AN ABSTRACT OF THE THESIS OF

(Major professor)

This study deals with the patterns of development and distribution of connective tissue fibers in the placentae and adnexa of the golden hamster (<u>Mesocricetus auratus</u> Waterhouse). The placentae and adnexa representing all days of gestation from six to term were stained for the presence of collagen, reticulin, and elastin. Additionally, both the non-pregnant and post-partum uteri were included for comparison. Although collagen becomes very widely distributed in the membranes of the gestation sac by mid-term, there is a progressive diminution of this element during later gestation but restricted to the endometrium. The distribution of reticulin follows that of collagen in large measure, but not always. In addition, a loss of reticulin occurs in only the labyrinth, during later gestation. Elastin is limited to the myometrium, to the walls of all umbilical cord vessels, and to the internal elastic membrane of medium and large arteries. Mechanisms involved in the deposition and maintenance of collagen in the uterus are reviewed. The relationship between reticulin and collagen as recorded in the literature, and also as observed in the developing hamster placenta, is discussed. A mention of both oxytalan and cellulose fibers is made in respect to their inter-relationships with the other connective tissue fibers.

THE PATTERNS OF DEVELOPMENT AND DISTRIBUTION OF COLLAGEN, RETICULIN, AND ELASTIN IN THE PLACENTAE OF THE GOLDEN HAMSTER (Mesocricetus auratus Waterhouse)

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THE PATTERNS OF DEVELOPMENT AND DISTRIBUTION OF COLLAGEN, RETICULIN, AND ELASTIN IN THE PLACENTAE OF THE GOLDEN HAMSTER (Mesocricetus auratus Waterhouse)

I. INTRODUCTION

In recent years, a number of papers have appeared concerning various aspects of development in the hamster placentae and adnexa. Graves (11, p. 219-251) in 1945, investigated both the embryo and fetal membranes through the first nine days of gestation; in 1946, Venable (39, p. 105-120) studied the pre-implantation stages; Ward (40, p. 231-275) in 1948, considered implantation and the associated endometrial changes into the fifth day of gestation; and in 1950, Adams and Hillemann (1, p. 363-383) published a paper on the morphogenesis of the vitelline and allantoic placentae. Orsini published a number of studies dealing with various subjects such as the trophoblastic giant cells and the endovascular cells associated with pregnancy (30, p. 273-331), the apposition and fusion of placentae (31, p. 454-466), the vascular knot of the uterus (32, p. 565-599), and ovo-implantation in cleared uterine tracts (33). The connective tissue elements however, of both the placenta and extra-embryonic membranes of the hamster have not been investigated. But a number of persons have studied connective tissue distribution in the membranes of other animals, and the pertinent accomplishments of these authors

are introduced below.

In 1942 Wolfe and Wright (44, p. 431-461) working with the rat, demonstrated that the reticular and collagenous fibers of the artificially induced maternal placenta are derived from the endometrial stroma, and that they are profoundly modified during pregnancy. Wislocki, Deane, and Dempsey (41, p. 281-346) in 1946, reported a diminution of the reticular fibers surrounding the fetal blood vessels in the labyrinth as gestation advances, in both the rabbit and guinea pig. Although the hamster placenta was included in their study, nothing on the connective tissues was mentioned. In the same year, Hunt and Evans (23) noted changes in the elastic tissues of the uterine wall in the rat during pregnancy and after parturition. In 1953 Wislocki and Padykula (42, p. 117-152), while investigating the structure and composition of Reichert's membrane of the rat, compared its staining properties with that of two basement membranes associated with the splanchnopleuric wall of the yolk sac. Harkness and Harkness (14) in 1954, studied the collagen content of the reproductive tract of the rat during pregnancy and lactation. The same authors (15) in 1955, published a preliminary account dealing with the growth of collagen, not only in the chorio-allantois and yolk sac, but also in the umbilical cord, the amnion, and the fetal tissues of the rat. In 1956, these same people (16) reported in greater detail on the changes in both the physical properties, and content

of collagen and hexosamine, in the fetal membranes of the rat during pregnancy. Harkness (18, p. 399-463) published an extensive review of the biological functions of collagen.

In 1960, Schoenberg, Hinman, and Moore (37) demonstrated both a gradual decrease in the number of argyrophilic fibers, and an increase in collagen bundles to term, in Wharton's jelly of the human umbilical cord. Maibenco (27, p. 59-72), in the same year, noted in the albino rat that the collagenic and elastic components of the uterine myometrium increase during gestation, and regress during involution.

Petry (34, p. 339-393) in 1961, published an extensive account on the embryonic membranes of the cat, and included details on the presence of collagen in the chorio-allantois. In the same year, Montfort and Pérez-Tamayo (28, p. 1240-1258) combined chemical and histological studies on the uterine changes occurring during both pregnancy and the puerperium in the rat, and concluded that the connective tissue picture of the endometrium changed from one of reticulin to one of collagen during gestation, and then returned to the reticular pattern after delivery. In 1961, Hillemann and Gaynor (19, p. 299-318) observed collagen, reticulin, and elastin in the definitive placentae of nutria. Bussche-Haddenhausen (2, p. 679-695), in the same year, described collagen fibers in the amnion of the armadillo.

In 1963, Petry and Kühnel (35, p. 625-662) working with the guinea pig, found both reticular and elastic fibers in the amnion. Mahlo (26, p. 237-248), in the same year, demonstrated that the connective tissues of the amnion of opossum consisted of a network of small argyrophil fibers. Fainstat (5, p. 337-369) in 1963 published on the disappearance of discrete collagen bundles along with the appearance of reticular fibers in their place, within the endometrial stroma during gestation in the rat. He also reported the regeneration of collagen bundles in the uterine stroma after parturition (6, p. 371-387). In the same year, Smith and Kaltreider (38) studied the collagen content of the non-pregnant uterus and observed its relation to the ovarian hormones. Woessner and Brewer (43) in 1963, by means of chemical analyses on human uteri, reported an increase in collagen and elastin during pregnancy, followed by a rapid breakdown of these elements after parturition. In 1963 and 1964, Hillemann and Ritschard (20, 21) reported preliminary accounts of the comparative fibroarchitecture in developing and mature mammalian placentae.

This study deals with the connective tissue fibers of the hamster placenta including their time of appearance, distribution, arrangement, and quantitative changes, beginning with the sixth day of gestation, and extending to term on day sixteen. Additionally, both the nonpregnant and post-partum (day one) uteri were included for comparison.

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II. MATERIALS AND METHODS

The placentae of 15 female hamsters were used, and the fetal age was determined by the lapse of time observed from coitus to sacrifice. The embryos were left intact in all cases except one (11-day placenta) to maintain the normal relationships among the various fetal and maternal membranes.

The material was placed in either ten percent formalin, Bouin's fluid, or Zenker's fixative. To facilitate fixation, these were injected either intravenously into the mesometrial vessels, or through the antimesometrial aspect of the loculi. Following dehydration in a graded series of alcohol (or dioxane), clearing in xylene, embedding in paraffin (56 to 58° C), the tissues were sectioned at seven to twelve microns. Infiltration of the larger loculi was carried out with the aid of a vacuum pump.

Staining methods involved the use of hematoxylin and eosin for general histology, Foot's method (25, p. 179-187) for reticulin (argyrophilic fibers), MacCallum's modification of Verhoeff's stain (24, p. 132), or the Orcinol-New fuchsin method (7) for elastic fibers, and a modification of Masson's trichrome stain (3, p. 245-246) for collagenous tissue. The improvised procedure consisted of substituting Biebrich scarlet for Ponceau 2R, and staining for one to two minutes. Rapid dehydration was necessary to prevent the removal

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of both the aniline blue and Biebrich scarlet stains. Finally, an original modification of the Humason-Lushbaugh combination stain (22) for elastin, reticulin, and collagen was employed with variable success in demonstrating the presence of these three elements simultaneously. This modification involved not only the substitution of ammoniacal silver (Foot's method) for reticulin in place of silver carbonate (Humason-Lushbaugh), but also the replacement of Knoeff-aniline blue with Masson-aniline blue.

III. OBSERVATIONS

Collagen

A layer of collagenic material surrounds the uterus beneath the perimetrium throughout the period of gestation. The myometrium contains a great deal of collagen distributed among the bundles of the smooth muscle. Although the muscles appeared to be hypertrophied during pregnancy, the relative amount of collagenous material did not appear to change. Within the stratum vasculare are numerous blood vessels covered by several layers of densely packed collagenic fibers. A progressive diminution of collagen occurs in the uterine endometrium as gestation proceeds. Although the blood vessels and glands of the endometrium are surrounded by dense bundles of collagen in the non-pregnant uterus, these structures are relatively free of this material in the post-partum condition. A layer of collagen forms a basement membrane under the uterine epithelium in all the stages under study.

Beginning on day nine, and continuing until term, densely arranged collagen fibers are present in the somatic mesodermal stroma of the chorionic plate as well as in the walls of the umbilical vessels in transit through the plate. The collagen content increased with the expansion of the somatic mesodermal tissue.

The membranes of the yolk sac splanchnopleure as distinguished

by Wislocki and Padykula (42, p. 117-152) demonstrate some interesting staining properties. Although the "serosal" basement membrane of the visceral yolk sac consists of heavy collagen fibers throughout gestation, no such collagenic material is found in the "visceral" basement membrane component until just before term (day 15); at this time a very thin layer of collagen becomes associated with it. The blood vessels contained within the splanchnic mesodermal stroma of this visceral yolk sac, are encircled by collagen fibers throughout the period of gestation.

Reichert's membrane, defined by Mossman (29, p. 137) as "the hyaline membrane between the trophoblast and the endoderm of the bilaminar wall of the yolk sac", shows a slight affinity for aniline blue. Collagen underlies the parietal endoderm cells for four days (12-15) of gestation; this site is identical with that described for Reichert's membrane.

The somatic mesoderm of the amnion, from its first appearance on day eight until term, is found to contain a very thin, finely arranged, collagenous network.

Collagen is absent during all stages of gestation from the network of allantoic vessels feeding the endothelial tubes, from the labyrinth proper, and from the vacuolated decidua basalis (containing giant cells). The umbilical cord, however, contains this element distributed within the matrix (Wharton's jelly) and surrounding

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all of its vessels throughout gestation.

Reticulin

The uterine wall of the hamster shows a marked affinity for silver. Within the myometrium, many reticular fibers are arranged among the circular and longitudinal smooth muscle bundles (Figs. 31, 32, 33). This fact is most apparent in the stratum vasculare (Fig. 36). With respect to both the glands and blood vessels of the myometrium as well as the endometrium, fine threads of reticulin regularly form four encircling layers. The reticulin of the non-pregnant endometrium is represented as short, frayed, and fragmented fibers (Fig. 3). But during gestation there is an obvious and progressive increase in this element along with a development of the characteristic network. Compacted reticular fibers comprise the basement membrane of the entire uterine epithelium throughout gestation. Fine processes of this reticular material extend from the membrane into the underlying endometrial stroma.

Reticular fibers are well represented both within the placental cone (Träger) as well as around the contained allantoic vessels (Figs. 11, 12) during the early stages of gestation (days six to eleven). Since after day 12 the placental cone itself is disrupted by the developing maternal lacunae, only traces of reticulin remain among these ectodermal cells. These cells which comprise the junctional zone are now distributed as a line (in section).

A wide band of reticulin is coextensive with the somatic mesodermal stroma of the chorionic plate (Fig. 9, 10); reticulin occurs also in the walls of the umbilical vessels passing through this plate (from day nine until term).

A similar wide band of reticulin comprises much of the splanchnic mesodermal stroma of the visceral yolk sac (Fig. 20); reticulin also encircles its contained vessels. Both the "visceral" and "serosal" basement membranes of the yolk sac splanchnopleure (as defined by Wislocki and Padykula) consist of a thickened layer of reticulin, similar in its arrangement and density from first appearance (on day nine) until term, irrespective of the increase in the absolute size of the yolk sac.

Since the somatic mesodermal stroma of the amnion is uniformly attenuated, the contained reticular fibers are proportionately diminished in amount (Fig. 21). This picture remains unchanged throughout gestation.

In the early stages (days nine to twelve), reticulin courses with the endothelial vessels of the labyrinth, but during later stages of gestation (day 13 to term) this material occurs in reduced amounts (Figs. 28, 29, 30), and becomes discontinuously distributed around these vascular tubes. The network of allantoic vessels feeding these endothelial tubes is surrounded by a mesh of fine reticular fibers (Figs. 13, 14, 26).

The mesometrially disposed giant cell layer contains only remnants of reticulin (Fig. 25). The same applies to the adjacent necrotic decidua basalis.

In addition to the reticular fibers making up a significant portion of the cord matrix, they also constitute a substantial part of the umbilical vessel walls. Here the reticular fibers occur in concentric circles and bind together the rings of smooth muscle (Figs. 17, 18).

Elastin

A layer of finely branched elastic fibers underlies the perimetrium of the uterus in all of the stages studied. Lightly stained elastic fibers occur sparsely among the muscle bundles of the myometrium and also in a very sporadic and indefinite pattern. These fibers appear to be much shorter than those directly beneath the perimetrium; they are more commonly found associated with the circular muscle bundles than with the longitudinal bundles. In the stratum vasculare elastic fibers occur in the walls of the maternal blood vessels near the chorio-allantoic disc. The internal elastic membrane occurs also in the medium and large arteries of the mesometrium.

Elastin is demonstrated to be present both in the walls of the umbilical vessels as well as around these vessels. Although the internal elastic membrane occurs in both the allantoic and vitelline arteries, it is absent from the corresponding veins.

The number and pattern of distribution of elastic fibers noted above remains unchanged throughout gestation.

Table I was prepared to summarize the first appearance, the persistence, and the disappearance of the three fibers in the developing hamster placentae and adnexa on a day to day basis, beginning on day six and extending through day 17 (post-partum). The nonpregnant uterus is included under day 0. Because of the difference of opinion concerning the derivation of Reichert's membrane (from either trophoblastic cells, or from parietal endoderm cells), this item is listed under the heading of ectoderm to circumvent the creation of an additional category.

	Day												
	0	6	7	8	9	10	11	12	13	14	15	16	17
ECTODERM			1.0										
Zone of junction	NA	R	R	R	R	R	NA						
Cells of placental cone	NA	R	R	R	R	R	R	NA	NA	NA	NA	NA	NA
Giant cell nests	NA	NA	NA	R	R	R	R	R	R	R	R	R	NA
Reichert's membrane	NA	NA	a	a	a	a	a	С	С	С	С	a	NA
MESODERM-somatic													
Stroma of chorionic plate		NA	NA	NA	CR	NA							
Stroma of amnion	NA	NA	NA	NA	CR	NA							
MESODERM-splanchnic													
Allantoic vessels feeding labyrinth	NA	NA	NA	NA	R	R	R	R	R	R	R	R	NA
Endothelium of labyrinth	NA	NA	NA	NA	R	R	R	R	R	R	R	R	NA
Walls of chorionic plate vessels	NA	NA	NA	NA	CR	NA							
Stroma of visceral yolk sac	NA	NA	NA	CR	NA								
Around vessels in yolk sac stroma	NA	NA	NA	CR	NA								
Stroma of umbilical cord	NA	NS	NS	NS	NS	CR	NA						
Walls of all umbilical cord vessels	NA	NS	NS	NS	NS	CRE	NA						
Internal elastic membrane of all													
umbilical cord arteries	NA	NS	NS	NS	E	E	E	E	E	E	E	E	NA
Internal elastic membrane of all													
umbilical cord veins	NA	NS	NS	NS	a	a	a	a	a	a	a	a	NA
MESODERM-uterus													
Under perimetrium	CE												
Among muscle bundles of myometrium	CRE												
Around vessels of both myo- and endometrium	CRE												
Internal elastic membrane of myometrial		_	_	_	_	-	_				_		
arteries	E	E	E	E	E	E	E	E	E	E	E	E	E
Walls of myometrial vessels near placental disc	CRE												
Around uterine glands	CR												
Basement membrane of uterine epithelium	CR												

TABLE I. CONNECTIVE TISSUES OF THE DEVELOPING HAMSTER PLACENTAE.

C=Collagen, R=Reticulin, E=Elastin, NA=not applicable, NS=not studied, and a=absent.

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IV. DISCUSSION

It is of interest to note that the hamster placenta is largely free of elastin, the exceptions comprising those fibers found in the walls of all umbilical cord vessels, and those which form the internal elastic membrane of all cord arteries. Harkness and Harkness (16, p. 488) in 1956 considered it unlikely that elastin played any part in determining the strength of the fetal membranes of the rat. Although Maibenco (27, p. 59-72) reported an increase of elastin in the myometrium of the rat, followed by a regression during involution, and Woessner and Brewer (43) observed that elastic tissues increased five to six fold (as measured by chemical analyses) in the human uterus during pregnancy, and then diminished after parturition, no such change was found in the hamster uterus during equivalent stages. The results obtained by Maibenco and by Woessner and Brewer, were both determined by chemical analyses of uteri from animals with a much longer gestation than that of the hamster. Therefore it is perhaps explained that elastic fibers should play a relatively minor role in the developmental picture of connective tissues in the hamster placenta during gestation, in view of its much shorter gestation.

Several authors have observed an increase of collagen in the uterus during gestation. Maibenco (27, p. 59-72) using chemical

determinations, noted an increase in collagen in the myometrium of the rat followed by a regression of it during post-partum involution; Harkness and Harkness (14) found by chemical means, an increase of collagen in the uterine horns during pregnancy in the rat and a diminution after parturition, but later these same authors (17, p. 498) noted that the collagenous material increased in greater measure in that portion of the uterine wall involved in distention, than in the part at the placental sites; using chemical analyses, an increase of seven fold in collagen content was reported by Woessner and Brewer (43) in human uteri during pregnancy, while after parturition this material diminished to its original concentration; Montfort and Perez-Tamayo (28, p. 1240-1258) observed an increase of collagen in the endometrium of both rat and human uteri due to an apparent change from reticulin to collagen during gestation, followed by a shift back to reticulin after parturition.

However, Fainstat (5, p. 337-369) using histological techniques, reported the disappearance of discrete collagen bundles in the endometrium of the rat during gestation along with the appearance of reticular fibers in their place. In agreement with Fainstat, a progressive diminution of collagen occurs in the hamster endometrium as gestation proceeds (histological methods). Although the blood vessels and glands of the non-pregnant uterus of the hamster are surrounded by dense bundles of collagen, these structures are

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relatively free of this material in the post-partum condition. The majority of the authors reporting an increase of collagen in the whole uterus used chemical analyses, which therefore measure both endometrial and myometrial changes. But Montfort and Pérez-Tamayo (28, p. 1240-1258) using both chemical and histological means in their studies, noted an increase in endometrial collagen during gestation in the rat, in contrast with both Fainstat and the writer. Smooth muscle hypertrophy in the uterine wall of the rat during pregnancy was reported by Maibenco (27, p. 59-72) and by Montfort and Pérez-Tamayo (28, p. 1240-1258). This condition occurs in the uterine wall of the hamster as well; this suggests that the increase in uterine collagen reported on the basis of chemical analyses, may occur exclusively in the myometrium. Smith and Kaltreider (38) intimated this idea in their studies of 1963 on the relation of the collagen content in non-pregnant rat uteri to the ovarian hormones. Unpublished findings of Hillemann and Ritschard demonstrate a pattern of collagen distribution in the term placentae and adnexa of chinchilla, nutria, and baboon such as one might expect as a result of a progressive loss in this material in late pregnancy.

Reichert's membrane in rodents was viewed by Wislocki, Deane, and Dempsey (41, p. 281-346) as consisting of compacted collagen fibers. Wislocki and Padykula (42, p. 117-152) in a later histochemical investigation of this membrane in the rat, described it as having a strong affinity for acid dyes and a lack of affinity for silver. These workers suggested that the membrane was similar to compacted collagen fibers. In the hamster, collagen underlies the parietal endoderm cells from day 12 to day 15 in a location identical with that described for Reichert's membrane.

A wide band of reticulin along with a layer of heavy collagen fibers comprises much of the splanchnic mesodermal stroma of the visceral yolk sac and its contained vessels in the hamster. Harkness and Harkness (16) studying the rat, and Hillemann and Gaynor (19, p. 299-318) investigating the nutria, reported collagen and reticulin in the visceral yolk sac.

In 1953, Wislocki and Padykula (42, p. 117-152) described two basement membranes associated with the splanchnopleuric wall of the yolk sac in the rat. These investigators found that the "visceral" basement membrane of the splanchnopleure was a characteristic reticular membrane except late in gestation, when the stroma of the villi developed an affinity for aniline blue, while the "serosal" basement membrane was a dense collagenous structure throughout pregnancy. In the development of the visceral yolk sac in the hamster, similar staining reactions are noted in the "visceral" basement membrane, which consists of a thickened layer of reticulin until just before term (day 15); at term a very thin layer of collagen becomes associated with it. However, the "serosal" basement membrane shows an affinity for silver as well as for collagen stains from first appearance (day nine) until term. This parallel between collagen and reticulin(which appear to comprise some of the same structures) suggests a chemical relationship between these two fiber components.

Bussche-Haddenhausen (2, p. 679-695) reported the presence of collagen fibers in the amnion of the armadillo, and Hillemann and Gaynor (19, p. 299-318) found this material to be common in the amnion of the nutria, while Harkness and Harkness (16), working with the rat, and Mahlo (26, p. 237-248), working with the opossum, have observed that the amnion consisted of a network of reticular fibers. Petry and Kühnel (35, p. 625-662) noted that the amnion of the guinea pig consisted not only of reticular fibers but also of elastic fibers in the navel region. The somatic mesodermal stroma of the hamster amnion contains a very thin, finely arranged collagenous network as well as a similar network of reticular material; it lacks any trace of elastic fibers.

Although Hillemann and Gaynor (19, p. 299-318) noted the presence of collagen, elastin, and reticulin, along the endotheliotrophoblastic tubes of the labyrinth in the nutria, reticulin alone was found in the labyrinth of the hamster placenta. It is suggested that the shorter length of gestation in the hamster, compared with that of the nutria, accounts for the lesser number of connective tissue

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fibers present. Wislocki, Deane, and Dempsey (41, p. 281-346) reported that as gestation advances in the guinea pig and rabbit, there is a diminution of reticular fibers around the fetal vessels in the labyrinth; a similar loss in reticular material occurs in the labyrinth of the hamster during pregnancy.

Harkness and Harkness (15) using chemical determinations, reported the presence of collagen in the rat umbilical cord, Hillemann and Gaynor (19, p. 299-318) working with the nutria, found collagen, elastin, and reticulin in all of the umbilical cord vessels, but only reticulin in the cord matrix, while Schoenberg, Hinman, and Moore (37) described an increase in the collagenous fibers of Wharton's jelly (human) during gestation along with a decrease in argyrophilic fibers. The walls of all umbilical cord vessels of the developing hamster possess collagen, elastin, and reticulin, and both collagen and reticulin are found in the cord matrix (Wharton's jelly); however there is no apparent change in the amount of any of these elements during gestation.

For convenience in surveying the literature reviewed above, Table II with Parts A and B, was prepared to emphasize the fate of collagen, reticulin, and elastin, and to classify the mechanisms suggested for the deposition and maintenance of collagen.

Mechanisms involved in the deposition and maintenance of collagen in the uterus include two conflicting points of view (Table

PART A. THE FATE OF COLLAGEN, RETICULIN AND ELASTIN IN UTERUS AND PLACENTA IN CONSEQUENCE OF GESTATION.

1. COLLAGEN and ELASTIN increase during gestation in the MYOMETRIUM, and BOTH regress during involution. Rat: Maibenco, 1960. Chemical determination.

- 2. COLLAGEN <u>increases</u> in greater part in the uterine area involved in distention, and very little in the uterine wall at the placental site. Rat: Harkness and Harkness. 1956b. Chemical determination.
- 3. COLLAGEN <u>increases</u> in the uterine horn during pregnancy, but <u>diminishes</u> precipitously after parturition. Rat: Harkness and Harkness, 1954. Chemical determination.
- 4. COLLAGEN increases seven fold, and ELASTIN increases five to six fold in the uterus during pregnancy; after parturition both diminish to original concentrations. Man: Woessner and Brewer, 1963. Chemical determination.

5. COLLAGEN increases in endometrium during gestation due to a shift from RETICULIN to COLLAGEN; the shift is back to RETICULIN after parturition. Rat: Montfort and Perez-Tamayo, 1961. Chemical and histological determination.

- 6. COLLAGEN regresses in the endometrium during gestation with the appearance of RETICULAR FIBERS; COLLAGEN regenerates in the uterine stroma after parturition. Rat: Fainstat, 1963a, 1963b. Histological determination.
- 7. COLLAGEN increases in Wharton's jelly of the cord during gestation, with the <u>decrease</u> in argyrophilic (RETICULAR) fibers. Man: Schoenberg, Hinman and Moore, 1960. Light and Electron Microscopy.
- 8. COLLAGEN does not change in amount in the FETAL MEMBRANES, but there is a change in the bonding with a resultant decrease in tensile strength, analogous with that in the <u>cervix</u>. Rat: Harkness and Harkness, 1956c. Chemical determination.
- 9. RETICULAR fibers around the FETAL VESSELS in the labyrinth diminish as gestation advances.

Rabbit; Guinea Pig: Wislocki, Deane, and Dempsey, 1946. Histochem.

determination.

PART B. SUGGESTED MECHANISMS INVOLVED IN THE DEPOSITION AND MAINTENANCE OF COLLAGEN IN UTERO

 ESTROGEN is necessary for the DEPOSITION and MAINTENANCE of dense COLLAGENOUS stroma in the rat ENDOMETRIUM. After parturition there are essentially no thick COLLAGENOUS bundles. PROGESTERONE is NOT required for this deposition and maintenance. Sucking prevents new collagen. Rat: Fainstat, 1962. Histological determination.

2. ESTROGEN and PROGESTERONE are needed for the deposition of COLLAGEN in the NON-PREGNANT uterus.

Rat: Smith and Kaltreider, 1963. Chemical determination.

II, B). Fainstat (4) demonstrated that estrogen is necessary for the deposition and maintenance of a dense collagenous stroma in the rat endometrium, and that progesterone is not required for this process, but Smith and Kaltreider (38) in a later study with non-pregnant rat uteri, reported that both estrogen and progesterone are needed for this deposition. Whereas Fainstat reached his conclusions from histological observations focused upon the aggregation of fibers in the endometrial stroma, Smith and Kaltreider used chemical analyses that measured both the endometrial and myometrial changes in the whole uterus. Even so, these authors demonstrated the importance of ovarian hormones in both the deposition and maintenance of a collagenous endometrial stroma.

It was noted above that collagen and reticulin are often found together in the hamster placentae and that these elements may comprise some of the same structures. In a similar vein, several authors have recognized a relationship between reticulin and collagen. Wolfe and Wright (44, p. 431-461) working with rat uteri, found that while the more attenuated fibers were argyrophilic, the thicker elements were collagenous; these authors implied that reticulin is a precollagenous type of material which may be transformed into collagen. Robb-Smith (36, p. 92-116) suggested that argyrophilia may be a reflection of the looser packing or aggregation of collagen fibrils. Along this same line, Schoenberg, Hinman, and Moore (37) in viewing sections of human umbilical cords with the electron microscope, found two type of fibers, the classical collagen fiber and also numerous finer fibrils. These authors suggested that the finer fibrils (argyrophilic) are not distinct entities, but represent the degree of aggregation of collagen fibrils. Smith and Kaltreider (38) implied a similar interpretation in their study of non-pregnant rat uteri. Glynn (10) felt that argyrophilia was due to a more intimate association between polysaccharides and the collagen fibers than normally exists in coarse collagen bundles. Harkness (18, p. 339-463) in a review of the biological functions of collagen, noted two types of fibers, those associated with particular tissues such as the basement membranes, and those that are found in rapidly growing tissues like those of embryos in which new formation of collagen is taking place.

A discussion of the relationship between collagen and reticulin as recorded in the literature, and observed in the developing hamster placentae, is beyond the scope of this paper, but the relationship is mentioned as a possible explanation of the similarities in staining properties for certain structures.

Although this study of connective tissue elements did not include either oxytalan or cellulose fibers, these elements have been described in the literature and therefore merit some mention in behalf of the relationships between connective tissue fibers. Oxytalan fibers, first described by Fullmer and Lillie (8) in a number of animals, have been established as a connective tissue fiber distinct from the collagenous, elastic, and reticular varieties. In a later publication Fullmer (9) indicated that oxytalan fibers are part of the elastic-like connective tissues, and as such represent immature or specially modified elastic fibers.

Also, a cellulose-protein complex was reported as a normal, although minor constituent of some mammalian connective tissues by Hall <u>et al.</u> (12, p. 497-516). After degrading collagen fibers by treatment with alkaline buffers, these authors observed the production of highly anisotropic fibers consisting of a protein-cellulose (polysaccharide) complex. Thus the literature suggests that there exists an interrelationship among various connective tissue fibers.

It is apparent that the hamster placenta (maternal and fetal) develops a connective tissue "skeleton" which undergoes alterations during pregnancy. Its relatively smaller content of connective tissue fibers in comparison with an animal such as nutria, is thought to be in consequence of its shorter gestation period (16 days).

V. SUMMARY

- 1. The placentae and adnexa of the golden hamster representing all days of gestation from six to term were stained for the presence of collagen, reticulin, and elastin. Additionally, both the non-pregnant and post-partum (day one) uteri were included for comparison.
- 2. Although collagen becomes very widely distributed in the membranes of the gestation sac by mid-term, there is a progressive diminution of this element during later gestation but restricted to the endometrium.
- 3. The distribution of reticulin follows that of collagen in large measure, but definitely not always. In addition, a loss of reticulin occurs in only the labyrinth during later gestation.
- 4. Elastin is limited to the myometrium, to the walls of all umbilical cord vessels, and to the internal elastic membrane of medium and large arteries.
- 5. Mechanisms involved in the deposition and maintenance of collagen in the uterus are reviewed.
- 6. The relationship between reticulin and collagen as recorded in the literature, and also as observed in the developing hamster placenta, is discussed.
- 7. Oxytalan and cellulose fibers were mentioned in respect to

their inter-relationships with other connective tissue fibers.

8. Charted data on the connective tissue fibers of the developing hamster placenta are presented in Table I.

EXPLANATION OF FIGURES

PLATE I

- Endometrium of non-pregnant uterus, with reticular material comprising the basement membrane of the uterine epithelium. X 170.
- A portion of the same region seen in Figure 1 but at a higher magnification. X 360.
- Non-pregnant uterus, demonstrating reticular fibers in the perimetrium and myometrium. Note the fragmented appearance of the fibers. X 170.
- Appearance of reticulin in uterine wall (perimetrium and myometrium) of seven-day placenta. X 170.
- A portion of the uterine wall of Figure 4 but at a higher magnification. X 360.
- Reticular fibers surrounding a blood vessel in the stratum vasculare (seven-day placenta). X 360.
- Reticular network in the endometrium of seven-day placenta.
 X 170.
- Same area as seen in Figure 7 but at a higher magnification.
 X 360.
- Ten-day chorionic plate, presenting the fine reticular network.
 X 170.

- 10. Ten-day chorionic plate at higher magnification. X 360.
- Reticulin surrounding an allantoic blood vessel in a ten-day placenta. X 170.
- A portion of the same area as presented in Figure 11 but at a higher magnification. X 360.
- Reticular network around a blood vessel leading to the labyrinth (13-day placenta). X 170.
- A portion of the same area featured in Figure 13 but at a [/]
 higher magnification. X 360.
- 15. Cross-section of a 13-day labyrinth with fine reticular fibers at the center of the figure; these line the endothelial tubes. There is a substantial loss of reticulin at this time. X 170.
- A portion of the same area seen in Figure 15 but at higher magnification. X 360.
- Concentric rings of reticulin surrounding a 13-day umbilical cord vessel near the embryo. X 360.
- Same area as seen in Figure 17 but at higher magnification.
 X 360.

PLATE II

 Visceral yolk sac of a 13-day placenta (in section) with fine reticular material surrounding the endodermal cells. X 170.
 Thirteen-day visceral yolk sac with an enclosed blood vessel. Note the wide band of reticulin associated with it. X 360.

- A network of fine reticular fibers in the somatic mesodermal stroma of the 13-day amnion. X 360.
- 22. Uterine wall of 15-day placenta, demonstrating reticulin among the bundles of smooth muscle (to the left) and beneath the uterine epithelium (to the right). X 170.
- 23. Fifteen-day uterine wall, showing the reticular pattern around a blood vessel of the stratum vasculare. X 170.
- 24. Higher magnification of the myometrium of the 15-day placenta.X 360.
- 25. Remnants of reticulin around a giant-cell nest. X 170.
- 26. Reticular network around a blood vessel leading to the labyrinth in a 15-day placenta. X 170.
- 27. Fifteen-day chorionic plate, showing the reticular pattern.X 360.
- Cross-sectional view of 15-day labyrinth. Note that only traces of reticulin remain. X 170.
- 29. A higher magnification of the 15-day labyrinth. X 360.
- Fifteen-day labyrinth, showing remnants of reticulin at the center of the Figure. X 360.
- Myometrium of post-partum uterus, demonstrating an extensive network of reticulin. X 170.

- 32. A portion of the same region featured in Figure 31 but at a higher magnification. Note the heavier reticular bands surrounding the smooth muscle bundles, and the finer fibers among the muscle cells. X 360.
- Reticular fibers among the longitudinal muscle bundles (postpartum uterus). X 360.
- 34. Endometrium of the post-partum uterus. Note the heavy line of reticular beneath the uterine epithelium. X 170.
- 35. The same area seen in Figure 34 but at a higher magnification.X 360.
- Reticular fibers surrounding a vessel in the post-partum endometrium. X 360.



PLATE I.



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