone 7β-hydroxydehydroepi-androsterone	Sulcova et al., 1968.
	dehydroepi-androsterone	homogenate	dehydroepiandrosterone sulfate, androstenedione	Shirley and Cooke, 1969.
	dehydroepi-androsterone	homogenate	dehydroepiandrosterone sulfate	Jaffe and Payne, 1971.
	testosterone	homogenate	testosterone sulfate	Jaffe and Payne, 1971.
Rhesus monkey	progesterone	slice	corticosterone	Kittinger and Beamer, 1971.

Guinea pig	pregnenolone	homogenate	cortisol, 11β-hydroxy-androstenedione	Bloch, 1969.
	progesterone		corticosterone	Bloch, 1969.
	progesterone	homogenate		
Armadillo	pregnenolone	homogenate	progesterone, cortisol corticosterone	Bloch, 1969.
	progesterone	slice	cortisol, corticosterone	Bloch and Benirschke, 1965.
Rat	pregnenolone, progesterone	homogenate	corticosterone	Bloch, 1969.
Dog	progesterone	homogenate	cortisol, corticosterone	Bloch, 1969.
Rabbit	pregnenolone	homogenate	deoxycorticosterone corticosterone, progesterone, cortisol, 17α-hydroxypregnenolone	Kurach et al., 1971.
Pig	progesterone	homogenate	corticosterone	Druzhinima, 1958.
Sheep	pregnenolone	mince	progesterone, 21-hydroxy-pregnenolone, deoxy-corticosterone, corticosterone	Vinson, 1967.
	pregnenolone	slice	deoxycorticosterone, corticosterone	Anderson et al., 1970b.
	progesterone		11-deoxycortisol, cortisol	
	progesterone	mince	deoxycorticosterone, corticosterone	Vinson, 1967.
Bovine	acetate	organ culture	cortisol, corticosterone 11-dehydrocorticosterone deoxycorticosterone	Chouraqui and Weniger, 1968.

structure of the substrate. Thus corticoids can be formed either from progesterone via the 'Δ^4-pathways' or from 21-hydroxypregnenolone, 17α, 21-dihydroxypregnenolone or 17α-hydroxypregnenolone via a 'Δ^5-pathway'. In this latter pathway the dehydrogenase/isomerase system acts at the last step of hormone formation (Fig. 5). The evidence which is now available (Pasqualini, 1970) suggests that corticosteroids are formed in the fetal adrenal gland mainly from pregnenolone via the 'Δ^5-pathway'. Other considerations have been advanced by Bloch (1968) who has suggested that the conversion of pregnenolone to progesterone may be inhibited by placental progesterone. Alternatively, adrenal sulphokinases may compete with Δ^5-3β-hydroxysteroid dehydrogenases for dehydroepiandrosterone and pregnenolone. Under in vitro conditions oxidation may be favored relative to sulphurylation. In vivo, however, the situation may be reversed. Studies of steroid content in cord blood have shown that the concentration of pregnenolone and dehydroepiandrosterone sulphates greatly exceeds that of free and conjugated 3-ketosteroids (Eberlein, 1965). Also steroids injected or perfused into the fetus are extensively sulphurylated whereas steroid sulphates are recovered unchanged (Diczfalusy, 1969). It should also be mentioned that Niemi and Baillie (1965) have demonstrated 3β-hydroxysteroid dehydrogenase activity in the human fetal adrenal gland by histochemical techniques using various 3β-hydroxy-Δ^5-steroids as substrates.

The human fetal adrenal, in addition to other fetal tissues, also possesses a very active steroid sulfokinase (Wengle, 1966; Villee and Loring, 1969) which is capable of sulphating the corticosteroids and dehydroepiandrosterone formed in the adrenal.

In conclusion, it can be stated that the human fetal adrenal is capable of forming all the biologically active corticosteroids, including aldosterone and dehydroepiandrosterone, which is the principal precursor for placental estrogen synthesis.

Recent studies with fetal sheep adrenal tissue in vitro by Anderson et al. (1970b, 1972) have established that the capacity of fetal adrenal tissue to form cortisol from pregnenolone and progesterone increases with gestational age or after stimulation of the fetal adrenal by infusion of a synthetic corticotropin (Synacthen, Ciba) into the fetus to induce premature labor. At 122 days of pregnancy (gestation period in sheep 146 days) fetal adrenal tissue yielded mainly progesterone from pregnenolone with only a small conversion to corticosteroids. The substrate, progesterone, was transformed mainly to deoxycorticosterone. A similar result was obtained after incubation of fetal adrenals from fetuses at 140 and 143 days of pregnancy, although the amount of progesterone converted to 11-deoxycorticoids was greatly increased. After incubation of adrenal tissue removed from fetuses infused with Synacthen, the extent of conversion of both pregnenolone

and progesterone to cortisol was significantly increased and was similar to the results observed after incubation of adrenal tissue from new born lambs. In addition, the formation of corticosterone from progesterone was significantly greater than that from pregnenolone. These results suggest that during the course of normal pregnancy, at least until the initiation of the sequence of events leading to parturition (see Section E), 11β-hydroxylation of C_{21} steroids by the sheep fetal adrenal is not particularly active. Similar studies using dehydroepiandrosterone as substrate (Anderson et al., 1972) have shown that the substrate is extensively converted to androstenedione by adrenal tissue removed at 116 days and 143 days of pregnancy. However, the conversion to 11β-hydroxyandrostenedione was considerably greater in the adrenals removed later in pregnancy. An interesting finding from these studies and also from studies with the adult adrenal (Anderson et al., 1970b) was the apparent inability of sheep adrenal tissue to form steroid sulphates. It appears, therefore, that the sheep fetal adrenal, in marked contrast to the human fetal adrenal, is unable to synthesize C_{21} sulphates or dehydroepiandrosterone sulphate, which is intimately involved in estrogen synthesis in the human feto-placental unit (Bolté et al., 1964; Solomon et al., 1967).

It is difficult to assess at this time the significance of fetal adrenal steroid synthesis and metabolism in the

other species (except for the sheep) listed in Tables 20 and 21. However, it does appear that in all the species studied, the fetal adrenal, which is the apparent source of fetal corticosteroids, has acquired the capacity for corticosteroid synthesis during fetal development. It is also apparent that the corticosteroids formed by the mammalian fetal adrenal are qualitatively the same same as those formed during adult life (Vinson and Whitehouse, 1970) although, quantatively, the ratio and amounts of the various corticosteroids secreted may be substantially different. However, the developmental stages in which the steroidogenic enzymes are first formed and active, and the rapidity with which the complete pathways of de novo synthesis of fetal corticoids are established remain to be defined.

2. Steroid production by mammalian fetal gonads.

The role of the mammalian fetal testis in the differentiation of the male reproductive systems has been investigated in a limited number of species (reviewed by Burns, 1961). The studies of several laboratories have shown that the fetal testes of rats (Wells and Fralick, 1951) and rabbits (Jost, 1953) stimulate proliferation of the Wolffian duct and also cause Mullerian duct regression. The means by which the testis brings about these changes is not well known, but experiments on castration and injection of steroid hormones (Jost, 1953, 1965; Price and Oritz, 1965) has suggested that

Wolffian duct differentiation could be caused by androgens secreted by the fetal testis. These results have been confirmed by in vitro studies (Price and Oritz, 1965). Furthermore, morphological studies (Roosen-Runge, 1955) and histochemical demonstration of Δ^5-3β-hydroxysteroid dehydrogenase activity in the fetal testis of the mouse (Hitzeman, 1962; Baillie and Griffiths, 1964; Hart et al., 1966), rat (Schlegel et al., 1965), human (Niemi and Baillie, 1965) and pig (Moon and Raeside, 1972) have provided additional evidence for a steroid-producing role of the mammalian fetal testis. However, it is now well documented from incubation studies and perfusion studies of fetuses with radioactive steroids that the fetal testis of several mammalian species are capable of synthesizing steroid hormones, particularly androgens. The results of these studies have been summarized in Table 22. From the results of histochemical studies, it is apparent that Δ^5-3β-hydroxysteroid dehydrogenase activity and the steroid biosynthetic capacity of the fetal testis become manifest before the time of sexual differentiation and appearance of Leydig cells. In a number of instances, the results reported in Table 22 also indicate that the extent of conversion of the substrate to testosterone increases with gestational age. Progesterone can be converted to androstenedione by fetal rat testis at 13.5 days (Noumura et al., 1966) while Leydig cells are not apparent until day 15 (Niemi and Ikonen, 1961). Rabbit testis can convert

TABLE 22

Biosynthesis of steroid hormones by mammalian fetal testes tissue

Species	Substrate	Tissue preparation	Metabolites identified	Reference
Human	acetate	organ culture	pregnenolone progesterone 17α-hydroxyprogesterone androstenedione testosterone	Rice et al., 1966.
	acetate	slice	cholesterol sulfate pregnenolone sulfate 17α-hydroxypregnenolone sulfate, dehydroepiandrosterone sulfate	Jaffe et al., 1968a.
	acetate	perfusion	cholesterol	Telegdy et al., 1969.
	acetate	mince	cholesterol testosterone	Serra et al., 1970.
	acetate	perfusion	pregnenolone pregnenolone sulfate dehydroepiandrosterone dehydroepiandrosterone sulfate, Δ^5-androstenediol testosterone, progesterone androstenedione	Mathur et al., 1972.
	pregnenolone	whole	dehydroepiandrosterone testosterone androstenedione	Acevedo et al., 1961.
	pregnenolone	mince	testosterone androstenedione dehydroepiandrosterone androst-5-en-3β, 16β, 17β-triol, 17α-hydroxypregnenolone	Acevedo et al., 1963.

pregnenolone	homogenate	dehydroepiandrosterone sulfate	Lamont et al., 1970.
pregnenolone	*in vitro*	pregnenediol sulfate progesterone androstenedione dehydroepiandrosterone 17α-hydroxyprogesterone 17α-hydroxypregnenolone testosterone	Schindler et al., 1971.
pregnenolone	homogenate	pregnenolone sulfate 17α-hydroxypregnenolone sulfate, dehydroepi-androsterone sulfate	Jaffe and Payne, 1971.
pregnenolone, pregnenolone sulfate	homogenate	progesterone 17α-hydroxyprogesterone 17α-hydroxypregnenolone dehydroepiandrosterone testosterone androstenedione	Lamont et al., 1970.
pregnenolone sulfate	homogenate	dehydroepiandrosterone sulfate 17α-hydroxypregnenolone sulfate 16α-hydroxydehydroepi-androsterone sulfate	Jaffe et al., 1968b.
pregnenolone sulfate	homogenate	17α-hydroxypregnenolone sulfate	Lamont et al., 1970.
17α-hydroxy-pregnenolone	slice	17α-hydroxyprogesterone dehydroepiandrosterone testosterone androstenedione androstenediol	Ikonen and Niemi, 1966.
progesterone	mince	17α-hydroxyprogesterone testosterone androstenedione	Bloch et al., 1962b.

	progesterone	mince	16α-hydroxyandrostene-dione 17α-hydroxyprogesterone 20α-dihydroprogesterone 20β-dihydroprogesterone deoxycorticosterone 11-deoxycortisol 17α, 20α-dihydroxypregn-4-ene-3-one 17α, 20β-dihydroxypregn-4-ene-3-one	Acevedo et al., 1963
	progesterone	homogenate	testosterone 16α-hydroxyprogesterone 17α-hydroxyprogesterone	Bloch, 1964.
	progesterone	slice	17α-hydroxyprogesterone androstenedione testosterone	Ikonen and Niemi, 1966.
	17-hydroxy-progesterone	perfusion	17α-hydroxyprogesterone 17α-hydroxy-20α-dihydro progesterone androstenedione testosterone	Coutts et al., 1969.
	dehydroepian-drosterone	homogenate	androstenedione	Bloch et al., 1962a.
	dehydroepian-drosterone	mince	androstenedione testosterone	Archer et al., 1969.
	dehydroepian-drosterone	homogenate	dehydroepiandrosterone sulfate, Δ^5-andro-stenediol-3-sulfate	Jaffe and Payne, 1971.
Rhesus monkey	pregnenolone	homogenate	testosterone androstenedione	Resko, 1970.
Rabbit	pregnenolone	mince	testosterone androstenedione	Lipsett and Tullner, 1965.
	androstene-dione	mince	testosterone	Lipsett and Tullner, 1965.

Armadillo, guinea pig, dog	pregnenolone	homogenate	testosterone androstenedione	Bloch, 1967.
Armadillo	pregnenolone	slice	17α-hydroxyprogesterone androstenedione testosterone	Bloch and Benirschke, 1965.
Rat	pregnenolone progesterone	homogenate	testosterone androstenedione	Bloch, 1967.
	progesterone	mince	17α-hydroxyprogesterone testosterone androstenedione	Noumura et al., 1966.
Mouse	pregnenolone progesterone 17α-hydroxy-pregnenolone	bisected intact	testosterone	Bloch et al., 1971.
Horse	acetate	slice	cholesterol dehydroepiandrosterone testosterone androstenedione 17α-hydroxyprogesterone estrone	MacArthur et al., 1967.
Cow	endogenous	culture	testosterone androstenedione	Weniger et al., 1967
Sheep	progesterone	slice, squash	testosterone androstenedione 17α-hydroxyprogesterone 17α-hydroxy-20α-dihydro progesterone 20α-dihydroprogesterone	Nancarrow and Seamark, 1972b.
	dehydroepi-androsterone	homogenate	testosterone androstenedione	Attal, 1969.

androstenedione to testosterone at day 15 of pregnancy even though Leydig cells cannot be distinguished until day 18 (Lipsett and Tullner, 1965). Moon and Raeside (1972) have demonstrated traces of Δ^5-3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase activities by histochemical techniques in gonads of pig fetuses at 1.5 cm crown-rump length, which is at a stage well before fetal pig testes become histologically recognizable. Furthermore, Attal (1969) has reported the presence of androstenedione and testosterone in the gonads of fetal sheep 5 days prior to the time sexual differentiation of the gonads is recognized. These results indicate that the mammalian fetal testis has the capacity to perform certain important steps in the biosynthesis of steroid hormones in both early and late fetal stages which agrees with the concepts put forward by Harris (1970) and Jost (1970) on the role of the fetal testis at various phases in sex differentiation.

In contrast to the recognized role of the mammalian fetal testis in mammalian male genital tract development, the fetal ovaries appear to exert little influence on these developmental processes in either the genetic male or female (Jost, 1953). An endocrine function for the ovary has not been demonstrated during fetal life. Nevertheless, the fetal ovary of a number of mammalian species appears to have a limited steroid biosynthetic capacity as judged by the results of several in vitro incubation studies which are sum-

marized in Table 23. Human ovarian tissue is capable of forming steroids from acetate and also androgens from C_{21} steroid precursors, as well as limited amounts of estrogen from C_{19} androgen precursors. In all cases, the principal metabolic transformation observed after incubations of mammalian fetal ovarian tissue with progesterone was the conversion to 20α-dihydroprogesterone. The conversion of androstenedione to testosterone by bovine and rabbit tissue has also been demonstrated. The results with fetal rabbit ovarian tissue have shown that ovarian synthesis of testosterone does not increase during gestation as it does in the testis, and there appears to be relatively little change in the capacity of the ovary to synthesize testosterone at varying stages of fetal development. The significance of the ring A reduction of progesterone by fetal sheep ovarian tissue is not known. However, ring A reduction of progesterone was not observed in comparable studies with sheep fetal testes tissue (Nancarrow and Seamark, 1972b). Although the steroidogenic role of the fetal ovary remains to be established it is of interest to note that in a variety of mammalian species the interstitial tissue of the fetal ovary shows considerable development during gestation eg. the horse (Cole et al., 1933) and the elephant (Perry, 1953). Also, Kellas et al. (1958) observed follicular development in the bovine and ovine fetal ovary and the development of large follicles and luteal bodies in the fetal ovary of the giraffe.

TABLE 23

Biosynthesis of steroid hormones by mammalian fetal ovarian tissue

Species	Substrates	Tissue preparation	Metabolites identified	Reference
Human	acetate	homogenate	lanosterol, cholesterol pregnenolone, progesterone	Jungmann and Schweppe, 1968.
	pregnenolone		progesterone	Schindler et al., 1971.
	progesterone	homogenate	20α-dihydroprogesterone	Bloch, 1964.
	progesterone	organ culture	20α-dihydroprogesterone	Bloch et al., 1965.
	7α-hydroxy-dehydroepi-androsterone	slice	7α-hydroxyestrone 7α-hydroxyestradiol 7-oxoestradiol	Starka et al, 1966.
	7α-hydroxy-dehydroepi-androsterone sulfate			
Cow	progesterone	mince	testosterone, androstene-dione 16α-hydroxyprogesterone 17α-hydroxyprogesterone 20α-dihydroprogesterone 17α-hydroxy-20α-dihydro progesterone	Roberts and Warren, 1964.
	androstene-dione	mince	testosterone, estradiol-17β	Roberts and Warren, 1964.
Sheep	progesterone	slice, squash	20α-dihydroprogesterone 6β-hydroxyprogesterone 5α-pregnane-3β-ol-20-one 5α-pregnane-3, 20-dione 5β-pregnane-3, 20-dione	Nancarrow and Seamark, 1972b.
Rabbit	pregnenolone	mince	testosterone	Lipsett and Tullner, 1965.
	androstene-dione	mince	testosterone	Lipsett and Tullner, 1965.

3. Steroid biosynthesis and metabolism by other fetal tissues.

The liver of the human fetus, like that of the adult, is the principal site of steroid metabolism and is capable of carrying out reduction, dehydrogenation, hydroxylation and conjugation reactions on all the major steroids secreted by the placenta and fetal endocrine glands (Diczfalusy, 1969; Jeffery and Klopper, 1970). The author does not consider it within the scope of this review to review in detail the extensive literature which has accumulated on the metabolism of steroids by the human fetal liver, except insofar as it relates to the formation of steroids or steroid precursors which are considered to be of importance in the maintenance of pregnancy. In this context one of the most important functions of the human fetal liver is to synthesize 16α-hydroxydehydroepiandrosterone, which is thought to be the principal precursor for placental estriol synthesis (Dell' Acqua et al., 1967b). The balance of evidence available at the present time indicates that placental pregnenolone is the most likely principal precursor of dehydroepiandrosterone and that the fetal adrenals represent the main site of its production (Diczfalusy, 1969). Dehydroepiandrosterone and its sulfate are exposed to an extensive 16α-hydroxylation in the fetal organism. This reaction takes place mainly, if not entirely in the fetal liver (Bolté et al., 1966). Although dehydroepiandrosterone and its 16α-hydroxylated deri-

vative are thought to be the principal precursors of estrogens elaborated by the feto-placental endocrine system, the fetus and particularly the fetal liver are also able to form estrogens from the α,β-unsaturated C_{19} steroids, androstenedione and testosterone. Using in situ perfusion techniques, Mancuso et al. (1965, 1968) have shown that the fetal liver is capable of forming estrone, estradiol-17β and estriol from the latter precursors. These authors suggested, however, that aromatization preceeded 16-hydroxylation in view of their inability to isolate 16α-hydroxylated androgens. Slaunwhite et al. (1965), Jungmann et al. (1966) and Jungmann and Schweppe (1967) have also demonstrated these conversions in vitro. However, in contrast to the in vivo results, Jungmann and Schweppe (1967) were also able to isolate 16α-hydroxyandrostenedione after incubation of fetal liver tissue with either androstenedione or testosterone. Other discrepancies between in vivo and in vitro results have arisen in relation to the enzyme activities that have been demonstrated in fetal liver tissue. In vivo perfusion studies have shown that fetal liver tissue lacks the 3β-hydroxysteroid dehydrogenase/isomerase enzyme system and therefore the ability to convert dehydroepiandrosterone to estrogen (Bolté et al., 1966; Mancuso et al., 1965). However, these conversions have been demonstrated in vitro (Slaunwhite et al., 1965; Jungmann and Schweppe, 1967). Diczfalusy and coworkers (Diczfalusy, 1969) have also reported the absence of 17α-hydroxy-

lase and $C_{17,20}$-desmolase systems using a variety of *in vivo* techniques. In addition, they found no hepatic 16α-hydroxylase activity for androstenedione, testosterone or progesterone, while active 16α-hydroxylation of dehydroepiandrosterone, pregnenolone and estrone has been observed. However, Jungmann and Schweppe (1967) using *in vitro* techniques demonstrated the presence of all these enzymes. These differences between *in vivo* and *in vitro* results are difficult to explain. It is recognized that fetal tissues undergo a process of progressive enzymatic changes and maturation from the early stages of pregnancy to full term, and that *in vivo* studies with fetuses at mid-term do not necessarily reflect the enzyme activities which may be present at later stages. However, *in vivo* perfusion studies will more accurately reflect the biosynthetic pathways of the intact cell at a particular stage of development.

Estrone and estradiol-17β have also been shown to undergo 16-hydroxylation in the human fetal organism, mainly in the liver. Thus, in the previable fetus both 16α and 16β-orientated hydroxylation products of estrone and estradiol-17β have been isolated from the fetal liver after perfusion (Schwers et al., 1965a; Bengiano et al., 1970). It has also been demonstrated that a considerable part of the fetal 16-hydroxylation of estrogens takes place in a conjugated form (Diczfalusy, 1969). Furthermore, it appears that an important function of the human fetal liver is to form considerable

quantities of 15α-hydroxyestradiol-17β; this has been demonstrated following perfusion of fetuses with estrone, estrone sulfate, androstenedione or testosterone (Diczfalusy, 1969). In addition, evidence indicates that considerable quantities of 15α-hydroxyestriol can also be synthesized by the human fetus (Gurpide et al., 1966). The view has formed that these 15α-hydroxylated estrogens formed during pregnancy and excreted in the maternal urine are characteristically fetal metabolic products (Gurpide et al., 1966).

The transformation of steroids by fetal liver tissue from other mammalian species are summarized in Table 24. In general, it appears that the principal function of the fetal liver in the species which have been studied is to metabolize the precursor to reduced metabolites. Apart from the rhesus monkey, which has been shown to produce 16α-hydroxydehydroepiandrosterone from dehydroepiandrosterone, fetal liver tissue from other mammalian species does not appear to possess a paraendocrine function.

The studies on the transformation of dehydroepiandrosterone by horse fetal liver tissue are of interest in that this steroid can be transformed via formation of 7α-hydroxydehydroepiandrosterone into 3β-hydroxyandrost-5, 7-diene-17-one and hence by placental enzymes into equilin and equilenin, the ring B unsaturated estrogens which are characteristic constituents of mares' pregnancy urine (Girard et al., 1932a, b, c; Gaudry and Glen, 1958).

TABLE 24

Biosynthesis of steroid hormones by mammalian fetal liver tissue.

Species	Substrate	Tissue preparation	Steroid identified	Reference
Baboon	acetate	perfusion	cholesterol	Khamsi et al., 1972.
	pregnenolone	perfusion	5α-pregnane-3β-ol-20-one 5α-pregnane-3α-ol-20-one 5β-pregnane-3α-ol-20-one pregn-5-en-3β, 20α-diol pregn-5-en-3β, 20β-diol pregn-5-en-3β, 17α, 20α-triol dehydroepiandrosterone androst-5-ene-3β, 17β-diol pregnenolone sulfate	Solomon and Leung, 1972.
	dehydroepi-androsterone	perfusion	androst-5-en-3β, 17β-diol	Solomon and Leung, 1972.
Sheep	pregnenolone	chopped	androstenedione pregnenolone 17α-hydroxypregnenolone dehydroepiandrosterone 5β-pregnane-3β-ol-20-one 5α-pregnane-3β-ol-20-one 5β-androstane-3β-ol-20-one pregnenolone sulfate 17α-hydroxypregnenolone sulfate dehydroepiandrosterone sulfate 20α-dihydropregnenolone sulfate	Anderson et al., 1970a.

	androstene-dione	chopped	androstenedione 5β-androstane-3β, 17α-diol 5β-androstane-3α, 17β-diol 5β-androstane-3α-ol-20-one 5β-androstane-3β-ol-17-one 5α-androstane-3α-ol-17-one 5α-androstane-3β-ol-17-one 5β-androstane-3β, 17α-diolsulfate 5β-androstane-3β-ol-17-one sulfate	Anderson et al., 1970a.
	estrone	homogenate	estrone, estradiol-17β estradiol-17α and corresponding sulfates.	Ainsworth, 1972b.
Horse	dehydroepi-androsterone	microsomes	7α-hydroxydehydroepiandrosterone androsta-5, 17-diene-3β-ol-17-one androsta-4, 7-diene-3, 17-dione	Starka and Breuer, 1966.

Transformations of steroids by peripheral fetal tissues have been examined only in the human fetus. Of these, the heart, lungs, kidney and intestine have been shown to contain reduced metabolites of both C_{21} and C_{19} steroid substrates, after administration to the fetus (Diczfalusy, 1969; Jeffery and Klopper, 1970). However, the significance of these transformations in relation to the overall fetal metabolism of steroids remains uncertain.

An unusual characteristic of ruminant fetuses is that fetal blood, particularly of the sheep and cow rapidly metabolizes progesterone in vitro to produce 20α-hydroxyprogesterone. This activity appears to be minimal in goat, human, rat and chicken blood (Nancarrow and Seamark, 1968). 20α-Hydroxysteroid dehydrogenase activity of fetal blood, although persisting in the neonate, declines with age and is not detectable in adult blood. In addition, three other enzymes involved in gestagen metabolism have been detected in ovine fetal blood viz a 3α-hydroxysteroid oxidoreductase, a 20β-hydroxysteroid oxidoreductase and a 6β-hydroxylase (Seamark et al., 1970) as well as a 17β-hydroxy-C-18-steroid oxidoreductase (Findlay and Seamark, 1969). The activity of these latter enzymes does not appear to be age dependent. Although the significance of this metabolic activity of sheep fetal blood has not been determined, Nancarrow and Seamark (1968) have suggested that fetal blood 20α-hydroxysteroid dehydrogenase may fulfil a protective role in regulating the level

of gestagens reaching the fetus. Further studies on the purification and properties of fetal blood 20α-hydroxy-steroid dehydrogenase have demonstrated that bicarbonate ion has a stimulatory effect on the enzyme activity and raises the possibility of a link between gestagen metabolism and fetal physiological homeostatic mechanisms (Seamark et al., 1972).

H. THE SYNTHESIS AND CLEAVAGE OF STEROID CONJUGATES BY THE MAMMALIAN FETUS AND PLACENTA.

The conjugation of steroids with sulphuric or glucuronic acid has been shown to be an important process in relation to the biosynthesis, metabolism and feto-placental transfer of steroids in the human feto-placental unit (Diczfalusy, 1969; Jeffery and Klopper, 1970). In general, in vivo experiments have demonstrated that the human fetus possesses significant steroid sulfokinase activity associated with little if any steroid sulfatase activity, whereas the placenta possesses extensive sulfatase activity with little or no sulfokinase activity. However, Pulkkinen (1961) has demonstrated a rather high estrogen sulfatase activity in the liver, gastrointestinal tract, pancreas, kidney and lungs of a large number of fetuses of different gestational ages using in vitro techniques. In contrast, little if any fetal hydrolysis of estrone sulfate can be observed in perfused previable fetuses (Emerman et al., 1965; Schwers et al., 1965b) or after the administration of estrone sulfate or estriol-3-sulfate into the intact umbilical circulation at laparotomy (Kirshner et al., 1966; Goebelsmann et al., 1966). In vitro studies have indicated the presence of very active steroid sulfokinase activities in the human fetus capable of converting β,γ-unsaturated steroids, such as dehydroepiandrosterone, and estrogens, such as estrone, into the corresponding

3-sulfates (Wengle, 1966). The steroid sulfate synthesizing activity appears to be localized mainly in the fetal adrenal and the fetal liver. Similar results have been obtained after the perfusion of previable fetuses with various radioactive steroids or after the injection of the radioactive steroids into the intact feto-placental circulation and subsequent isolation of radioactive metabolites from the various fetal organs (Diczfalusy, 1969). In vivo studies have also indicated the ability of the midterm human fetus to synthesize and hydrolyze estrogen glucosiduronates (Diczfalusy and Mancuso, 1969). These activities have not been demonstrated in the mid-term placenta in situ. Furthermore, it has been demonstrated that unconjugated estrogens are transferred across the placenta much more rapidly than conjugated forms (Diczfalusy and Mancuso, 1969). Part of the estrogen reaching the placenta as sulfates is transferred after hydrolysis, whereas estrogen glucosiduronates are transferred in an unchanged form without hydrolysis.

Little information is available at the present time on the distribution and relative activity of enzymes capable of synthesizing and cleaving steroid sulfates in fetal or placental tissues of other mammalian species. Steroid sulfate synthesis has been demonstrated in the placenta of the cow (Holcenberg and Rosen, 1965) and the guinea pig (Levitz et al., 1960) and in the placenta and fetal liver of the sheep (Anderson et al., 1970a; Pierrepoint et al., 1971a;

Ainsworth, 1972b). Sulfate conjugation of steroids by the fetal tissue of the rat and guinea pig is low compared to the activity observed in corresponding human tissues (Pulkkinen, 1966). It also appears that the sheep fetal adrenal, in marked contrast to the human fetal adrenal, does not synthesize dehydroepiandrosterone sulfate which is the principal precursor of estrogen synthesis in the human feto-placental unit (Bolté et al., 1964; Anderson et al., 1970b). The capacity of fetal rat tissues to hydrolyze dehydroepiandrosterone sulfate or estrone sulfate is very low or absent in utero, but hydrolytic activity increases steadily from birth until about 80 days of age, then tends to level off (Pulkkinen, 1961; Burstein and Westort, 1967). In contrast, sheep and pig fetal liver, kidney and placental preparations have been shown to hydrolyze dehydroepiandrosterone sulfate and estrone sulfate (Ainsworth, 1972a). Activity was found to be greatest in the sheep placenta, whereas in the pig, fetal liver preparations were the richest source of hydrolytic enzymes. These results suggest that in the pig feto-placental endocrine system, steroid sulfate hydrolysis appears to be a fetal rather than a placental function.

Although the studies on the synthesis and cleavage of steroid conjugates, particularly sulfates, by the human feto-placental endocrine system have indicated the importance of steroid conjugates in the biosynthesis and metabolism of steroids, the significance of these reactions has not yet

been established. It may be that conjugation of steroids with sulfate may represent the transport form of the steroid in fetal blood, since recent studies by Huhtaniemi and Vihko (1970) have shown that of the endogenous neutral steroids present in fetal blood at early and mid-pregnancy only progesterone was present as an unconjugated steroid, the remaining steroids being present as either mono- or disulfates. Studies by Findlay and Cox (1970) on the estrogens present in sheep fetal blood have also shown that these compounds are present mainly in the form of sulfate conjugates.

I. THE FETO-PLACENTAL UNIT CONCEPT AND ITS ROLE IN MAINTAINING PREGNANCY IN MAMMALS.

1. The human feto-placental unit

During pregnancy in the human species the urinary excretion of estriol as well as that of pregnanediol shows a progressive increase until parturition. Until the last decade it was believed that these large quantities of steroids were formed by the temporary endocrine gland, the placenta, from low molecular weight precursors such as acetate, by de novo synthesis via cholesterol, in a manner similar to that taking place in the adrenals, ovaries and testis (Dorfman and Ungar, 1965).

This theory has now been replaced largely as a result of the work of Diczfalusy and his colleagues, by the concept of a feto-placental endocrine unit. According to this concept the increasing levels of steroid hormones elaborated by the pregnant women are not formed from acetate by the placenta, but rather from circulating sterol and steroid precursors by the feto-placental endocrine system. As a result of the extensive work on the endocrine capabilities of the human placenta and fetus (reviewed in Sections, C, G and H) it is apparent that at midpregnancy the placenta and fetus represent incomplete steroidogenic systems, due to the lack of, or non functioning of certain essential enzymes. However, the enzymes absent from the fetus are present in

the placenta and those not functioning in the placenta are operative in the fetus, thus allowing the feto-placental endocrine system to synthesize all types of steroid hormones.

In Fig. 6 an attempt has been made to integrate into a general scheme the major steroid biogenetic reactions which have been described in previous sections of this review and to show the interrelationships between them and the various compartments in which the various transformations take place.

It has now been established that maternal cholesterol is the principal precursor of the steroids formed in the feto-placental unit (Hellig et al., 1970). However, recent evidence by Telegdy et al. (1970a, b) and by Mathur et al. (1970) based on the results of perfusion of the complete feto-placental unit with radioactive acetate has established that the midgestation human placenta is not capable of synthesizing cholesterol from acetate, but that the conversion of acetate to cholesterol is a quantitatively significant metabolic pathway in the fetus, and that part of this cholesterol may be transferred to the placenta. After passing the maternal-placental barrier the maternal cholesterol is transformed by the feto-placental endocrine unit into a variety of C_{21}, C_{19} and C_{18} steroids.

Furthermore, it is postulated that there is a specific distribution of steroidogenic enzyme systems between the fetus and placenta in the midgestation human fetus. On the basis of the <u>in vivo</u> studies described or summarized in the

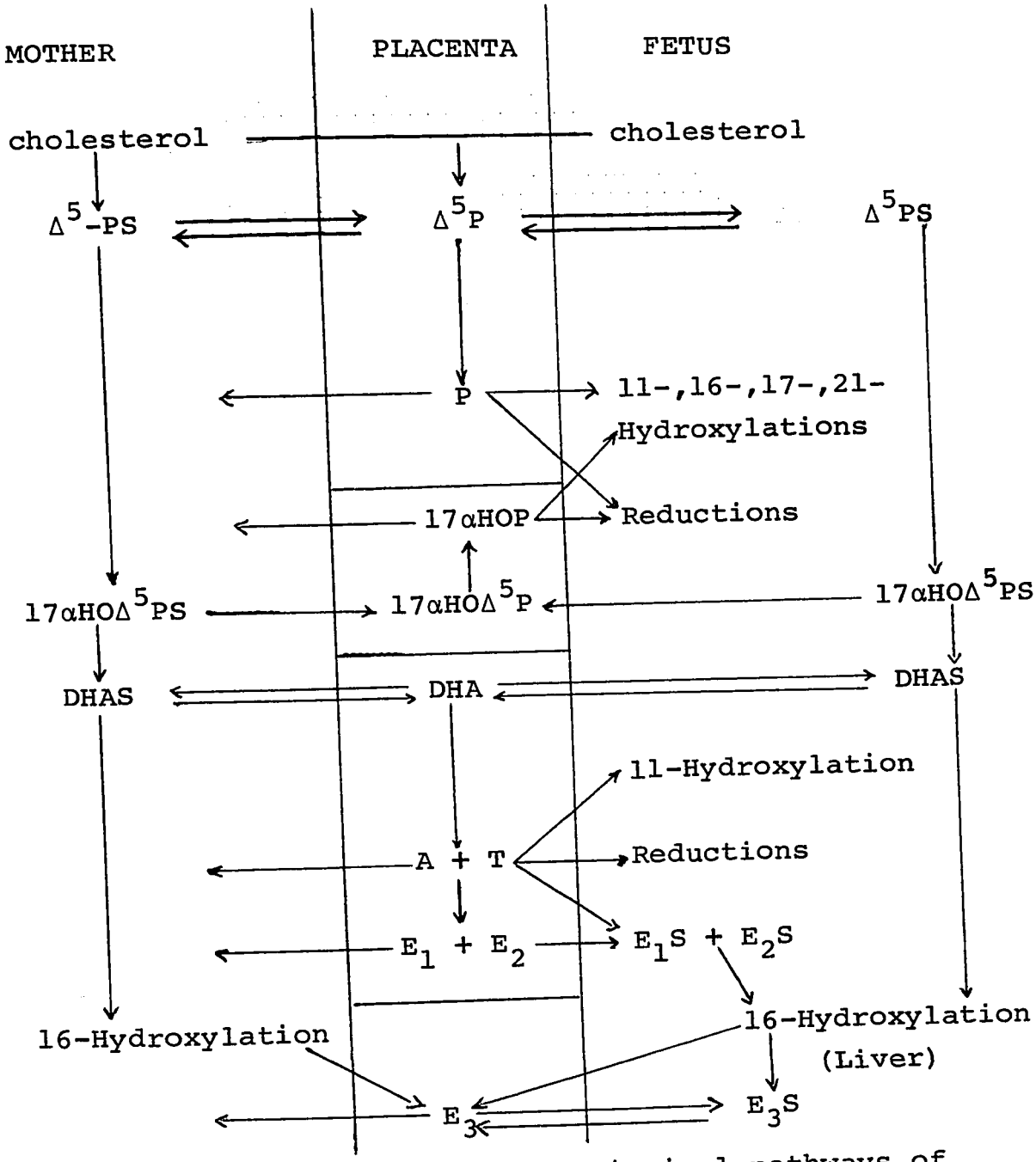


Fig. 6. Suggested scheme of the principal pathways of steroidogenesis in the human feto-placental unit at midpregnancy. (Reproduced from Diczfalusy, 1967). Δ^5P indicates pregnenolone, Δ^5PS is pregnenolone sulphate. 17αHOΔ^{5}P is 17α-hydroxypregnenolone and 17αHOΔ^{5}PS is 17α-hydroxypregnenolone sulphate. P indicates progesterone, 17α-HOP is 17α-hydroxy-progesterone. DHA is dehydroepiandrosterone and DHAS is dehydroepiandrosterone sulfate. A indicates androstenedione. T is testosterone, E_1 is estrone, E_2 is 17β-estradiol, E_3 is estriol, E_1S, E_2S and E_3S indicate the 3-sulphates of estrone, 17β-estradiol and estriol, respectively.

previous sections, Table 25 summarizes the distribution of the enzyme systems present in the human fetus and placenta at midgestation.

The past decade has witnessed a concentration of research effort on the elucidation of the various pathways of steroid biosynthesis and metabolism in the human feto-placental unit. Although a great deal still remains to be done in this respect, the next decade may result in an intensification of effort on investigations aimed at the elucidation of the various mechanisms by which steroidogenesis is regulated in the feto-placental unit, and of the physiological role of the different types of steroids produced.

2. The feto-placental endocrine system in other species of mammals.

Comparable information on the interaction and steroid biosynthetic capacity of the fetus and placenta in species other than the human is lacking. However, over the past several years work on various species of primates has indicated that one of these species, the baboon, may possess a feto-placental unit resembling that found in human pregnancy. Solomon and co-workers (reviewed by Solomon and Leung, 1972) have studied the biosynthesis and metabolism of steroids in the feto-placental unit of the baboon by injection of labeled steroids into the umbilical vein and subsequent analysis of fetal and placental tissues for labeled metabolites. After

TABLE 25

Distribution between the fetus and placenta of certain enzyme activities involved in steroid metabolism at midpregnancy as suggested by the results of various *in vivo* experiments.*

Enzyme (Trivial names)	Placenta	Fetus
Squalene cyclohydroxylase	+	+
Desmolases		
20α-22 (C_{27})	+	a
17α-20 (C_{21})	-	+
Hydroxysteroid dehydrogenases		
3α-	-	+
3β-	+	b
11β-	+	?
17α-	-	+
17β-	+	+
20α-	+	+
20β-	-	+
Δ^4-Δ^5-isomerase	+	b
Δ^4-5α-reductase	-	+
Δ^4-5β-reductase	-	+
Steroid hydroxylases		
2-	c	+
6α-	c	+
6β-	+	+
11β-	-	+
15α-	-	+
16α-	-	+
16β-	-	+
17α-	-	+
18-	-	+
21-	-	+
Aromatizing enzyme system	+	+
O-Methyltransferase	-	+
Arylsulphotransferase	a	+
Hydroxysteroid sulphotransferases		
3β-	-	+
21-	-	+
UDP-Glucuronyltransferase	-	+
β-Glucuronidase	-	+
Arylsulphatase	+	-
Sterolsulphatase	+	+

* Table reproduced from Diczfalusy, 1967.

a Traces

b With the probable exception of the testis, fetal tissues do not convert 3β-hydroxy Δ^5-steroids into α,β-unsaturated 3-ketosteroids. However, they are able to reduce C_{21} α,β-unsaturated 3-ketosteroids into 3β-hydroxy-compounds.

c Demonstrated so far only *in vitro*.

injection of a mixture of pregnenolone-7α-^{3}H and dehydroepiandrosterone-4-^{14}C into the umbilical vein in late pregnancy, it was found that pregnenolone could be hydroxylated at 11β-, 17α-, and 21-positions by the fetal adrenal as well as being metabolized to C_{19} steroids. Pregnenolone was not converted to C_{19} steroids by the placenta, but this tissue was capable of converting dehydroepiandrosterone to estrogen, a situation analogous to that in the human. In a separate study, cholesterol-4-^{14}C and progesterone-7α-^{3}H were injected into the umbilical vein of the pregnant baboon in the third trimester. It was shown that the fetal adrenal was capable of forming cortisol from progesterone, but that cholesterol was not metabolized to neutral steroids. In the human mid-term fetus a very small conversion of cholesterol to neutral steroids has been demonstrated in the adrenals and perfusates with the use of perfusion techniques (Solomon et al., 1967; Telegdy et al., 1970b; Archer et al., 1971).

Merkatz and Beling (1969) have found that the predominant estrogen excreted in the pregnancy urine of the baboon is estrone, with estradiol-17β and estriol being excreted in significantly smaller amounts. However, after the incubation of placental tissue with dehydroepiandrosterone, androstenedione or testosterone, estradiol-17β was the predominant estrogen isolated (Ainsworth et al., 1969; Milewich and Axelrod, 1971; 1972a). Estradiol-17β was also the principal estrogen isolated from placental tissue after injection of a

mixture of dehydroepiandrosterone-4-^{14}C and pregnenolone-7α-^{3}H into the umbilical circulation (Solomon and Leung, 1972). In order to explain the difference between the predominance of estrone in urine and estradiol-17β in the placenta Leung et al. (1972) studied the fate of estradiol-17β-4-^{14}C injected intravenously into the maternal compartment of the pregnant baboon. These authors were able to isolate the following radioactive steroids from the urine after injection of estradiol-17β; estrone and estradiol-17β from the unconjugated fraction; estrone, 2-methoxyestrone, estradiol-17β, 15α-hydroxyestradiol-17β, 16-oxoestradiol-17β and estriol from the sulfate fraction; and estrone, 2-methoxyestrone, 16α-hydroxyestrone, estradiol-17β, 16-oxoestradiol-17β and estriol from the glucosiduronate fraction. Estrone was the most abundant estrogen in all three fractions. These results confirm the observation that estrone is the principal estrogen present in pregnancy urine of the baboon and suggests that estradiol-17β formed in the placenta is transported to the maternal circulation, where it is converted to a large extent to estrone, which is then excreted in the maternal urine. Furthermore, all of the metabolites isolated in this study have also been isolated from human pregnancy urine and emphasizes the qualitative similarity between the estrogen metabolites excreted in the urine of the two species.

Khamsi et al. (1972) studied the conversion of acetate to cholesterol in the fetus of the baboon and the transfer

of cholesterol from mother to fetus by injection of cholesterol-7α-^{3}H into the maternal circulation and acetate-1-^{14}C into the fetus. Their results indicated several similarities, with respect to the conversion of acetate to cholesterol and the transfer of cholesterol from mother to fetus, between the pregnant baboon and pregnant human female. The pregnant baboon like the human is able to form cholesterol from acetate in the fetus and incorporate maternal cholesterol into the fetus. Also, the placenta in either species appears to be unable to form cholesterol from acetate to any significant extent.

Kling et al. (1972) have evaluated the baboon as a model for studying the control of estrogen synthesis during human pregnancy. Using techniques similar to those described by Siiteri and MacDonald (1963; 1966) these authors showed that the efficiency of conversion of maternal circulating dehydroepiandrosterone-^{3}H or dehydroepiandrosterone sulfate-^{3}H to urinary estrogens increased with gestational age. However, the extent of conversion of the precursors into urinary estrogens was lower and the pattern of incorporation into urinary estrogens was different from that observed in pregnant women. In pregnant women the majority of radioactivity recovered in the urinary phenolic fraction is associated with estriol (Baulieu and Dray, 1963) while in the baboon, incorporation of the precursors into estrone was predominant. These results, however, agree with those of Merkatz and Beling

(1969) and Leung et al. (1972). Moreover, the authors showed that in pregnant baboons the conversion of dehydroepiandrosterone sulfate-^{3}H to estradiol-17β at the tissue level (about 4%) was about one tenth of that observed in the human female at a similar stage of gestation (Siiteri and MacDonald, 1966). They also demonstrated that placental enzyme activities involved in estrogen synthesis decreased in the order steroid-3-sulfatase < 3β-hydroxysteroid dehydrogenase-isomerase < aromatase. These activities were similar to those observed for the human placenta, except that the baboon aromatase activities were relatively lower (Townsley et al., 1970). The structure-activity correlations for inhibition of baboon and human placental sulfatase by naturally occurring steroids were also similar.

From the above results it appears that the pregnant baboon may offer a suitable model for studying endocrine aspects of human pregnancy. The evidence suggests that the baboon possesses a feto-placental unit similar to that found in human pregnancy. However, this feto-placental unit is centred on the formation of estrone and estradiol-17β rather than estriol and more work needs to be done in this species in order to determine its suitability as a model for human pregnancy.

A second primate species, the rhesus monkey, has been investigated as to its suitability as a model for human pregnancy. The rhesus monkey has been used extensively for

studies on reproduction, but little is known concerning the biosynthesis and metabolism of steroids by this species during pregnancy. The temporal relationships between the plasma levels of estrogens, progesterone and the urinary level of chorionic gonadotropin during pregnancy have been studied in detail (Hodgen et al., 1972). The plasma level of estrogens increases with advancing pregnancy as does the urinary excretion (Hopper and Tullner, 1967). However, in contrast to the high levels of estriol in human pregnancy urine, the excretion of estrone exceeds that of the combined urinary excretion of estradiol-17β and estriol (Hopper and Tullner, 1967). The plasma concentration of cholesterol in the rhesus monkey declines at about one month after conception and this level is maintained until about one month after parturition, when the level returns to that observed in the non-pregnant animal (Wolf et al., 1967). In pregnant women, the plasma cholesterol decreases slightly during the first trimester and this is followed by a gradual increase to a peak value at or near parturition (Green, 1966). The plasma levels of progesterone are much lower than those observed in pregnant women at comparable stages of pregnancy (Neill et al., 1969; Hodgen et al., 1972). Pregnanediol and the 5α-isomer have been detected in the urine of pregnant rhesus monkeys by gas-liquid chromatography (Chamberlain et al., 1964).

Recently, the metabolism of progesterone in the fetus and placenta of the rhesus monkey has been studied by Leung

and Solomon (1972) by injection of progesterone-4-^{14}C into the umbilical vein of two pregnant monkeys in the 20th and 21st week of pregnancy, followed by isolation of radioactive metabolites from the placenta and various fetal tissues. A series of metabolites was isolated from the various tissues, all in the unconjugated form. Allopregnanedione was isolated from the placenta, 5β-pregnane-3α-ol-20-one from the liver and intestine, 5α-pregnane-3α-ol-20-one and pregnanediol from the liver, 5α-pregnane-3β-ol-20-one from the placenta, lungs and testes, 20α-hydroxyprogesterone from the lungs and intestine, 17α-hydroxyprogesterone from the adrenals and testes and androstenedione from the adrenals. The authors concluded from these data that progesterone is metabolized mainly to reduced products in the fetus and that conjugation with either sulfuric and/or glucuronic acid occurs to a very limited extent. They further suggest that the metabolism of progesterone by the fetus and placenta of the rhesus monkey does not resemble that found in the human feto-placental unit and therefore, this species cannot be used as a model for the investigation of endocrine aspects of human pregnancy.

In domestic species, indirect evidence to suggest the concept of a feto-placental endocrine system has been demonstrated for the sow, cow, sheep and goat. Erb et al. (1968b) studied the excretion of urinary estrogens following ovariectomy of cows at various stages of pregnancy and demonstrated that the excretion rate of estrogens was not significantly

lowered as a result of ovariectomy. However, the levels of progesterone dropped significantly, which is in accordance with the view that the corpus luteum is required for pregnancy maintenance and normal parturition in this species (Estergreen et al., 1967). In a similar study with the pregnant sow, Fèvre et al. (1968) showed that the estrogen excretion of sows, ovariectomized in early pregnancy and maintained on 300 mg progesterone to prevent abortion, followed a pattern similar to that of intact sows. They concluded that the placenta and fetus contain the necessary enzymes required for synthesis of estrogens. Studies by Bedford et al. (1972a) with the pregnant sheep and Thorburn et al. (1972) with the pregnant goat have shown that the principal site of estrogen production in late pregnancy is the uterus, as evidenced by the correlation between the uterine secretion rate and production rate of estrogens in the sheep and the arteriovenous difference in estrogen concentration between the uterine artery and vein in the goat. Furthermore, in each of these species the placenta has been shown to be capable of synthesizing estrogens from androgen precursors (Ainsworth and Ryan, 1966; 1970). Although these studies with the domestic species indicate that the probable source of estrogens during pregnancy is the uterus, definitive experiments on the capacity of the fetus to supply estrogen precursors has not yet been investigated to any significant extent. In a preliminary study on the perfusion of the iso-

lated sheep fetus with pregnenolone-7α-^{3}H and progesterone-4-^{14}C, Pierrepoint et al. (1971b) isolated dehydroepiandrosterone and its sulfate and androstenedione from the perfusate, indicating that the sheep fetus has the capacity to supply C_{19} estrogen precursors from C_{21} steroids.

CHAPTER II

MATERIALS AND METHODS

A. MATERIALS AND APPARATUS

1. Reference standards

Crystalline 18-hydroxycorticosterone was presented by Dr. T. Sandor, Hospital Notre-Dame, Montreal, Quebec, Canada.

The remaining crystalline steroid standards (see Table 26) were obtained from either Sigma Chemical Co., St. Louis, Mo., U.S.A., or from Steraloids Inc., Pawling, N.Y., U.S.A. Purity was assessed by thin-layer chromatography and, if necessary, the steroids were recrystallized before use.

2. Radioisotopes

Acetic acid-1-^{14}C, sodium salt (S.A. 61 mCi/mM), pregnenolone-7α-^{3}H (S.A. 500 mCi/mM) and progesterone-7α-^{3}H (S.A. 255 mCi/mM) were purchased from Amersham/Searle Corporation, Des Plaines, Illinois, U.S.A. The acetic acid was used as received whereas the pregnenolone and progesterone were checked for purity by thin-layer chromatography in the systems methylene chloride-acetone (80:20 v/v) and chloroform-acetone (98: 2 v/v) immediately before use.

3. Solvents and reagents

All solvents employed conformed to A.C.S. specifications and were used without prior distillation.

All reagents were of analytical grade unless otherwise stated.

TABLE 26

Systematic and trivial names of crystalline steroids used

Trivial	Abbreviation	Systematic
adrenosterone		androst-4-ene-3, 11, 17-trione
aldosterone		11β, 21-dihydroxy-3, 20-dioxopregn-4-en-18-al
androstenedione	Δ^4	androst-4-ene-3, 17-dione
corticosterone	B	11β, 21-dihydroxypregn-4-ene-3, 20-dione
18-hydroxycorticosterone		11β, 18, 21-trihydroxypregn-4-ene-3, 20-dione
deoxycorticosterone	DOC	21-hydroxypregn-4-ene-3, 20-dione
cortisol	F	11β, 17α, 21-trihydroxypregn-4-ene-3, 20-dione
11-deoxycortisol	S	17α, 21-dihydroxypregn-4-ene-3, 20-dione
cortisone	E	17α, 21-dihydroxypregn-4-ene-3, 11, 20-trione
dehydroepiandrosterone	DHEA	3β-hydroxyandrost-5-en-17-one
pregnenolone		3β-hydroxypregn-5-en-20-one
17α-hydroxypregnenolone		3β, 17α-dihydroxypregn-5-en-20-one
progesterone		pregn-4-ene,-3, 20-dione
11β-hydroxyprogesterone		11β-hydroxypregn-4-ene-3, 20-dione
17α-hydroxyprogesterone		17α-hydroxypregn-4-ene-3, 20-dione
20α-dihydroprogesterone		20α-hydroxypregn-4-en-3-one

4. Chromatography

a) Thin layer chromatography

Thin layer chromatography was performed on Silica Gel G (Mackerey, Nagel & Co., obtained through Canlab, Montreal, Canada).

Using a Desaga thin-layer spreader (Hauptstrasse 60, Heidelberg, Germany, obtained through C.A. Brinkmann, Long Island, N.Y., U.S.A.), thin layer chromatoplates were prepared by spreading a slurry of 30 g of silica gel and 60 ml water on 20 x 20 cm glass plates, at a thickness of 250 μ. The plates were allowed to air dry and then activated at 110^{o}C for 60 minutes, after which they were kept over anhydrous silica gel in a dessicator cabinet at room temperature until used. Chromatography was carried out by ascending chromatography in tanks in which three of the inner walls were lined with filter paper dipping into the solvent at the bottom of the tank. Samples were placed on the plates, approximately 3 cm from the edge of the plate, as a series of spots using a fine tip pipette. Samples on the chromatograms were dried with an electric fan and the plates developed in the tank. In cases of bulky extracts, the material was placed on the starting line as a strip rather than as a series of discontinuous spots. The running time in the systems employed varied between 45-75 minutes.

b) Paper chromatography

Paper chromatograms were developed on Whatman # 1 filter paper. The apparatus, method of application of the samples and development of the chromatograms was essentially as described by Bush (1961). The running time in the systems used varied between 3-5 hours.

5. Radioactivity measurements

Areas of radioactivity on thin layer chromatograms were located by scanning in a Panax thin-layer Radiochromatogram Scanner (Panax Equipment Ltd., Redhill, Surrey, England) and on paper chromatograms in a Packard, Model 7201, Radiochromatogram Scanner (Packard Instrument Co. Inc., Downers Grove, Illinois, U.S.A.). Radioactivity was measured in either an Ansitron Model (obtained through Dero Research and Dev. Corp., Hungtington, N.Y., U.S.A.) or a Beckman Model LS-250 liquid scintillation counter. Samples were counted in 10 ml toluene ('scintanalyzed' toluene Fisher Scientific Co. Ltd., Ottawa) containing 4 g Omnifluor/litre (New England Nuclear, Boston, Mass., U.S.A.) for sufficient time to ensure a counting error no greater than 3%.

B. METHODS

1. Studies on the biosynthesis of sterols and cholesterol by sheep and pig fetal liver and placental tissue <u>in vitro</u>.

a) Collection and preparation of tissues

Fetal liver and placenta were removed from a pregnant sheep at 127 days gestation and from a pregnant pig at 112 days gestation, within 20 minutes after slaughter. The tissues were transported in ice chilled saline to the laboratory where samples of each tissue were cut into approximately 0.7 mm thick slices with a razor blade.

b) Incubation and extraction procedures

Duplicate samples of each tissue slice preparation were incubated, initially for 2 hours at 37°C in a Dubnoff incubator with air as the gas phase, with acetate-1-^{14}C in Krebs-Ringer phosphate buffer, pH 7.4, containing 200 mg % glucose, 10 µ mole ATP and 10 µ mole NAD. After 2 hours the medium was removed by decantation and replaced with fresh medium containing additional acetate-1-^{14}C. Incubation was continued for another 2 hours, then the medium was again decanted and the two media from each incubation combined. The combined incubation media were extracted three times with twice their volume of ethyl acetate. The ethyl acetate was evaporated and the residue partitioned between hexane-90% methanol. After incubation, the tissue slices were homogenized in 10 ml ethanol-acetone-ether (4:4:1 v/v/v), centrifuged at 200 xg for 10 minutes and the supernatants decanted. This procedure was repeated. The combined tissue slice extracts were evaporated almost to dryness and saponified for 1 hour at 60°C in 5 ml of 15% potassium hydroxide

in 50% methanol. The saponified mixture was extracted with ether, the ether evaporated and the residue partitioned between hexane-90% methanol.

c) Fractionation and identification of radioactive sterols and cholesterol.

The hexane extracts from each incubation were combined and evaporated to dryness. The residue was chromatographed on Silica Gel G in the system benzene-ethyl acetate (5:1 v/v), as recommended by Avigan et al. (1963). This system gives a complete separation of squalene, lanosterol and cholesterol. Zones of radioactivity corresponding in mobility to each of these compounds were eluted from the silica gel with chloroform-methanol (2:1 v/v).

The cholesterol fractions were diluted with 200 µg reference cholesterol and subjected to digitonin precipitation, as outlined by Butt et al. (1948). The digitonin precipitable material was further diluted with 100 mg reference cholesterol, the dibromide formed and free cholesterol regenerated (Schwenk and Werthessen, 1952). The cholesterol was then recrystallized to constant specific activity.

The squalene fractions were diluted with 25-100 mg. carrier squalene and subjected either to bromination (van Leusden et al., 1971) or to hexahydrochloride formation (Loud and Bucher, 1958). The crystals of squalene dodecabromide or squalene hexahydrochloride were subsequently recrystallized to constant specific activity from different

solvent systems.

The lanosterol fractions were diluted with 50 mg carrier lanosterol and recrystallized to constant specific activity from different solvent systems, initially as the free compound and finally as the acetate derivative.

2. Studies on the biosynthesis of corticosteroids by fetal and maternal adrenal preparations from pregnant sheep and pigs in vitro.

a) Collection and preparation of tissues.

Fetal and maternal adrenal tissues were removed from pregnant sheep, at 70, 105 and 140 days of pregnancy, and from pregnant pigs, at 56 and 112 days of pregnancy, within 30 minutes after slaughter. The glands were transported in ice chilled saline to the laboratory where they were cleaned of adhering fat, the capsule removed, and in the case of maternal tissue, the cortical tissue was dissected free of medulla.

b) Incubation and extraction procedures.

Equivalent quantities of fetal and maternal sheep and pig adrenal tissue were homogenized in a glass homogenizer at 600 rpm in 0.05 M phosphate buffer, pH 7.0, containing 0.25 M sucrose and 0.04 M nicotinamide. The homogenates were incubated with either pregnenolone-7α-^{3}H or progesterone-7α-^{3}H, together with 10μ moles DPN and 10μ moles ATP for 1 hour at 37^{o}C in a Dubnoff incubator with air as the gas phase. After incubation the tissue and medium were extracted

with 3 x 10 ml methylene chloride and 3 x 10 ml ethyl acetate. The crude solvent extracts were filtered through Whatman # 3 filter paper, pooled, evaporated and stored in 10 ml methanol at -5°C until required.

c) Fractionation and identification of radioactive steroid metabolites.

The radioactive methanolic extracts were separated and purified by a series of chromatographic steps employing the following solvent systems: paper chromatography (PC),

a) chloroform-formamide (Zaffaroni and Burton, 1951)

b) benzene-methanol-water (2:1:1 v/v/v) (Bush, 1952)

c) acetone-benzene-water (1:2:2 v/v/v) (Sandor, pers. comm.)

Thin-layer chromatography (TLC),

1) chloroform (Hagerman, 1964)

2) chloroform-methanol (200: 9 v/v) (Vandenheuvel, 1969).

3) benzene-ethyl acetate (18:2 v/v) (Fahmy et al., 1968).

4) chloroform-acetone (185:15 v/v) (Fahmy et al., 1968).

5) ethyl acetate-cyclohexane (50:50 v/v)

6) chloroform-absolute ethanol (90:10 v/v)

7) benzene-absolute ethanol (40:10 v/v)

8) benzene-absolute ethanol (90:10 v/v).

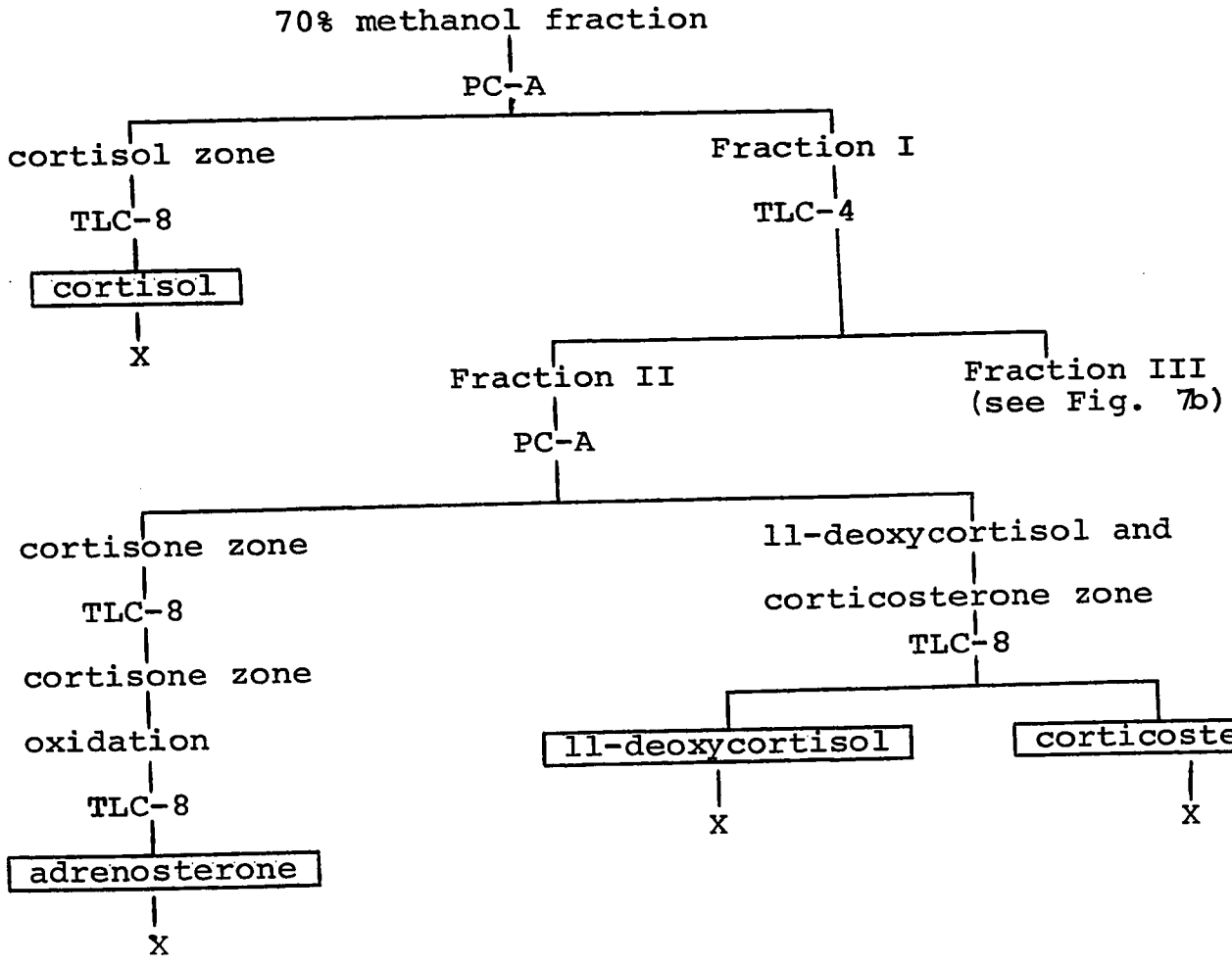
Systems 5 to 8 were used as described by Lisboa (1963; 1966a, b). All systems were used at room temperature. Equilibration and development of the chromatograms were carried out according to Bush (1952) and Lisboa (1966a).

Phosphomolybdic acid was used to locate authentic reference Δ^5-3β-hydroxy steroids on both paper strips and thin layer chromatograms. Δ^4-3-oxosteroids were located on both paper strips and thin layer chromatograms by exposure to ultraviolet light at 254 mμ. Radioactive areas on either paper strips or thin layer chromatograms were located as described in section A-5.

The schemes used to separate individual steroid metabolites from extracts of sheep and pig adrenal tissue preparations after incubation with either pregnenolone or progesterone are shown in Fig. 7 and Fig. 8. Final identification of each of the steroid metabolites isolated was carried out by recrystallization of the isolated radioactive material and a derivative with authentic reference material to constant specific activity (Axelrod et al., 1965). Constant specific activity was admitted when the specific activity of successive crystals and mother liquors agreed within ±5%.

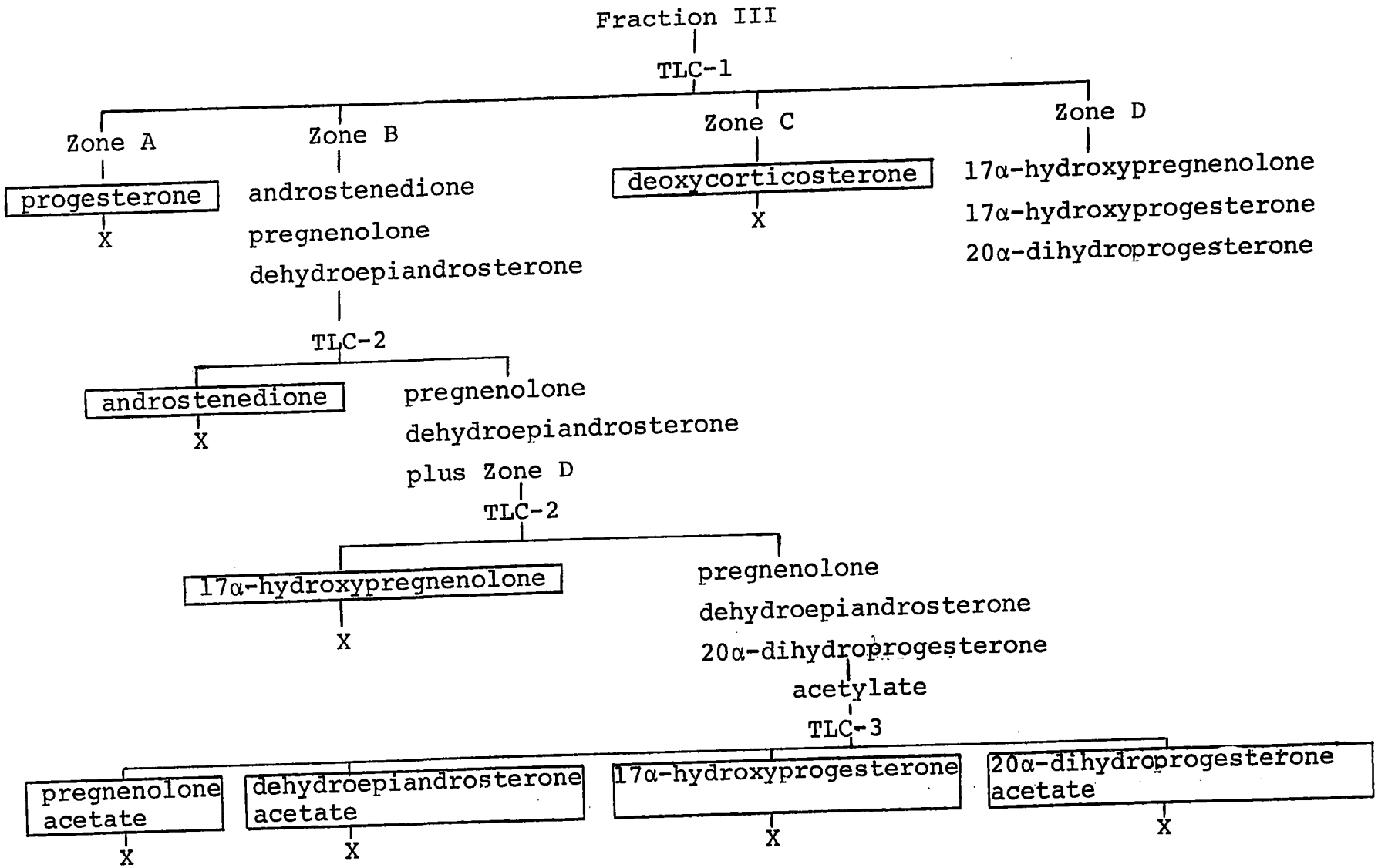
3. Derivative formation.

The procedure used for derivative formation were as follows:



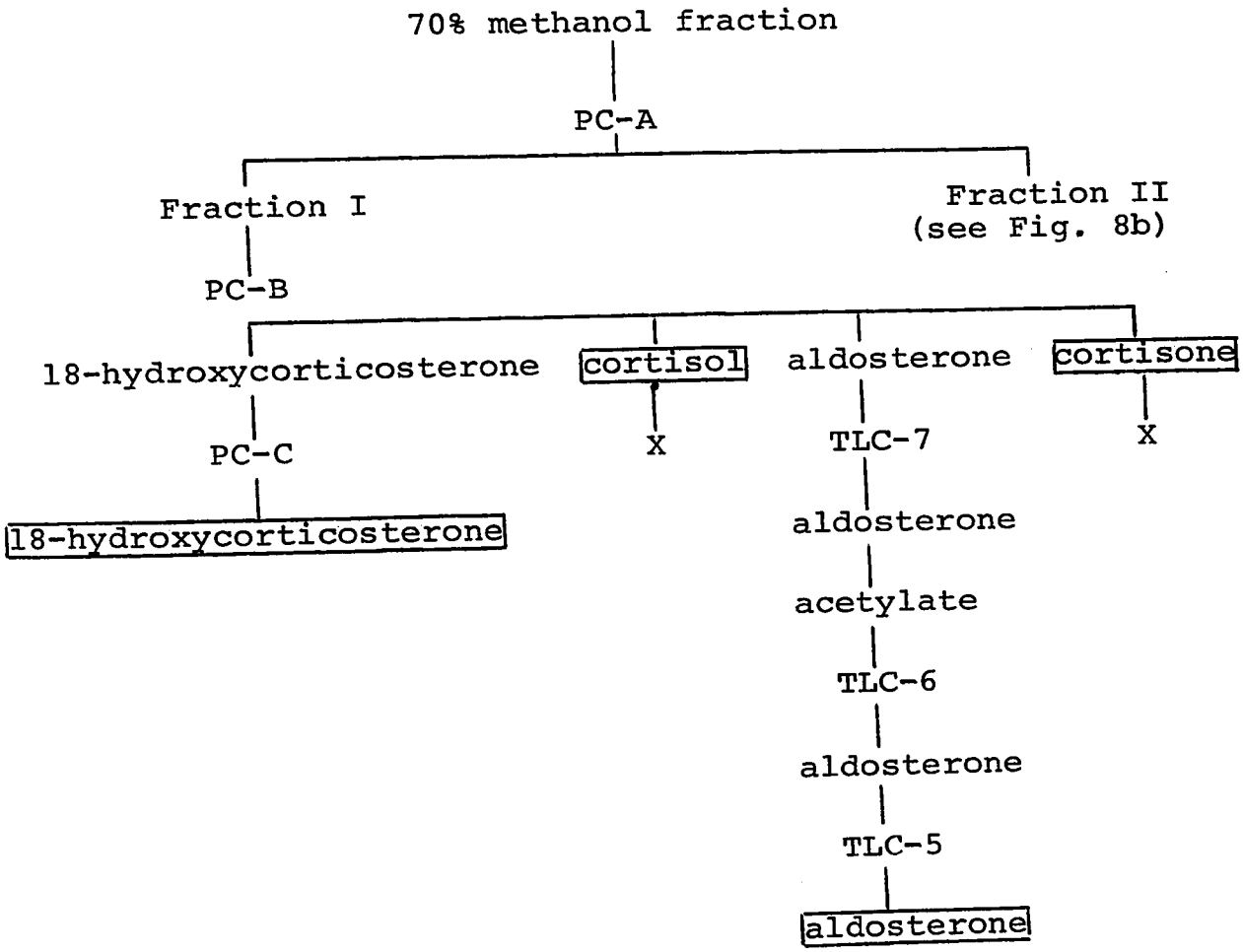
X = Crystallization to constant specific activity

Fig. 7a. Scheme used to separate and identify radioactive steroid metabolites in extracts from sheep fetal and maternal adrenal glands after incubation with pregnenolone-7α-^{3}H and progesterone-7α-^{3}H.



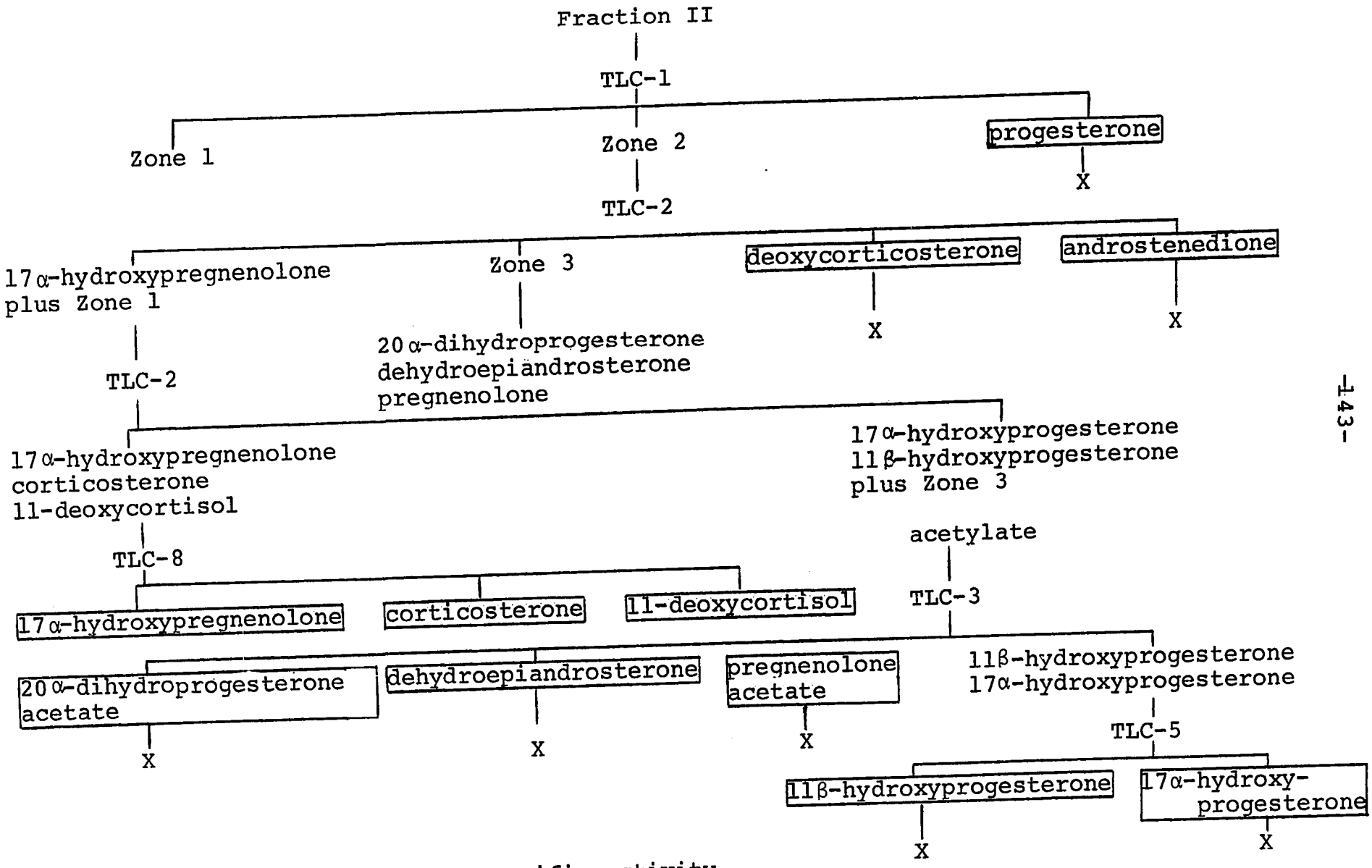
X = Crystallization to constant specific activity.

Fig. 7b. Scheme used to separate and identify radioactive steroid metabolites in extracts from sheep fetal and maternal adrenal glands after incubation with pregnenolone-7α-^{3}H and progesterone-7α-^{3}H.



X = Crystallization to constant specific activity.

Fig. 8a. Scheme used to separate and identify radioactive steroid metabolites in extracts from pig fetal and maternal glands after incubation with pregnenolone-7α-^{3}H and progesterone-7α-^{3}H.



X = Crystallization to constant specific activity

Fig. 8b. Scheme used to separate and identify radioactive steroid metabolites in extracts from pig fetal and maternal adrenal glands after incubation with pregnenolone-7α-^{3}H and progesterone-7α-^{3}H.

a) cholesterol dibromide formation.

The material purified through the digitonide was dissolved in 3 ml of ether in a 25 ml. Erlenmeyer flask. The flask was cooled in ice water and bromine added in fine drops until the solution remained orange-colored. The flask was kept in the ice water for 30 minutes, and 2-3 ml of glacial acetic acid was added. Within a few minutes crystals of 5, 6-dibromo-3-cholestanol appeared. Ten minutes later, the crystals were filtered over suction and washed with cold methanol. The crystals should be white and silky. They were transferred to a 25 ml beaker and 2 gm zinc dust was added. The contents were thoroughly mixed with a small glass rod. Then 3 ml of glacial acetic acid were added and contents mixed until the mixture became a magna. The contents of the beaker were stirred occasionally for the next 30 minutes. Ten to 15 ml of ether were added and mixed with the contents of the beaker. The ether was decanted into a separatory funnel and this was repeated 4 times. The combined ether solution was washed twice with water, twice with 10% NaOH solution and again twice with water. The ether was evaporated under nitrogen and the residue dissolved in methanol with a drop of ethyl acetate. The methanol was filtered and left in the cold to recrystallize. Cholesterol obtained in this fashion was brilliantly white and has a melting point of 148-149^{o}C (Schwenk and Werthessen, 1952).

b) Squalene dodecabromide formation.

The squalene fractions were diluted with 25-100 mg of carrier squalene. The mixture was dissolved in peroxide free ether at 0-4°C and brominated, after which a few ml. of glacial acetic acid were added. The crystals of the squalene dodecabromide formed were washed twice with ether and twice with absolute methanol (van Leusden et al., 1971).

c) Squalene hexahydrochloride formation.

The squalene fractions were diluted with 20 mg. carrier squalene and treated for 30 minutes with 10 ml of dry acetone previously saturated with HCl at -5 to -10°C. HCl gas was continuously bubbled through the mixture in order to maintain saturation and to provide stirring. A flocculent precipitate of crude squalene hexahydrochloride began to appear in 3 to 5 minutes. Still at the same temperature, the solvent was evaporated under a vigorous flow of nitrogen. The solid residue was washed twice with 0.3 ml cold absolute ethanol and twice with 0.2 ml cold ether. Crystals were centrifuged down after each washing and solvent drawn off with a Pasteur pipette. The colorless residue was digested for 5 minutes at 50°C in 1.0 ml acetone and rapidly centrifuged for a couple of minutes. The purified product was crystallized from the supernatant fluid by leaving in the refrigerator at -10°C (Loud and Bucher, 1958).

d) Acetylation.

The material to be acetylated was dissolved in 0.2 ml pyridine. An equal volume of acetic anhydride was added and the mixture was left overnight, in the dark, at room temperature. The reagents were removed with a stream of nitrogen (Bush, 1961).

e) Sodium bismuthate oxidation.

The material to be oxidized was dissolved in 0.5 ml glacial acetic acid and 0.5 ml water and 25 mg of sodium bismuthate were added. The mixture was shaken for 1-2 hr. in the dark at room temperature and extracted with 10 ml of ethyl acetate. The ethyl acetate was filtered and the filter was washed twice with 5 ml ethyl acetate. The extract was washed with aqueous sodium bicarbonate (0.1 vol., saturated) and water (0.1 vol.), and dried with sodium sulphate and evaporated (Bush, 1961).

CHAPTER III

The de novo Synthesis Of Sterols And Cholesterol From Acetate-1-^{14}C By Sheep and Pig Placental And Fetal Liver Slices in vitro.

1. Introduction

A systematic investigation of de novo cholesterol biosynthesis by the human placenta and fetus in vivo has established that the midgestation human placenta, in contrast to the midgestation human fetus, does not appear to be capable of synthesizing cholesterol from acetate (Mathur et al., 1970; Teledy et al., 1970a). Based on these studies and those of van Leusden et al. (1971), the balance of evidence suggests that the de novo synthesis of sterols and cholesterol by the midgestation human placenta is of little significance. However, human term placental preparations are capable of converting acetate to squalene, lanosterol and cholesterol in vitro (van Leusden and Villee, 1965; Zelewski and Villee, 1966; Villee and Tsai (1969), which indicates that the placenta may acquire the capacity to synthesize limited amounts of these compounds at a later stage of gestation. Furthermore, the principal source of placental progesterone is probably derived from circulating maternal cholesterol (Hellig et al., 1969; Diczfalusy, 1969). As demonstrated by Ainsworth and Ryan (1969) and Ryan (1969), a number of mammalian placental preparations are capable of the in vitro conversion

of pregnenolone to progesterone. However, apart from the demonstration of acetate to cholesterol conversion by the fetal liver of the rabbit (Popjak and Beechams, 1950) and the mare (Heard et al., 1954), literally nothing is known of acetate to cholesterol conversion by the placenta and fetus of other mammalian species.

The present experiments were performed to evaluate the ability of sheep and pig placental and fetal liver tissues to carry out the de novo synthesis of cholesterol from acetate-1-^{14}C in vitro.

2. Materials and Methods

Fetal liver and placental tissue were removed from a pregnant sheep at 127 days gestation and from a pregnant pig at 112 days gestation, after slaughter. Samples of each tissue were cut into approximately 0.7 mm thick slices with a razor blade. Duplicate samples (approximately 750 mg) of each tissue slice preparation were incubated initially for 2 hr at 37^{o}C in a shaking water bath with air as the gas phase and with a 20 µCi acetate-1-^{14}C in 5.2 ml Krebs-Ringer phosphate buffer (ph 7.4) containing 200 mg % glucose, 10µ mole & 10µ mole NAD. After 2 hr the medium was decanted and replaced with fresh medium containing a further 20 µCi acetate-1-^{14}C. Incubation was continued for another 2 hr, then the medium was again decanted and the two media from each incubation were combined. Extraction of the incubation media and tissues and subsequent separation and identification of sterols

and cholesterol in the extracts were carried out as described in the methods section.

3. Results and discussion

The crystallization data for squalene, lanosterol and cholesterol isolated from the hexane fractions after incubation of acetate-1-^{14}C with sheep and pig placental and fetal liver slices are presented in Tables 27-29. Each compound was isolated in a radiochemically homogenous form from all the tissues incubated. In each case radiochemical purity of the isolated metabolite was established by crystallization to constant specific activity of the parent compound and/or derivative. The percentage conversion of acetate-1-^{14}C to each metabolite after recrystallization to constant specific activity is presented in Table 30. The figures presented represent minimal conversions as no account was taken of the losses incurred during the isolation procedures.

The study shows that sheep and pig placental and fetal liver tissue slices are capable of converting acetate-1-^{14}C into sterols and cholesterol in vitro. These data also shows, particularly in the case of the sheep, that placental tissue appears to be considerably more effective than the fetal liver in converting acetate to cholesterol. This observation is in sharp contrast to that observed by corresponding human tissues incubated in vitro (van Leusden and Villee, 1965; Zelewski and Villee, 1966; Villee and Tsai, 1969; Givner and Jaffe, 1971; Telegdy et al., 1972) where fetal

TABLE 27

Crystallization data for radioactive squalene[a,b] isolated following incubation of sheep and pig fetal liver and placental tissue with sodium acetate-1-^{14}C (S.A. expressed as d.p.m./mg).

Species	Tissue	Number of Crystallizations[c] 1	2	3	4
Sheep	Placenta	201	183	175	-
		179	191	181	-
	F. Liver	20	20	20	19
		56	31	20	22
Pig	Placenta	148	151	133	140
		200	162	144	139
	F. Liver	473	443	470	482
		565	453	472	482

a) The material isolated from the sheep tissues was recrystallized as the dodecabromide derivative. Material crystallized from 1) acetone-chloroform 2) toluene-methanol-chloroform 3) benzene-methanol-chloroform 4) toluene.

b) The material isolated from the pig tissues was recrystallized as the hexahydrochloride derivative. Material recrystallized from ethyl acetate-chloroform.

c) In each series of crystallizations upper figure represents the specific activity of the crystals and lower figure the specific activity of the corresponding mother liquor.

TABLE 28

Crystallization data[a] for radioactive lanosterol[b] isolated following incubation of sheep and pig fetal liver and placental tissue with sodium acetate-1-^{14}C (S.A. expressed as d.p.m./mg).

Species	Tissue	Number of Crystallizations 5	6	7	8
Sheep	Placenta	1078[d]	1031	1083	1045[c]
		1520	1184	1193	1106
		4	5	6	7
	F. Liver	89	87	94	89[c]
		136	118	98	90
		6	7	8	9
Pig	Placenta	184	165	166	155[c]
		334	244	177	167
		9	10	11	12
	F. Liver	332	331	338	315[c]
		394	373	369	332

a) In each case the crystallization data for the last four crystallizations only are shown.

b) Material crystallized from 1) chloroform-methanol 2) acetone-methanol 3) chloroform-ethanol 4) acetone-ethanol.

c) Final crystallization carried out after formation of acetate derivative.

d) In each series of crystallizations upper figure represents the specific activity of the crystals and lower figure the specific activity of the corresponding mother liquor.

TABLE 29

Crystallization data for radioactive cholesterol[a] isolated following incubation of sheep and pig fetal liver and placental tissue with sodium acetate-1-^{14}C (S.A. expressed as d.p.m./mg).

Species	Tissue	Number of Crystallizations 1	2
Sheep	Placenta	2045[b]	2041
		2052	1933
	F. Liver	31	28
		30	30
Pig	Placenta	161	153
		157	160
	F. Liver	58	57
		62	59

a) Material crystallized from chloroform-methanol.

b) In each series of crystallizations upper figure represents the specific activity of the crystals and lower figure the specific activity of the corresponding mother liquor.

TABLE 30

Percentage conversion of sodium acetate-1-^{14}C into radioactive squalene, lanosterol and cholesterol following incubation with sheep and pig fetal and placental tissues.

Species	Tissue	% Conversion		
		Squalene	Lanosterol	Cholesterol
Sheep	Placenta	0.16	0.14	0.18
	F. Liver	0.03	0.002	0.003
Pig	Placenta	0.002	0.003	0.004
	F. Liver	0.004	0.006	0.002

liver tissue preparations were found to have considerably greater capacity than placental tissue in transforming acetate into cholesterol. In fact, it has been suggested by Telegdy et al (1972) that cholesterol biosynthesis and metabolism in the various compartments of the human feto-placental unit may be highly specialized. The human fetal liver is able to synthesize considerable quantities of cholesterol from acetate both in vitro and in vivo (Telegdy et al., 1970a; Telegdy et al., 1972; Givner and Jaffe, 1971) but does not appear to convert the cholesterol formed to steroids. The placenta, however, is unable to synthesize cholesterol from acetate in vivo (Telegdy et al., 1970a; van Leusden et al., 1971) but is capable of transforming cholesterol to C-21, C-19 and C-18 steroids (Telegdy et al., 1970b) and finally, the fetal adrenals and testes are capable of synthesizing cholesterol from acetate in vitro, as well as metabolizing it to a variety of steroids (Jaffe et al., 1968a, Sherr Serra et al., 1970).

In the present study this small but definitive conversion of acetate to cholesterol by each of the tissues investigated indicates the potential for de novo synthesis of cholesterol by the sheep and pig feto-placental endocrine system. However, the contribution of this potential source of cholesterol to the overall availability of cholesterol in the feto-placental endocrine system of each of these species remains to be determined.

CHAPTER IV

Studies on the Metabolism of Pregnenolone-7α-^{3}H and Progesterone-7α-^{3}H by Sheep Fetal and Maternal Adrenal Tissue Preparations at Different Stages of Gestation

Introduction

Over the past several years a number of lines of evidence have suggested that the fetus, and more particularly, the fetal adrenal makes a significant contribution to the events leading to parturition. The study of Liggins et al. (1967), Drost and Holm (1968), Liggins and Kennedy (1968) and Liggins (1969b) have shown that prolonged pregnancy in sheep is associated with fetal adrenal hypoplasia induced experimentally by intra-uterine fetal hypophysectomy or pituitary stalk section and bilateral fetal adrenalectomy. Moreover, Liggins (1968; 1969b) has demonstrated that premature parturition can be induced in sheep by infusion of either synthetic corticotropin or the glucocorticoids cortisol or dexamethasone into the fetus. These results indicate the importance of a functional fetal pituitary - adrenal axis for the occurrence of normal parturition in sheep. Further evidence in support of this concept has been provided by Bassett and Thorburn (1969) and by Nathanielsz et al. (1972) who have shown that there is a several-fold increase in the levels of fetal plasma corticoids over the

week preceding parturition. Alexander et al. (1971) have also shown an elevation of fetal plasma ACTH levels over this period.

It has also been established that the adrenal cortex of the fetal and adult sheep secretes cortisol and corticosterone (Blair-West et al. 1963; Chester Jones et al., 1964; Alexander et al., 1968). However, until fairly recently little attempt had been made to study the biosynthesis of these compounds. In a kinetic study of the synthesis of corticosterone from pregnenolone-16-^{3}H and progesterone-4-^{14}C by fetal and adult sheep adrenal glands, Vinson (1967) showed that the compound 21-hydroxypregnenolone was formed only from pregnenolone and the author suggested that this compound might act as an alternative intermediate to progesterone for corticosterone formation. With both substrates deoxycorticosterone was shown to be an intermediate in the formation of corticosterone. Anderson et al. (1970) compared the formation of corticoids from pregnenolone-7α-^{3}H and progesterone-4-^{14}C by adult and fetal sheep adrenal tissue, the latter being taken from a sheep at 122 days of pregnancy. They showed that both substrates were almost completely metabolized by the adult tissue over a 2 hour incubation period, whereas with fetal tissue only pregnenolone showed extensive metabolism. The adult adrenal converted pregnenolone mainly to cortisol, corticosterone and 11-deoxycortisol, while corticosterone was the principal product formed from progesterone.

The fetal adrenals yielded mainly progesterone from pregnenolone with only a small conversion to 11-deoxy- and 11-hydroxycorticoids; progesterone was transformed mainly to deoxycorticosterone. The compound 21-hydroxypregnenolone was not isolated during this study, although the formation of corticoids from pregnenolone via a pathway not involving progesterone could not be ruled out.

In view of the paucity of information regarding fetal adrenal steroidogenesis in sheep, the present studies were initiated to investigate the capacity of the fetal adrenal gland to synthesize steroids, particularly corticosteroids from pregnenolone and progesterone at different stages of gestation. The metabolism of these substrates by maternal and fetal adrenal glands removed at the same time from sheep at various stages of gestation has been compared. It was hoped that such an approach would provide valuable information regarding the development and activity of adrenal enzyme systems during gestation and would allow an evaluation and comparison of the principal biosynthetic pathways involved in corticosteroid formation by fetal and maternal adrenal tissue.

Method

Adrenal glands were removed from both the fetus and the mother of pregnant sheep at the following times during apparently normal pregnancies: a) 70 days (fetal adrenals

only), b) 105 days and c) 140 days. The preparation and processing of the tissues before incubation and subsequent extraction, separation and identification of the metabolites formed after incubation of the adrenal tissue preparations with either pregnenolone-7α-^{3}H or progesterone-7α-^{3}H have been described in the Methods section. Details of the incubations carried out are shown in Table 31. It will be noted that in each series of incubations approximately equal amounts of fetal and maternal adrenal tissue were incubated with approximately equimolar amounts of each substrate under identical incubation conditions. Under these conditions it was hoped that any differences in metabolism of the substrates by corresponding fetal and maternal tissue preparations might be reflected in the distribution of the radioactivity among the metabolites isolated.

Results

The recovery of radioactivity from the incubations after extraction and removal of the lipid from the extracts by precipitation with 70% methanol is shown in Table 32. It can be seen that an average loss of radioactivity of 13.8% (range 9.3-18.1) occurred on precipitation of the lipid. The reason for this loss has not been investigated but it is possible that it occurred as a result of binding of steroids to the lipid material. The percentage of radioactivity in the various metabolites from each of the incu-

TABLE 31

Summary of incubations carried out with fetal and maternal adrenal tissue of sheep

Tissue	Gestation age (Days)	Weight (g)	Buffer (ml)	Substrate
F.*Adrenal	70	0.0520	5.0	2µc pregnenolone-7α-^{3}H
F. Adrenal	70	0.0450	4.5	2µc progesterone-7α-^{3}H
F. Adrenal	105	0.0867	5.0	2µc pregnenolone-7α-^{3}H
M.*Adrenal	105	0.0867	5.0	2µc pregnenolone-7α-^{3}H
F. Adrenal	105	0.0860	5.0	4µc progesterone-7α-^{3}H
M. Adrenal	105	0.0861	5.0	4µc progesterone-7α-^{3}H
F. Adrenal	140	0.1047	5.0	2µc pregnenolone-7α-^{3}H
M. Adrenal	140	0.1040	5.0	2µc pregnenolone-7α-^{3}H
F. Adrenal	140	0.1720	3.4	4µc progesterone-7α-^{3}H
M. Adrenal	140	0.1710	3.4	4µc progesterone-7α-^{3}H

* F = fetal; M = maternal

TABLE 32

Percentage recovery of radioactivity from incubations after extraction and removal of lipid material from the extracts by precipitation with 70% methanol. (Recovery in all cases expressed as a percentage of the radioactivity incubated).

Tissue	Gestational age (Days)	Substrate	% Radioactivity After extraction	% Radioactivity After lipid precipitation
F.*Adrenal	70	pregnenolone	89.9	75.0
F. Adrenal	70	progesterone	80.5	66.4
F. Adrenal	105	pregnenolone	87.8	76.1
M.*Adrenal	105	pregnenolone	81.6	66.0
F. Adrenal	105	progesterone	90.4	72.3
M. Adrenal	105	progesterone	80.4	65.1
F. Adrenal	140	pregnenolone	92.1	78.2
M. Adrenal	140	pregnenolone	90.9	76.9
F. Adrenal	140	progesterone	84.7	73.4
M. Adrenal	140	progesterone	83.4	74.1
		Average	86.2	72.4

* F = fetal; M = maternal.

bations after isolation and identification by recrystallization to constant specific activity of the metabolite and/or derivative is shown in Tables 33 and 34. The figures in each of these tables represent minimal recoveries and conversions as no account was taken of the lossed incurred during the isolation procedures. The crystallization data for each of the metabolites isolated from the various incubations shown in Tables 33 and 34 are summarized in Tables I and II presented in Appendix II. It will be noted from Table 33 that all fetal and maternal adrenal preparations studied extensively metabolized pregnenolone. However, at 70 and 105 days of gestation, progesterone was a major metabolite formed from pregnenolone. It is evident that a greater transformation of pregnenolone to corticosteroids took place in the maternal adrenal glands and fetal adrenal glands removed at 140 days gestation, apparently by further conversion of the progesterone formed. The metabolism of progesterone (Table 34) by sheep adrenals showed many features in common to those reported for pregnenolone.

If the results in Tables 33 and 34 are expressed as shown in Table 35, it will be noted that over 90% of the radioactivity recovered in the isolated steroids can be accounted for by progesterone, 11-deoxycorticoids and 11-oxycorticoids. It is also evident that the decrease in amount of radioactivity associated with progesterone is accompanied by a proportionate increase in the radioactivity

TABLE 33

Percentage radioactivity found in isolated steroids after incubation of pregnenolone-7α-^{3}H with fetal and maternal adrenal tissue from sheep at different stages of gestation. (Results expressed as a percentage of the radioactivity incubated).

Steroid isolated	% Radioactivity found in isolated steroids				
	F.*Adrenal 70 days	F. Adrenal 105 days	M.*Adrenal 105 days	F. Adrenal 140 days	M. Adrenal 140 days
Pregnenolone	0.10	1.44	0.06	0.05	0.06
17α-Hydroxypregnenolone	0.10	1.12	1.36	2.32	1.58
Progesterone	32.97	17.05	0.44	0.39	1.70
17α-Hydroxyprogesterone	0.08	0.37	0.27	0.36	0.48
20α-dihydroprogesterone	0.07	0.12	0.07	0.09	0.07
Deoxycorticosterone	9.47	28.86	13.74	19.03	15.15
Corticosterone	0.60	0.58	2.04	3.86	1.18
11-Deoxycortisol	0.68	0.79	5.87	2.28	6.22
Cortisol	0.42	0.48	1.42	1.29	0.78
Cortisone	0.03	0.03	1.16	0.54	0.47
Dehydroepiandrosterone	0.06	0.09	0.05	0.06	0.06
Androstenedione	0.27	0.15	0.20	0.23	0.69
TOTAL RECOVERY	44.85	51.08	26.68	31.50	28.44

* F = fetal; M = maternal.

TABLE 34

Percentage radioactivity found in isolated steroids after incubation of progesterone-7α-^{3}H with fetal and maternal adrenal tissue from sheep at different stages of gestation. (Results expressed as a percentage of the radioactivity incubated).

Steroid isolated	% Radioactivity found in isolated steroids				
	F.*Adrenal 70 days	F. Adrenal 105 days	M.*Adrenal 105 days	F. Adrenal 140 days	M. Adrenal 140 days
Progesterone	29.92	4.52	0.13	9.29	0.52
17α-Hydroxyprogesterone	0.07	0.50	0.92	0.75	0.80
20α-dihydroprogesterone	0.07	0.05	0.03	0.04	0.03
Deoxycorticosterone	9.74	21.42	0.77	7.15	1.39
Corticosterone	0.33	3.02	2.77	3.08	3.21
11-Deoxycortisol	0.37	0.85	2.55	2.26	2.38
Cortisol	0.37	0.76	3.04	1.21	3.36
Cortisone	-	0.17	0.27	0.30	0.39
Androstenedione	0.25	0.71	0.06	0.15	0.80
TOTAL RECOVERY	41.12	32.00	10.54	24.23	12.88

* F = fetal; M = maternal.

TABLE 35

Distribution of radioactivity between progesterone, 11-deoxycorticosteroids and 11-oxycorticosteroids after incubation of sheep adrenal glands with pregnenolone-7α-^{3}H or progesterone-7α-^{3}H. (Results expressed as a percentage of the radioactivity recovered after isolation and identification of metabolites. See recoveries in Tables 33 and 34.

Substrate	Metabolites	F^{*}_{70}	F_{105}	M^{*}_{105}	F_{140}	M_{140}
Pregnenolone-7α-^{3}H	progesterone	73.5	33.4	1.7	1.3	6.0
	11-deoxycorticosteroids	22.6	58.0	73.5	67.6	75.1
	11-oxycorticosteroids	2.3	2.1	17.3	18.1	8.5
Progesterone-7α-^{3}H	progesterone	72.8	14.1	1.2	38.3	4.0
	11-deoxycorticosteroids	24.6	69.6	31.5	38.8	29.3
	11-oxycorticosteroids	1.7	12.3	57.6	18.9	54.0

* F = fetal adrenal; M = maternal adrenal; subscript in all cases indicates stage of gestation.

associated with corticosteroids. In addition, the distribution of radioactivity between the various corticosteroids also changes with gestational age, a greater proportion being associated with 11-oxycorticosteroids at 140 days gestation than at 70 or 105 days. The studies also show that several compounds in addition to corticosteroids were formed after incubation of sheep adrenal tissue with pregnenolone or progesterone. 17α-Hydroxyprogesterone, 20α-hydroxyprogesterone and androstenedione were formed from both substrates while 17α-hydroxypregnenolone and dehydroepiandrosterone were formed from pregnenolone.

Discussion

The results of the present investigation indicate that sheep fetal adrenal glands are able to metabolize pregnenolone and progesterone to a variety of steroids even at 70 days of pregnancy. This is not surprising in view of the fact that chicken, rat and human secretory activity has been demonstrated after histological and anatomical establishment of the adrenal cortex (See Bloch, 1968 for pertinent references). However, steroid metabolism may proceed even as early as embryonic differentiation. This is suggested from the work of Huff and Eik-Ness (1966) who demonstrated steroid biosynthetic activity in six-day-old rabbit blastocysts and the demonstration of steroid dehydrogenase activities in a 1.4 cm human embryo (Baillie et al., 1966). As shown in

Tables 33, 34 and 35 fetal adrenal tissue incubated with pregnenolone or progesterone was able to convert both these substrates to corticosteroids, the proportion of corticosteroids formed increasing with gestational age. It is apparent from these studies that the increase in corticosteroid formation from both pregnenolone and progesterone by fetal adrenal tissue was due to a proportionate decrease in the amount of radioactivity associated with progesterone, suggesting that the metabolic route from pregnenolone to corticosteroid proceeds through progesterone. The extensive conversion of pregnenolone to progesterone by sheep adrenal tissue at 70 days gestation would tend to support this concept and supports the findings of Anderson et al. (1970b; 1972). However, the formation of cortisol from pregnenolone via the pathway pregnenolone → 17α-hydroxypregnenolone → 17α-hydroxyprogesterone → 11-deoxycortisol → cortisol cannot be ruled out although it would appear that this pathway does not make as significant a contribution to cortisol synthesis in the fetal adrenal as it does in the adult. In the adult sheep adrenal, Anderson et al. (1970b) have assessed the relative activity of the alternative routes to cortisol synthesis from pregnenolone and concluded that the major pathway is by the route involving 17α-hydroxypregnenolone, which is similar to that determined for adult human adrenal tissue (Griffiths and Cameron, 1970). The kinetic study by Vinson (1967) showed that the compound 21-hydroxypregnenolone

was formed only from pregnenolone and although progesterone was also formed, the results indicated that progesterone was not necessarily an obligatory intermediate in the production of corticosterone from pregnenolone. The formation of 21-hydroxypregnenolone is of interest in this respect because the structure is such that it could conceivably be formed as an alternative intermediate to progesterone in the formation of corticosterone. The latter findings also showed that there was little difference between the adrenals of adult and fetal sheep with respect to corticosterone formation.

It is not possible to assess the relative significance of pregnenolone and progesterone as precursors of cortisol and corticosterone respectively in the present studies because steroid standards were not added to correct for losses during isolation of the various metabolites. However, Anderson et al. (1970b) have suggested that pregnenolone and progesterone are transformed as efficiently to corticosterone and cortisol by sheep fetal adrenal tissue. These authors also demonstrated an apparent lack of both 17α- and 11-hydroxylase activity in fetal adrenal glands when compared to adult tissues. In a further study Anderson et al. (1972) demonstrated that both of these enzyme activities increased with gestational age or after infusion of synthetic ACTH (Synacthen) into fetuses to induce premature delivery. The most interesting aspect of this study was the marked inactivity of 11β-hydroxylase in fetal adrenals incubated with

pregnenolone and progesterone at 140 and 143 days of pregnancy. Active 11β-hydroxylase activity was apparent, however, after stimulation of the fetal adrenals with Synacthen or after removal from fetuses during spontaneous labor and the immediate new-born. The present results tend to confirm this finding, since the percentage of radioactivity associated with corticosterone and cortisol increased with gestational age (Table 35). These results, together with the results of Anderson et al. (1970b; 1972), Bassett and Thorburn (1969) and Nathanielsz et al. (1972) are in keeping with the hypothesis that the 11β-hydroxylase enzyme activity in the sheep fetal adrenal may increase very rapidly in the final days of gestation, thus increasing the synthesis of cortisol, which, in turn, may initiate the sequence of events leading to parturition. That ACTH may be responsible for the increase in 11β-hydroxylase activity has been indicated by the findings of Anderson et al. (1972) and Liggins (1968) who showed that the increase in 11β-hydroxylase activity and premature parturition could be induced by infusion of Synacthen into the fetus <u>in utero.</u> Although information is still lacking regarding the levels of fetal ACTH immediately before labor, Alexander et al. (1971) have demonstrated an increase of fetal plasma ACTH, particularly after 130 days gestation, further indicating the importance of a functional fetal pituitary-adrenal axis for the occurrence of natural parturition in sheep.

The formation of dehydroepiandrosterone and androstenedione from pregnenolone and of androstenedione from progesterone by fetal adrenal glands, at all three stages of gestation studied, is significant in view of the fact that one of the essential pre-requisites for a feto-placental endocrine unit, at least as defined in the human, is that the fetus is able to provide C_{19} steroid precursors for placental estrogen synthesis (Diczfalusy, 1969). The sheep placenta appears to be unable to synthesize estrogens from C_{21} precursors (Ainsworth and Ryan, 1966) and should, like the human, be incapable of de novo synthesis of estrogens. Sheep placental tissue is, however, capable of utilizing C_{19} precursors for estrogen synthesis (Ainsworth and Ryan, 1966; Pierrepoint et al., 1970; 1971a; Harvey et al., 1972) and, therefore, it is likely that estrogen synthesis by placental tissue occurs as a result of the provision of C_{19} precursors from the fetus. Further support for this concept in the sheep has come from a study by Davies et al. (1970) who showed that although estrogens could be synthesized from pregnenolone by combined homogenates of sheep fetal adrenal and placental tissue, similar incubations of placental tissue or adrenal tissue alone did not yield estrogens.

It has also been demonstrated that the maintained isolatedsheep fetus is capable of forming dehydroepiandrosterone and androstenedione from pregnenolone and progesterone respectively (Pierrepoint et al., 1971a). Of interest in these

experiments was the observation that a large proportion of the dehydroepiandrosterone present in the perfusate was in the form of its sulfate conjugate. It has been well documented that dehydroepiandrosterone sulfate is the principal precursor of estrogens in the human and is formed mainly in the fetal adrenal from pregnenolone (Diczfalusy, 1969). In vitro and in vivo studies have shown that the human fetal adrenal possesses a very potent steroid sulfokinase activity (Bolté et al., 1966; Wengle, 1966; Villee and Loring, 1969). In contrast, steroid sulfokinase activity in the sheep fetal adrenal appears to be lacking (Anderson et al., 1970b; 1972). This observation raises the question as to the site of formation of dehydroepiandrosterone sulfate in the sheep fetus. Recent evidence has suggested that the fetal liver may be the principal site of steroid sulfokinase activity in the sheep. Anderson et al. (1970a) have shown that fetal liver preparations are capable of sulfating pregnenolone and other steroids, while Ainsworth (1972b; 1973) has demonstrated that dehydroepiandrosterone and estrogens undergo active sulfation by sheep fetal liver tissue in vitro. These latter observations, together with the demonstration that sheep placental tissue is also able to convert C_{19}-steroids to estrogen sulfates (Pierrepoint et al., 1971a) explain the observations of Findlay and Cox (1970), who showed that the estrogens present in the plasma of the sheep fetus at various stages of gestation were principally in the form of sulfate conjugates.

In conclusion, it would appear that the enzymic capacity of the sheep fetal adrenals is established early in fetal life. This enzymic capacity once established is similar to that of the adult. The expression of this enzymic capacity appears to be regulated by several factors, one of which is the stimulation of 11β-hydroxylase activity by fetal ACTH resulting in an increase in fetal plasma cortisol which in turn appears to initiate the sequence of events leading to parturition (Liggins et al., 1972). In contrast to the human fetal adrenal, where the total fetal environment appears to limit adrenal secretion to sulphurylated Δ^5-3β-hydroxy-steroids (Diczfalusy, 1969) the sheep adrenal appears to be lacking in steroid sulphokinase activity and also appears to possess an active 3β-ol-dehydrogenase/isomerase enzyme system capable of converting pregnenolone to progesterone and dehydroepiandrosterone to androstenedione (Anderson et al., 1972). The physiological significance of the differences between sheep and human fetal adrenal enzymic activity are not yet apparent. However, it is hoped that as more information becomes available on the feto-placental endocrine relationships of steroids in the sheep during pregnancy, an insight into the mechanisms regulating adrenal cortical activity and elucidation of the physiological needs for the steroid hormones elaborated by the fetal adrenal and the feto-placental endocrine system may become apparent.

CHAPTER V

STUDIES ON THE METABOLISM OF PREGNENOLONE-7α-^{3}H AND PROGESTERONE-4-^{14}C BY FETAL AND MATERNAL ADRENAL GLANDS OF PIGS AT DIFFERENT STAGES OF GESTATION

1. Introduction

By the use of <u>in vitro</u> techniques it has been established by several authors that the principal corticoids elaborated by the adult pig adrenal are cortisol and corticosterone (Table 36). In addition, Dobriner et al. (1954), Heard et al. (1956) and Aprile et al. (1956) have reported the isolation of cortisol and cortisone from the adrenal glands of adult pigs. The studies of Marchut and Rembiesa (1967) with neonatal pig adrenal tissue incubated with progesterone-4-^{14}C have further demonstrated that 18-hydroxylation can occur with the formation of 18-hydroxycorticosterone and aldosterone. In contrast to the sheep, there are no reports of studies, as far as this author is aware, on the development of fetal adrenal enzymic activity or biosynthetic capacity of pig fetal adrenal glands. However, a recent report by Dvorak (1972) has demonstrated that the fetal blood plasma levels of 17-hydroxycorticosteroids increased with gestational age, the maximum being reached at the time of parturition. The levels then decreased progressively, the greatest decrease being noted over the initial ten days postpartum. A similar rapid increase in fetal plasma

TABLE 36

Metabolites isolated after incubation of pig adrenal tissue preparations with various substrates.

Substrate	Products	System	Reference
Acetate	cortisol cortisone corticosterone deoxy-corticosterone	adrenal slice	Haines, 1952
Pregnenolone	17-deoxy-corticosteroids 17-hydroxy-corticosteroids	homogenate	Yudaev and Pankov, 1964
Progesterone	cortisol cortisone aldosterone 11-deoxycortisol corticosterone 16α-hydroxy-progesterone deoxy-corticosterone 11β-hydroxy-progesterone 17α-hydroxy-progesterone 11-ketoproges-terone	adrenal cell free extract	Rao and Heard, 1957
Progesterone	cortisol corticosterone	adrenal slice	Druzhinina, 1958; Li, 1962
Progesterone	17-deoxy-corticosteroids	homogenate	Yudaev and Pankov, 1964

Progesterone	cortisol corticosterone deoxy-corticosterone 18-hydroxy-corticosterone aldosterone	adrenal mince (neonates)	Marchut and Rembiesa, 1967
Deoxy-corticosterone	corticosterone deoxy-corticosterone 6β-hydroxy-deoxycorticosterone	adrenal slice	Haines, 1952
11-Deoxy-cortisol	cortisol cortisone	adrenal slice	Haines, 1952

cortisol just prior to parturition, followed by a decline in the neonatal period has also been observed in the sheep (Paisay and Nathanielsz, 1971). In the cow and the guinea pig, Nathanielsz et al. (1972) have shown a significant decrease in the plasma levels of cortisol in the neonate over the first 10-12 days of life. These studies suggest that 17-hydroxycorticosteroids, particularly cortisol, may also be involved in the sequence of events leading to parturition in other species including the pig, although definitive evidence implicating fetal corticoids in the initiation of parturition in this species have yet to be established.

In view of the above observations, it became of interest to carry out similar experiments to those described in Chapter IV using pig adrenal tissue preparations. In this study, the spectrum of metabolites formed after incubation of pig fetal and maternal adrenal tissue removed from animals at different stages of pregnancy with pregnenolone-7α-^{3}H and progesterone-7α-^{3}H have been compared. The objectives of this study were similar to those for the sheep experiments, namely, to obtain information on the development and activity of adrenal enzyme systems during pregnancy and to evaluate and compare the biosynthetic steroid ability of fetal and maternal adrenal tissue of the pig.

2. Method

Adrenal glands were removed from both the fetus and the mother of pregnant pigs at the following times during apparently normal pregnancies: a) 56 days and b) 112 days. The preparation and processing of the tissues before incubation and subsequent extraction, separation and identification of the metabolites formed after incubation of the adrenal tissue preparations with either pregnenolone-7α-^{3}H or progesterone-7α-^{3}H have been described in the Methods section. Details of the incubations carried out are shown in Table 37. It will be noted that in each series of incubations approximately equal amounts of fetal and maternal adrenal tissue were incubated with approximately equimolar amounts of each substrate under identical incubation conditions. Under these conditions it was hoped that any differences in metabolism of the substrates by corresponding fetal and maternal tissue preparations would be reflected in the distribution of the radioactivity among the metabolites isolated.

3. Results

The recovery of radioactivity from the incubations after extraction and removal of the lipid from the extracts by precipitation with 70% methanol is shown in Table 38. An average loss of radioactivity of 13.4% (range 9.1-20.9) was apparent on precipitation of the lipid material, which is similar to that incurred with the sheep incubations

TABLE 37

Summary of incubations carried out with pig fetal and maternal adrenal tissue preparations at different stages of pregnancy

Tissue	Gestation age (Days)	wt(g)	Buffer (ml)	Substrate
F.*Adrenal	56	0.0541	5.0	2µc pregnenolone-7α-^{3}H
M.*Adrenal	56	0.0540	5.0	2µc pregnenolone-7α-^{3}H
F. Adrenal	56	0.0534	5.0	4µc progesterone-7α-^{3}H
M. Adrenal	56	0.0534	5.0	4µc progesterone-7α-^{3}H
F. Adrenal	112	0.1838	3.6	2µc pregnenolone-7α-^{3}H
M. Adrenal	112	0.2034	4.0	2µc pregnenolone-7α-^{3}H
F. Adrenal	112	0.1892	3.8	4µc progesterone-7α-^{3}H
M. Adrenal	112	0.1911	3.8	4µc progesterone-7α-^{3}H

* F = fetal; M = maternal.

TABLE 38

Percentage recovery of radioactivity from incubation after extraction and removal of the lipid material from the extracts by precipitation with 70% methanol. (Recovery in all cases expressed as a percentage of the radioactivity incubated).

Tissue	Gestational age (Days)	Substrate	% Radioactivity	
			After extraction	After lipid precipitation
F.*Adrenal	56	pregnenolone	85.5	76.2
M.*Adrenal	56	pregnenolone	83.4	71.1
F. Adrenal	56	progesterone	80.9	71.8
M. Adrenal	56	progesterone	83.6	62.7
F. Adrenal	112	pregnenolone	93.3	78.4
M. Adrenal	112	pregnenolone	92.9	81.0
F. Adrenal	112	progesterone	88.9	72.8
M. Adrenal	112	progesterone	85.9	73.0
		Average	86.8	73.4

* F = fetal; M = maternal.

(Chapter IV). The percentage of radioactivity in the various metabolites from each of the incubations after isolation and identification by recrystallization to constant specific activity of the metabolite and/or a derivative is presented in Tables 39 and 40. The figures in each of these tables represent minimal recoveries and conversions, as no account was taken of the losses incurred during the isolation procedures. The crystallization data for each of the metabolites isolated from the various incubations shown in Tables 39 and 40 are summarized in Tables III and IV presented in Appendix II. The data as presented in Tables 39 and 40 shows many features in common to that reported for the sheep incubations (Chapter IV). Pregnenolone was extensively metabolized by both fetal and maternal adrenals. At 56 days of pregnancy, however, progesterone was the principal metabolite isolated, whereas in the maternal adrenal incubations and the fetal adrenal incubations at 112 days of pregnancy, the percentage of radioactivity present in progesterone was small compared to that present in the various corticosteroids. A similar distribution of radioactivity was observed after incubation of pig adrenal tissues with progesterone (Table 40).

If the results in Tables 39 and 40 are expressed as shown in Table 41 it will be noted that the decrease in percentage of radioactivity in the progesterone fraction is largely accounted for by an increase in the proportion of

TABLE 39

Percentage radioactivity found in isolated steroids after incubation of pregnenolone-7α-^{3}H with fetal and maternal adrenal tissue from pigs at different stages of gestation

Steroid isolated	% Radioactivity found in isolated steroids			
	F.* adrenal 56 days	M.* adrenal 56 days	F. adrenal 112 days	M. adrenal 112 days
Pregnenolone	1.98	1.44	0.18	0.11
17α-Hydroxypregnenolone	0.90	1.23	0.52	0.95
Progesterone	37.23	1.53	1.85	0.90
17α-Hydroxyprogesterone	1.55	0.90	0.61	0.47
20α-dihydroprogesterone	0.24	0.24	0.13	0.12
Deoxycorticosterone	11.64	23.27	1.32	1.60
Corticosterone	1.18	3.17	10.13	4.48
11-Deoxycortisol	1.67	8.23	4.79	8.55
Cortisol	-	0.88	4.87	5.79
Cortisone	-	0.45	1.37	1.32
Dehydroepiandrosterone	-	-	-	-
Androstenedione	1.48	2.10	1.69	1.57
18-Hydroxycorticosterone	-	-	-	-
Aldosterone	-	-	-	-
11β-Hydroxyprogesterone	0.61	2.51	4.76	8.99
TOTAL RECOVERY	58.48	45.95	32.22	34.85

* F = fetal; M = maternal.

TABLE 40

Percentage radioactivity found in isolated steroids after incubation of progesterone-7α-^{3}H with fetal and maternal adrenal tissue from pigs at different stages of gestation

Steroid isolated	% Radioactivity found in isolated steroids			
	F.* adrenal 56 days	M.* adrenal 56 days	F. adrenal 112 days	M. adrenal 112 days
Progesterone	30.34	1.40	0.15	1.14
17α-Hydroxyprogesterone	1.22	0.80	1.17	0.55
20α-dihydroprogesterone	0.15	0.21	0.10	0.11
Deoxycorticosterone	10.89	24.16	1.30	1.44
Corticosterone	1.25	2.21	8.00	3.08
11-Deoxycortisol	0.99	6.29	3.17	7.17
Cortisol	-	0.75	8.85	5.62
Cortisone	-	0.55	0.71	1.23
Dehydroepiandrosterone	-	-	-	-
Androstenedione	4.87	4.62	6.92	3.31
18-Hydroxycorticosterone	-	-	-	-
Aldosterone	-	-	-	-
11β-Hydroxyprogesterone	0.63	1.90	7.47	2.71
TOTAL RECOVERY	50.24	42.89	37.84	26.36

* F = fetal; M = maternal.

TABLE 41

Distribution of radioactivity between various steroids after incubation of pig adrenal tissue preparations with pregnenolone-7α-^{3}H or progesterone-7α-^{3}H. (Results expressed as a percentage of the radioactivity recovered after isolation and identification of metabolites. See recoveries in Tables 39 and 40).

Substrate	Metabolite	F^{*}_{56}	M^{*}_{56}	F_{112}	M_{112}
Pregnenolone-7 -^{3}H	progesterone	63.7	3.3	5.7	2.6
	11-deoxycorticosteroids	22.8	68.6	19.0	29.1
	11-oxycorticosteroids	2.0	9.8	50.8	33.3
	11β-hydroxyprogesterone and androstenedione	3.5	10.0	20.0	30.3
Progesterone-7 -^{3}H	progesterone	60.4	3.3	0.4	4.3
	11-deoxycorticosteroids	23.6	71.0	11.8	32.7
	11-oxycorticosteroids	2.5	8.2	46.4	37.7
	11β-hydroxyprogesterone and androstenedione	10.9	15.2	38.0	22.8

* F = fetal adrenal; M = maternal adrenal; subscript in all cases indicates stage of gestation.

radioactivity associated with 11-deoxy and 11-oxycorticosteroids. Further, the proportion of radioactivity associated with 11-oxycorticosteroids is significantly greater in both maternal and fetal adrenal glands at 112 days of pregnancy than at 56 days. In all the incubations a large proportion of the radioactivity not accounted for by progesterone or corticosteroids can be seen to be associated with 11β-hydroxyprogesterone and androstenedione. In addition to these latter two compounds and corticosteroids several other compounds were isolated in small amounts from the incubation extracts. 17α-Hydroxyprogesterone and 20α-hydroxyprogesterone were formed from both pregnenolone and progesterone while 17α-hydroxypregnenolone was formed from pregnenolone. It is of interest that dehydroepiandrosterone could not be isolated from the pregnenolone incubations. Similarly, although some radioactivity was found to have a polarity similar to that of aldosterone and 18-hydroxycorticosterone on the initial paper chromatograms, further purification established that the radioactive material was not identical with either of these compounds.

4. Discussion

The results of this study have established that pig fetal adrenal glands, like those of the sheep, are able to metabolize pregnenolone and progesterone to a variety of steroids, particularly corticosteroids, even at mid-gestation.

It is also apparent that cortisol formation from pregnenolone, at least in the fetal adrenal glands, proceeds via a pathway utilising progesterone as the principal intermediate. However, the formation of cortisol via other pathways, such as that involving 17α-hydroxypregnenolone cannot be ruled out. In both the fetal and maternal adrenal incubations with pregnenolone, 17α-hydroxypregnenolone was isolated as a metabolite, indicating that cortisol may be formed via a pathway not involving progesterone as an intermediate. The occurrence of 11β-hydroxyprogesterone as a metabolite of pregnenolone and progesterone in both fetal and maternal adrenal incubations raises the possibility of a further alternative pathway in the formation of corticosteroids, other than through the classical sequence of reactions in which hydroxylation at C-21 precedes hydroxylation at C-11. The early studies by Levy et al. (1954) and by Eickhorn and Heckter (1958) with mammalian adrenals suggested that 11β-hydroxyprogesterone was not incorporated to any significant extent into 21-hydroxysteroids by either perfused bovine adrenals or bovine adrenal homogenates. These results led to the concept that 11β-hydroxyprogesterone was an end product of steroid biosynthesis. However, evidence to the contrary has been forthcoming from experiments using both mammalian and lower vertebrate adrenal gland preparations. Brownie et al. (1954) demonstrated the formation of 11β-hydroxyprogesterone by bovine adrenal mitochon-

drial preparations and suggested its possible intermediary role in corticosteroid formation. 11β-hydroxyprogesterone has also been isolated as a metabolite from duck, chicken and rat adrenal tissue incubated with pregnenolone or progesterone as substrates (Sandor et al., 1963; Tsang and Carballeira, 1966). Other investigators have shown that incubation of 11β-hydroxyprogesterone with bovine, rat, frog and duck adrenals consistently leads to an increased yield of corticosterone and aldosterone (Stachenko and Giroud, 1964; Kraulis and Birmingham, 1964; Tsang and Stachenko, 1969; Sandor and Lanthier, 1970). From the experiments with the duck adrenal (Sandor and Lanthier, 1970) and with the rat adrenal (Tsang and Stachenko, 1969) it was concluded that both C-21 → C-11 and C-11 → C-21 hydroxylation sequences were equally important in corticosteroid formation, under the in vitro conditions used. Although the results of the present study do not allow similar conclusions to be reached, the isolation of 11β-hydroxyprogesterone from all the incubations suggests that this compound may be formed as an intermediate in corticosteroid formation.

In the sheep and goat it has been established that activation of the fetal hypothalamus-pituitary-adrenal axis followed by a progressive increase in fetal plasma cortisol levels plays an integral part in initiating the sequence of events leading to parturition (Liggins et al., 1972; Thorburn et al., 1972). That fetal corticosteroids may play a similar

role in the pig is indicated from the results of the present study which suggest that fetal adrenal 11β-hydroxylase activity increases with gestational age (see Table 41) and from the study by Dvorak (1972) who has shown a significant rise in fetal plasma levels of 17α-hydroxycorticosteroids over the last 10 days of intra-uterine life. In addition, Dvorak (1972) demonstrated that pig fetal adrenals in late pregnancy were capable of responding markedly to stimulation with ACTH and that the increase in 17α-hydroxycorticosteroid secretion was accompanied by a significant increase in adrenal weight.

Although the work of Diczfalusy and his co-workers (Diczfalusy, 1969) has established that the human feto-placental unit is essential for the production and maintenance of estrogens and progesterone required for the continuation of normal pregnancy, the interrelationships between the fetus and placenta in terms of hormone production has not been established to the same extent for other mammalian species. In the sow, it should be noted that the ovary is required throughout gestation for pregnancy maintenance (du Mesnil du Buisson and Dauzier, 1957). Furthermore, the total luteal content of progesterone is maintained throughout pregnancy at a level comparable to that observed in the luteal phase of the estrus cycle (Duncan et al., 1960; Rombauts et al., 1965; Masuda et al., 1967). This latter observation is consistent with the fact that the ovaries are the principal source of progesterone during pregnancy in this species.

Thus, in this species the dominant function of a feto-placental endocrine system, if one exists, would be related to production and control of estrogen synthesis. Indirect evidence to support the concept of a feto-placental endocrine system in the pig has been provided by Rombauts and duMesnil du Buisson (1964) who showed that urinary estrogen secretion remained very low following hysterectomy of pregnant sows. Rombauts (1964) also showed that the estrogen content of placental tissue increased progressively with gestational age. In a further study using ovariectomized pregnant sows injected with 300 mg progesterone daily to prevent abortion, Fèvre et al. (1968) showed that the urinary excretion of estrone followed a pattern similar to that of intact sows. From these experiments it can be concluded that the estrogens excreted are not of ovarian origin. Furthermore, it is known that in this species the placenta is capable of forming estrogens from androgens but not from C-21 steroids (Ainsworth and Ryan, 1966). Fèvre (1970) also established that C-19 steroids were more efficiently converted to urinary estrogens when injected into the fetal rather than the maternal circulation. These results suggest that placental estrogen production in the sow, may like the human be dependent on a supply of androgen precursors from the fetus. If this is indeed the case, the role of the fetal adrenal as a source of fetal androgens becomes important, particularly as it has been demonstrated that the fetal adrenal has the capacity

to form C-19 steroids notably androstenedione, from pregnenolone and progesterone (Tables 39 and 40).

CHAPTER VI

SUMMARY

1. The ability of sheep and pig placental and fetal liver slices to convert acetate-1-^{14}C to sterols and cholesterol was investigated *in vitro*. Each preparation was incubated initially for 2 hr at 37°C in duplicate, with 20μCi acetate-1-^{14}C in 5.2 ml Krebs-Ringer phosphate buffer containing 200 mg glucose/100 ml, 10 μmole ATP and 10 μmole NAD. After 2 hr the medium was removed by decantation and replaced with fresh medium containing a further 20μCi acetate-1-^{14}C. Incubation was then continued for an additional 2 hr. After extraction of both tissues and incubation media with organic solvents, radioactive cholesterol was isolated in a radiochemically homogenous form from each of the incubations after parturition of the extracts between hexane-90% methanol, digitonide precipitation, dibromide formation and regeneration of free cholesterol and finally crystallization of the regenerated cholesterol to constant specific activity. Radioactive squalene and lanosterol were isolated from each series of incubations by thin-layer chromatography, purified by derivative formation and finally characterized by recrystallization to constant specific activity. This small but definitive formation (range 0.003-0.19%) of cholesterol by all the tissues examined indicates the potential for *de novo* synthesis of cholesterol by the sheep and pig feto-placental endocrine system and suggests that cholesterol synthesis from acetate occurs via the classical biosynthetic pathway involving squalene and lanosterol.

2. A comparison of the capacity of sheep and pig fetal and maternal adrenal glands removed at different stages of gestation to metabolize pregnenolone-7α-^{3}H and progesterone-7α-^{3}H was investigated in vitro. Fetal and maternal adrenal glands were removed from sheep at 70 days (fetal adrenals only), 105 days and 140 days of pregnancy and from pigs at 56 days and 112 days of pregnancy. Approximately equal amounts of fetal and maternal adrenal tissue were homogenized in a phosphate-sucrose-niacinamide buffer and 800 xg supernatant fractions were prepared and incubated with equimolar amounts of pregnenolone-7α-^{3}H and progesterone-7α-^{3}H for 1 hr at 37°C. After extraction of the incubation mixtures with organic solvents the radioactive steroid metabolites present in the extracts were purified and separated by paper and thin-layer chromatography. Final identification of all metabolites was carried out by recrystallization of the metabolite and/or a derivative to constant specific activity after addition of the appropriate non-radioactive reference compound.

Both substrates were metabolized extensively by maternal adrenal glands of both species, the principal metabolites isolated being cortisol, corticosterone, 11-deoxycortisol, and deoxycorticosterone. In addition, 17α-hydroxyprogesterone, 20α-dihydroprogesterone, cortisone and androstenedione were formed from both substrates, whereas 17α-hydroxypregnenolone was formed only from pregnenolone. 11β-Hydroxyprogesterone was also formed from both substrates by pig maternal adrenals.

Dehydroepiandrosterone was isolated only from sheep adrenals incubated with pregnenolone. Although the same metabolites were isolated after incubation of fetal adrenal preparations, the proportion of corticosteroids formed increased with the stage of gestation at which the adrenals were removed. Pregnenolone was metabolized extensively by all fetal adrenals. Progesterone was formed as a major metabolite after incubation of adrenals removed at mid-gestation from both species. The extent of conversion of pregnenolone to corticosteroids was increased significantly in fetal adrenals removed at later stages of gestation, presumably by further metabolism of the progesterone formed. The results suggest that the major metabolic route from pregnenolone to corticosteroids, at least in the fetal adrenal incubations, proceeds via progesterone, although alternative pathways not involving progesterone cannot be ruled out. The metabolism of progesterone by fetal adrenals showed many features in common to those reported for pregnenolone. Of greatest significance in this study was the apparent increase in fetal adrenal 11β-hydroxylase activity with gestational age in both species, as judged by the extent of conversion of both substrates to 11-oxygenated corticosteroids. This observation supports the concept that a functional fetal hypothalamic-pituitary-adrenal axis plays a significant role in the events leading to parturition by stimulation of fetal adrenal 11β-hydroxylase activity which results in an increase of fetal plasma cortisol levels. The formation of C_{19} compounds by fetal adrenals of both species indicates that the fetus of

both species has the potential to provide C_{19} precursors for placental estrogen synthesis. Furthermore, by comparing the spectrum of metabolites formed by fetal and maternal adrenal glands of both species it may be concluded that the steroidogenic capacity of fetal adrenal glands is established during intra-uterine life.

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APPENDIX I

APPENDIX I

Glossary of trivial names and corresponding systematic names used in this thesis

Trivial name	IUPAC (1968) systematic name
19-Aldoandrostenedione	4-androstene-3, 17, 19-trione
Aldosterone	11β, 21-dihydroxy-3, 20-dioxo-4-pregn-en-18-al (11 → 18) - lactol
19-Aldotestosterone	17β-hydroxy-4-androstene-3-one, 19-al
Allo pregnanedione; Allopregnanetriol; Allopregnanolone	5α-pregnane-3, 20-dione; 5α-pregnane-3β, 17α, 20α-triol; 5α-pregnane-3β-ol-20-one
$\Delta^{4,7}$-Androstadien-19-ol-3, 17-dione	19-hydroxy-4, 7-androstadiene-3, 17-dione
Δ^{5}-Androstenediol	5-androsten-3β, 17β-diol
Δ^{5}-Androstenediol-3-sulfate	3β, 17β-dihydroxy-5-androsten-3β-yl sulfate
Androstenedione	4-androstene-3, 17-dione
Δ^{7}-Androstenolone	3β-hydroxy-5, 7-androstadien-17-one
Cholesterol	5-cholesten-3β-ol
Cholesterol sulfate	5-cholesten-3β-yl sulfate
Corticosterone	11β, 21-dihydroxy-4-pregnene-3, 20-dione
Corticosterone sulfate	11β, 21-dihydroxy-4-pregnene-3, 20-dione-21-yl sulfate
Cortisol	11β, 17α, 21-trihydroxy-4-pregnene-3, 20-dione
Cortisone	17, 21-dihydroxy-4-pregnene-3, 11, 20-trione
Dehydroepiandrosterone	3β-hydroxy-5-androsten-17-one

Dehydroepiandrosterone sulfate
Deoxycorticosterone
Deoxycorticosterone sulfate
11-Deoxycortisol
3-Desoxyequilenin
Dexamethasone

Dihydroequilenin-17α
Dihydroequilenin-17β
Dihydroequilin-17α
Dihydroequilin-17β
17α, 20α-Dihydroxycholesterol
17α, 21-Dihydroxypregnenolone
20α-Dihydroxypregnenolone sulfate
17α-hydroxy-20α-Dihydroprogesterone
16-Epiestriol
16, 17-Epiestriol
17-Epiestriol
Epipregnanolone
Epitestosterone
Equilenin
Equilin
Estradiol-17α
Estradiol-17αsulfate
Estradiol-17β

3β-hydroxy-5-androsten-17-one-3β-yl sulfate
21-hydroxy-4-pregnene-3, 20-dione
21-hydroxy-4-pregnene-3, 20-dione-21-yl sulfate
17α, 21-dihydroxy-5-pregnene-3, 20-dione
1, 3, 5(10), 6, 8-estrapentaen-17-one
9α-fluoro-11β, 17, 21-trihydroxy-16α-methyl-1, 4-pregnadiene-3, 20-dione
1, 3, 5(10), 6, 8-pentaene-3, 17α-diol
1, 3, 5(10), 6, 8-pentaene-3, 17β-diol
1, 3, 5(10), 7-estratetraene-3, 17α-diol
1, 3, 5(10), 7-estratetraene-3, 17β-diol
5-cholestene-3β, 17α, 20α-triol
3β, 17α, 21-trihydroxy-5-pregnen-20-one
3β, 20α-dihydroxy-5-pregnen-20-one-3β-yl sulfate
17α, 20α-dihydroxy-4-pregnene-3, 20-dione
1, 3, 5(10)-estratriene-3β, 16β, 17β-triol
1, 3, 5(10)-estratriene-3β, 16β, 17α-triol
1, 3, 5(10)-estratienne-3β, 16α, 17α-triol
5β-pregnane-3α-ol-20-one
17α-hydroxy-4-androsten-3-one
3-hydroxy-1, 3, 5(10), 6, 8-estrapenta-17-one
3-hydroxy-1, 3, 5(10), 7-estratetraen-17-one
1, 3, 5(10)-estratriene-3β, 17α-diol
1, 3, 5(10)-estratriene-3β, 17α-diol-3-yl sulfate
1, 3, 5(10)-estratriene-3β, 17β-diol

Estradiol-17β sulfate

$\Delta^{5,7,9}$-Estratrienol-3-one-17

Estriol

Estrone

Estrone sulfate

$\Delta^{5,7,9(10)}$-Estratrienol-3-one-17

11β-Hydroxyandrostenedione

15α-Hydroxyandrostenedione

16α-Hydroxyandrostenedione

19-Hydroxyandrostenedione

7α-Hydroxyandrosterone sulfate

20α-Hydroxycholesterol

18-Hydroxycorticosterone

7α-Hydroxydehydroepiandrosterone

7β-Hydroxydehydroepiandrosterone

16α-Hydroxydehydroepiandrosterone

16α-Hydroxydehydroepiandrosterone sulfate

19-Hydroxydehydroepiandrosterone

2-Hydroxyestradiol

7α-Hydroxyestradiol

15α-Hydroxyestradiol

2-Hydroxyestrone

7α-Hydroxyestrone

15α-Hydroxyestrone

1, 3, 5(10)-estratriene-3β, 17β-diol-3-yl sulfate

3β-hydroxy-5, 7, 9-estratien-17-one

1, 3, 5(10)-estratriene-3β, 16α, 17β-triol

3β-hydroxy-1, 3, 5(10)-estratrien-17-one

3β-hydroxy-1, 3, 5(10)-estratrien-17-one-3-yl sulfate

3β-hydroxy-5, 7, 9(10)-estratrien-17-one

11β-hydroxy-4-androstene-3, 17-dione

15α-hydroxy-4-androstene-3, 17-dione

16α-hydroxy-4-androstene-3, 17-dione

19-hydroxy-4-androstene-3, 17-dione

3α, 7α-dihydroxy-5α-androstan-17-one-3α-yl sulfate

5-cholesten, 3β, 20α-diol

11β, 18β, 21-trihydroxy-4-pregnene-3, 20-dione

3β, 7α-dihydroxy-5-androsten-17-one

3β, 7β-dihydroxy-5-androsten-17-one

3β, 16α-dihydroxy-5-androsten-17-one

3β, 16α-dihydroxy-5-androsten-17-one-3β-yl sulfate

3β, 19-dihydroxy-5-androsten-17-one

1, 3, 5(10)-estratriene-2, 3 , 17β-diol

1, 3, 5(10)-estratriene-3, 7α, 17β-triol

1, 3, 5(10)-estratriene-3 , 15α, 17β-triol

2, 3 -dihydroxy-1, 3, 5(10)-estratrien-17-one

3 , 7α-dihydroxy-1, 3, 5(10)-estratrien-17-one

3 , 15α-dihydroxy-1, 3, 5(10)-estratrien-17-one

16α-Hydroxyestrone
16α-Hydroxyhydrocortisone

16α-Hydroxypregnenolone
17α-Hydroxypregnenolone
17α-Hydroxypregnenolone sulfate
21-Hydroxypregnenolone
6β-Hydroxyprogesterone
16α-Hydroxyprogesterone
17α-Hydroxyprogesterone
20α-Hydroxyprogesterone
20β-Hydroxyprogesterone
21-Hydroxyprogesterone
2β-Hydroxytestosterone
16α-Hydroxytestosterone
19-Hydroxytestosterone
16-Ketoestradiol-17β
6-Ketoprogesterone
16-Ketoprogesterone
2-Methoxyestrone

19-Oxoandrostenedione
19-Oxodehydroepiandrosterone
7-Oxoestradiol
Pregnanediol

3 , 16α-dihydroxy-1, 3, 5(10)-estratrien-17-one
11β , 16α, 17α, 21-tetrahydroxy-4-pregnene-3, 20-dione
3β , 16α-dihydroxy-5-pregnen-20-one
3β , 17α-dihydroxy-5-pregnen-20-one
3β , 17α-dihydroxy-5-pregnen-20-one-3β-yl sulfate
3β , 21-dihydroxy-5-pregnen-20-one
6β-hydroxy-4-pregnene-3, 20-dione
16α-hydroxy-4-pregnene-3, 20-dione
17α-hydroxy-4-pregnene-3, 20-dione
20α-hydroxy-4-pregnen-3, 20-dione
20β-hydroxy-4-pregnen-3-one
21-hydroxy-4-pregnene-3, 20-dione
2β , 17β-dihydroxy-4-androsten-3-one
16α, 17β-dihydroxy-4-androsten-3-one
17β , 19-dihydroxy-4-androsten-3-one
3 , 17β-dihydroxy-1, 3, 5(10)-estratriene-16-one
4-pregnene-3, 6, 20-trione
4-pregnene-3, 16, 20-trione
2, 3 -dihydroxy-1, 3, 5(10)-estratrien-17-one-2-methyl ether
3β , 17β-dihydroxy-5-androsten-19-al
3β-hydroxy-5-androstene-17-one-19-al
3 , 17β-dihydroxy-1, 3, 5(10)-estratrien-7-one
5β-pregnane-3α, 20α-diol

Pregnanedione	5β-pregnane-3, 20-dione
Pregnanediol sulfate	3α, 20α-dihydroxy-5-pregnen-3β-yl sulfate
Pregnanolone	5β-pregnane-3β-ol-20-one
Pregnenolone	3β-hydroxy-5-pregnen-20-one
Pregnenolone sulfate	3β-hydroxy-5-pregnen-20-one-3β-yl sulfate
Progesterone	4-pregnene-3, 20-dione
Testosterone	17β-hydroxy-4-androsten-3-one
Δ^7-Testosterone	17β-hydroxy-4, 7-androstadiene-3-one
Testosterone sulfate	17β-hydroxy-4-androsten-3-one-17β-yl sulfate
3β, 17α, 21-Trihydroxypregnenolone	3β, 17α, 21-trihydroxy-5-pregnen-20-one

APPENDIX II

TABLE I

Crystallization data for radioactive steroid metabolites[b] isolated following incubation of fetal and maternal sheep adrenal tissue with pregnenolone-7α-^{3}H (S.A. expressed as d.p.m./mg)

Steroid isolated	Material crystallized	Gestational age (days)	Fetal adrenal Number of crystallizations			Maternal adrenal Number of crystallizations		
			1	2		1	2	
Pregnenolone	pregnenolone acetate	70	155[c] 166	150 161		-	-	
		105	2133 2185	2123 2178		96 92	93 89	
		140	78 83	76 79		92 96	90 92	
			1	2	3	1	2	3
17α-Hydroxypregnenolone	17α-hydroxypregnenolone	70	861 773	811 794	806[a] 789	-	-	-
		105	8642 7929	8173 8010	8167[a] 8001	11618 13348	12817 13002	12800[a] 12995
		140	17449 16629	17085 16926	17080[a] 16919	12408 10877	11084 10787	11079[a] 10779
			1	2		1	2	
Progesterone	progesterone	70	36261 57679	38756 40326		-	-	
		105	19541 15714	18008 16940		508 374	470 431	
		140	453 374	410 389		1982 1726	1891 1799	
			1	2		1	2	
17α-Hydroxyprogesterone	17α-hydroxyprogesterone	70	182 227	187 202		-	-	
		105	809 922	848 859		582 685	598 615	
		140	898 791	861 844		1047 1384	1159 1203	
			1	2		1	2	
20α-Dihydroprogesterone	20α-Dihydroprogesterone acetate	70	577 567	580 59		-	-	
		105	1013 1040	1025 1029		644 683	633 661	
		140	812 797	801 803		539 566	527 543	
			1	2	3	1	2	3
Deoxycorticosterone	deoxycorticosterone	70	14669 9449	12376 11962	12361[a] 11984	-	-	-
		105	43891 38750	41740 40618	41731[a] 40834	21276 18827	19257 18962	19243[a] 18989
		140	29306 26512	27503 26216	27492[a] 26751	23054 21585	22305 21559	22289[a] 21786

			3	4	5	3	4	5
Corticosterone	corticosterone	70	490	496	490[a]	-	-	-
			570	505	499			
		105	412	419	422[a]	987	992	1000[a]
			529	432	425	1762	1043	1008
		140	2736	2800	2810[a]	820	812	820[a]
			3094	2917	2831	1091	851	829
			2	3	4	2	3	4
11-Deoxycortisol	11-deoxycortisol	70	611	608	615[a]	-	-	-
			691	615	622			
		105	1093	1080	1083[a]	6074	6010	6033[a]
			1148	1103	1100	6552	6103	6100
		140	2892	2851	2871[a]	5591	5338	5411[a]
			2998	2887	2897	6007	5498	5392
			3	4	5	3	4	5
Cortisol	cortisol	70	305	306	308[a]	-	-	-
			313	299	304			
		105	303	308	312[a]	817	838	856[a]
			319	316	319	830	855	865
		140	865	854	859[a]	404	393	384[a]
			873	854	868	407	415	502
			1	2	3	1	2	3
Cortisone	adrenosterone	70	58	59	57	-	-	-
			95	62	59			
		105	70	68	65	1034	1091	1075
			119	75	70	1354	1114	1081
		140	680	654	661	441	436	434
			1097	691	668	1253	499	438
			1	2		1	2	
Dehydroepiandrosterone	dehydroepiandrosterone acetate	70	82	78		-	-	
			83	80				
		105	132	126		79	73	
			135	130		75	77	
		140	80	78		85	89	
			89	83		92	89	
			1	2		1	2	
Androstenedione	androstenedione	70	406	388		-	-	
			362	374				
		105	230	225		299	275	
			218	213		265	270	
		140	364	340		1117	1012	
			317	324		956	964	

a. Final crystallization carried out after formation of the acetate derivative

b. See Table V for solvent pairs used for crystallization of steroid hormones and/or their derivatives.

c. In each series of crystallizations upper figure represents the specific activity of the crystals and lower figure the specific activity of the corresponding mother liquor.

TABLE II

Crystallization data for radioactive steroid metabolites[b] isolated following incubation of fetal and maternal sheep adrenal tissue with progesterone-7α-^{3}H (S.A. expressed as d.p.m./mg).

Steroid isolated	Material crystallized	Gestational age (days)	Fetal adrenal Number of crystallizations			Maternal adrenal Number of crystallizations		
			1	2		1	2	
Progesterone	progesterone	70	35343[c] 25533	31976 29544		-	-	
		105	10585 7773	9378 8956		304 256	282 270	
		140	21650 17854	20097 19620		1222 908	1106 1061	
			1	2		1	2	
17α-Hydroxyprogesterone	17α-hydroxyprogesterone	70	188 131	179 164		-	-	
		105	2122 2909	2342 2506		4393 3771	4182 3982	
		140	3803 2880	3348 3092		3808 3444	3782 3693	
			1	2		1	2	
20α-Dihydroprogesterone	20α-Dihydroprogesterone acetate	70	686 641	675 652		-	-	
		105	821 825	829 822		518 504	514 509	
		140	781 739	776 754		572 632	563 581	
			1	2	3	1	2	3
Deoxycorticosterone	deoxycorticosterone	70	14827 12743	13841 13062	13832[a] 13180	-	-	-
		105	66203 62723	64183 63348	64173[a] 63592	2431 2143	2305 2213	2299[a] 2242
		140	22477 20192	21774 20291	21759[a] 20420	4216 3812	4002 3888	3990[a] 3899
			3	4	5	3	4	5
Corticosterone	corticosterone	70	201 258	210 219	206[a] 211	-	-	-
		105	3985 4372	3991 4018	3981[a] 3995	3919 4732	3942 4037	3932[a] 4001
		140	3179 5292	3569 4109	3580[a] 3599	3414 4399	3571 3719	3582[a] 3591

			2	3	4	2	3	4
11-Deoxycortisol	11-deoxycortisol	70	396 420	391 399	384[a] 393	-	-	-
		105	2407 2801	2393 2412	2401[a] 2429	5593 5890	5621 5700	5602[a] 5629
		140	4173 5934	4258 4470	4098[a] 4105	4163 4910	4212 4310	4290[a] 4299
			3	4	5	3	4	5
Cortisol	cortisol	70	142 150	153 157	154[a] 160	-	-	-
		105	1316 1322	1303 1331	1251[a] 1285	3993 4134	4054 4109	4011[a] 4082
		140	1752 1775	1741 1791	1737 1752	4728 4585	4777 4427	4771[a] 4465
			1	2	3	1	2	3
Cortisone	adrenosterone	70	-	-	-	-	-	-
		105	454 518	460 470	458 460	611 1000	598 620	601 608
		140	598 1065	584 599	580 583	727 1388	710 750	716 721
			1	2		1	2	
Androstenedione	androstenedione	70	397 327	350 234		-	-	
		105	2192 1910	2012 1921		214 187	197 189	
		140	473 411	447 420		2521 1944	2208 2094	

a. Final crystallization carried out after formation of the acetate derivative.

b. See Table V for solvent pairs used for crystallization of steroid hormones and/or their derivatives.

c. In each series of crystallizations upper figure represents the specific activity of the crystals and lower figure the specific activity of the corresponding mother liquor.

TABLE III

Crystallization data for radioactive steroid metabolites[b] isolated following incubation of fetal and maternal pig adrenal tissue with pregnenolone-7α-^{3}H (S.A. expressed as d.p.m./mg).

Steroid isolated	Material crystallized	Gestational age (days)	Fetal adrenal Number of crystallizations			Maternal adrenal Number of crystallizations		
			1	2		1	2	
Pregnenolone	pregnenolone acetate	56	2256[c] 2466	2281 2391		1666 1660	1642 1653	
		112	378 408	371 393		246 292	250 279	
			1	2		1	2	
17α-Hydroxypregnenolone	17α-hydroxypregnenolone	56	2455 2910	2478[a] 2609		3794 4342	3725[a] 4004	
		112	1561 1904	1539 1797		3232 3750	3200 3569	
			1	2	3	1	2	3
Progesterone	progesterone	56	56826 67053	58269 62173	58976 60017	3137 3816	3208 3646	3372 3600
		112	4224 4163	4191 4204	4150 4193	2020 1992	2259 2329	2185 2292
			1	2		1	2	
17α-Hydroxyprogesterone	17α-hydroxyprogesterone	56	3430 2713	3193 3077		1592 1704	1614 1785	
		112	1063 1327	1130 1226		844 1018	976 993	
			1	2		1	2	
20α-Dihydroprogesterone	20α-Dihydroprogesterone acetate	56	1056 1128	1043 1109		1015 1099	1009 1023	
		112	440 412	435 423		415 463	429 451	
			1	2		1	2	3
11β-Hydroxyprogesterone	11β-hydroxyprogesterone	56	2562 2712	2588 2682		11808 12311	12006 12111	12071 12101
		112	23144 22923	23008 22950		42325 41388	41972 41282	

			2	3	4	2	3	4
Deoxycorticosterone	deoxycorticosterone	56	22008 23774	23002 23736	22871[a] 23191	40791 50218	43228 46004	43899[a] 45136
		112	2497 2576	2402 2495	2478[a] 2505	3257 3706	3491 3617	3411[a] 3582
			1	2	3	1	2	3
Corticosterone	corticosterone	56	5224 6234	5633 5724	5678[a] 4711	14511 16207	15220 15519	15242[a] 15482
		112	49033 51763	50217 50537	50289[a] 50505	19994 20148	19880[a] 20284	
			1	2		1	2	
11-Deoxycortisol	11-deoxycortisol	56	3616 3604	3585[a] 3615		16258 15176	16314[a] 15228	
		112	10918 10938	10708[a] 10996		17478 17601	17306[a] 17662	
			1	2	3	1	2	3
Cortisol	cortisol	56	-	-	-	2087 1869	1992 1907	1955[a] 1934
		112	16322 22524	19291 20786	19412[a] 20132	20079 24072	21716 22573	21140[a] 22070
			2	3	4	2	3	4
Cortisone	cortisone	56	-	-	-	1109 1736	1336 1417	1303[a] 1409
		112	5048 5792	5246 5417	5233[a] 5401	4051 4908	4386 4429	4343[a] 4415
			1	2	3	1	2	3
Androstenedione	androstenedione	56	3002 4404	3277 3514	3303 3442	4032 6546	4168 5317	4230 4801
		112	3090 4956	3206 4472	3829 3706	2829 4389	2893 3541	2912 3101

a. Final crystallization carried out after formation of the acetate derivative.

b. See Table V for solvent pairs used for crystallization of steroid hormones and/or their derivatives.

c. In each series of crystallizations upper figure represents the specific activity of the crystals and lower figure the specific activity of the corresponding mother liquor.

TABLE IV

Crystallization data for radioactive steroid metabolites[b] isolated following incubation of fetal and maternal pig adrenal tissue with progesterone-7α-^{3}H (S.A. expressed as d.p.m./mg).

Steroid isolated	Material crystallized	Gestational age (days)	Fetal adrenal Number of crystallizations			Maternal adrenal Number of crystallizations		
			1	2	3	1	2	3
Progesterone	progesterone	56	98594[c] 111342	101724 109248	102990 105972	6109 7152	6342 7006	6561 6879
		112	667 707	674 690	680 665	3583 4729	3799 4420	3983 4320
			1	2		1	2	
17α-Hydroxyprogesterone	17α-hydroxyprogesterone	56	5484 4672	5180 4908		3900 2771	3531 3268	
		112	4514 8788	5984 6105		2035 2269	2105 2203	
			1	2		1	2	
20α-Dihydroprogesterone	20α-dihydroprogesterone acetate	56	1480 1434	1466 1451		1662 1760	1653 1706	
		112	831 863	839 855		1175 1070	1132 1093	
			1	2	3	1	2	3
11β-Hydroxyprogesterone	11β-hydroxyprogesterone	56	5485 6090	5659 5821	5691 5808	17394 18631	17881 18131	17900 18007
		112	68335 70669	69244 69796	69331 69662	23756 25689	24565 24818	24621 24800
			2	3	4[a]	2	3	4[a]
Deoxycorticosterone	deoxycorticosterone	56	41306 50993	43969 46703	44116 45581	94507 100556	96234 97119	96739 97582
		112	4281 4414	4269 4300	4292[a] 4321	5333 7058	5908 6774	6116[a] 6613
			1	2	3[a]	1	2	3[a]
Corticosterone	corticosterone	56	11775 13436	12547 12636	12572 12619	20716 22946	21530 21979	21489 21832
		112	76731 76539	76792[a] 76589		28688 29653	29088 29125	29101[a] 29152

			1	2		1	2		
11-Deoxycortisol	11-deoxycortisol	56	3954 4467	4016[a] 4301		22084 23652	22257[a] 23049		
		112	12959 13634	12785[a] 13396		29213 31340	29732[a] 30832		
			1	2	3	1	2	3	
Cortisol	cortisol	56	-	-	-	3911 3092	3781 3376	3595[a] 3458	
		112	68929 78090	71193 72993	71579[a] 72335	47296 53719	49573 51073	49860[a] 50542	
			2	3	4	2	3	4	
Cortisone	cortisone	56	-	-	-	2951 3673	3281 3303	3263[a] 2997	
		112	5213 5888	5406 5512	5399[a] 5508	9107 10152	9612 9726	9600[a] 9717	
			1	2	3	1	2	3	
Androstenedione	androstenedione	56	17388 29558	20837 22851	21174 22016	19966 23672	20118 22392	20651 21734	
		112	27893 36712	28318 31995	29062 30011	9296 13808	10106 12880	10565 11374	

a. Final crystallization carried out after formation of the acetate derivative.

b. See Table V for solvent pairs used for crystallization of steroid hormones and/or their derivatives.

c. In each series of crystallizations upper figure represents the specific activity of the crystals and lower figure the specific activity of the corresponding mother liquor.

TABLE V

Solvent pairs used for crystallization of steroid hormones and/or their derivatives

Steroid	Solvent pair	Crystals dissolved in
Pregnenolone acetate	acetone/pentane benzene/hexane	chloroform/methanol (2:1 v/v)
17α-Hydroxypregnenolone	acetone/pentane benzene/hexane	methanol
Progesterone	acetone/pentane benzene/hexane	chloroform/methanol (2:1 v/v)
11β-Hydroxyprogesterone	acetone/pentane chloroform:methanol (2:1 v/v)/ pentane chloroform:methanol (2:1 v/v)/ hexane	chloroform/methanol (2:1 v/v)
17α-Hydroxyprogesterone	chloroform/hexane chloroform/pentane	chloroform/methanol (2:1 v/v)
20α-Dihydroprogesterone	acetone/pentane benzene/hexane	chloroform/methanol (2:1 v/v)
Deoxycorticosterone	acetone/pentane benzene/hexane	chloroform/methanol (2:1 v/v)
Corticosterone	chloroform:methanol (2:1 v/v)/ hexane chloroform:methanol (2:1 v/v)/ pentane	chloroform/methanol (2:1 v/v)
11-Deoxycortisol	chloroform/hexane chloroform/pentane chloroform/benzene: hexane (1:1 v/v) chloroform:methanol (2:1 v/v)/ pentane	chloroform/methanol (2:1 v/v)
Cortisol	methanol ethanol chloroform:methanol (2:1 v/v)/ toluene	chloroform/methanol (2:1 v/v)
Cortisone	acetone/pentane	chloroform/methanol (2:1 v/v)
Adrenosterone	chloroform/hexane chloroform/pentane acetone/hexane chloroform:methanol (2:1 v/v)/ hexane	chloroform/methanol (2:1 v/v)
Dehydroepiandrosterone	acetone/pentane benzene/hexane	chloroform/methanol (2:1 v/v)
Androstenedione	acetone/pentane benzene/hexane	chloroform/methanol (2:1 v/v)

APPENDIX III

APPENDIX III

Note added before final submission of thesis to the Department of Graduate Studies.

At the time of the defence of the thesis one of the external examiners, Dr. T. Sandor, Research Professor of Medicine, Laboratoire d'Endocrinologie, Hôpital Notre-Dame, Montréal, criticized the omission of exogenous NADPH and metal ions from the incubation media of the adrenal experiments involving fetal and maternal adrenals. It is now well established that the electron transport system associated with the oxygen activator, cytochrome P-450, is necessary for both mitochondrial and microsomal steroid hydroxylation reactions (Harding et al., 1968; Cammer et al., 1968). As cytochrome P-450 requires reducing equivalents, most conveniently furnished in the form of NADPH in a cell free system, it could be argued that increase in 11β-hydroxylating activity with increasing fetal age was due not to an increase in the hydroxylating system itself but to the increased biosynthesis of either NADPH or cytochrome P-450. It was also pointed out that, in addition to NADPH, metal ions are required for optimal 18-hydroxylase activity (Raman et al., 1966; Touitou and Legrand, 1970; Sandor et al., 1972). The lack of NADPH and metal ions may account, to a large extent, for the failure to isolate any aldosterone or 18-hydroxycorticosterone from the adrenal incubations.

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