

AN ABSTRACT OF THE THESIS OF

James Walter York for the M.A. in Zoology
(Name) (Degree) (Major)

Date thesis is presented May 12, 1966

Title AN HISTOCHEMICAL STUDY OF THE CHANGING PATTERNS
OF GLYCOGEN DISTRIBUTION IN THE UTERUS AND EXTRA-EMBRY-
ONIC MEMBRANES OF THE GOLDEN HAMSTER (Mesocricetus
auratus Waterhouse)

Abstract approved 
(Major professor)

This study deals with the histochemistry of the changing patterns of glycogen in the uterus and extra-embryonic membranes of the golden hamster (Mesocricetus auratus Waterhouse). The uterus and extra-embryonic membranes of the golden hamster representing days of gestation from six to term were stained with periodic acid-Schiff and Best's carmine. Diastase was used to check the glycogen-staining efficiency of these two procedures. Non-pregnant and post-partum uteri (day one) were also stained and checked with diastase for comparison. Glycogen is present in great quantities in the Träger, trophospongium, myometrium, uterine glands, and blood vessel tunica media. No observable increase or decrease in glycogen content was observed in these tissues. Glycogen appears in the visceral yolk sac on day eleven and remains in great quantities until term.

Glycogen may be observed in small quantities in the parietal yolk sac entoderm near the visceral yolk sac, and in the visceral yolk sac splanchnic mesoderm from day eleven to term. Endometrial glycogen concentration appears to decrease as gestation proceeds, and as the endometrial cells become compressed. There appears to be slightly less glycogen in interocular than in locular uterine regions. The residual trophoblast cells in the labyrinth stain for glycogen throughout gestation. Glycogen may occasionally appear in uterine epithelium. Some theories on glycogen storage and transport are reviewed.

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(Mesocricetus auratus Waterhouse)

by

JAMES WALTER YORK

A THESIS

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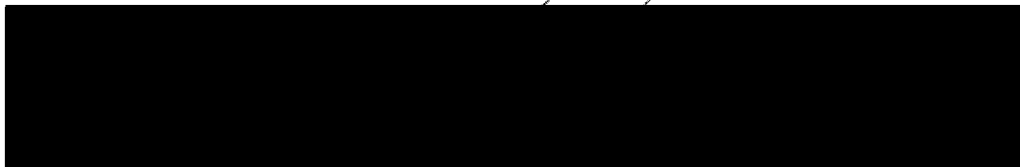
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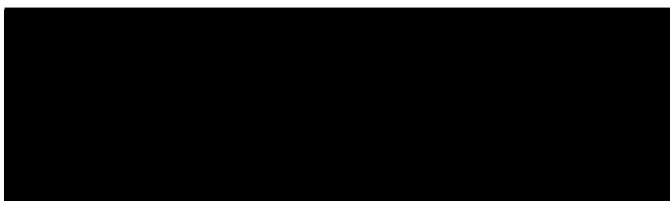
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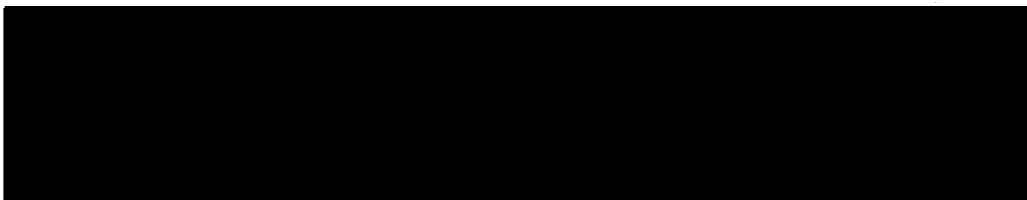
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ACKNOWLEDGMENT

Expressed here is an acknowledgment of my appreciation and gratitude to Dr. Howard H. Hillemann of Oregon State University, who suggested this study and gave freely of his time and laboratory facilities so that it could be properly completed. I also thank Dean Henry P. Hansen for the use of his excellent microscope.

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AN HISTOCHEMICAL STUDY OF THE CHANGING PATTERNS
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INTRODUCTION

A number of investigators have studied the morphology of the hamster placenta. Graves (13, p. 219-251) in 1945 studied the development of the golden hamster during the first nine days; Venable (28, p. 105-120) in 1946 examined the pre-implantation stages; Ward (32, p. 231-275) in 1948 observed early development and implantation and associated endometrial changes, and Adams and Hillemann (1, p. 263-283) in 1950 studied the morphogenesis of vitelline and allantoic placentae. Orsini (19, p. 273-331; 20, p. 454-466; 21, p. 565-599; 22, p. 288-293) in 1954, 1956, 1957, and 1961 studied the giant cells and endovascular cells associated with pregnancy, the apposition and fusion of placentae, the placental arterial supply and its changes during gestation and postpartum involution (vascular knot), and ovoimplantation in the hamster. Hillemann and Ritschard (14, p. 346) 1963 studied the connective tissue fibers in the placenta and adnexa, and Enders (11, p. 29-67) in 1965 demonstrated that the hamster placenta was hemo-trichorial.

Glycogen distribution has not hitherto been investigated in the hamster. Wislocki, Deane, and Dempsey (34,

p. 281-346) in 1946 found glycogen mainly in the syncytial trophoblast, basal decidua, and yolk sac epithelium of the mouse, rat, and guinea pig. They did not investigate the hamster placenta with respect to glycogen. In 1958, Graumann, as cited in Padykula and Richardson (24), studied the influence of several fixatives on the preservation of decidual glycogen in the eleven-day placenta of the hamster.

Many studies on the location and distribution of glycogen in pregnant and non-pregnant animals other than the hamster have been undertaken. Corner (8, p. 117-146) in 1921 found no glycogen in the uterine mucosa of the non-pregnant sow during any stage of the estrous cycle. In 1945 and 1946, Wislocki and Dempsey (36, p. 365-403 and 38, p. 181-225) found glycogen to be absent from the gravid uterus of the sow as well as from the non-gravid uterus, but present in the chorionic fossae, ridges, areolae, and in mesenchymal elements and blood vessel walls of the chorio-allantois.

In 1949 Wimsatt (33, p. 63-142) found glycogen in the endometrial connective tissue, decidua basalis, and glandular epithelium of the uterus, chorionic trophoblast cells, mesenchyme cells of the labyrinth, yolk sac entoderm and mesenchyme, and amniotic ectoderm of the bat. He also noted a diminution of glycogen in the trophoblast and in the yolk sac of the bat later in pregnancy.

The hedgehog uterus and placenta were investigated in 1957 by Morris (18, p. 184-200) who saw glycogen in the trophospongium, adjacent decidual tissue, uterine epithelial cells, and entoderm and mesenchyme of the membrane of the yolk sac. Glycogen was absent from the endometrium of the non-pregnant uterus.

Glycogen was present only in the maternal tissues of the mouse placenta according to Driessen in 1908, as cited by Dempsey and Wislocki (10, p. 409-429), but Wislocki, Deane, and Dempsey (34) in 1946 noted glycogen appearing in the yolk sac by day ten and persisting in undiminished quantities until term, and in the maternal decidua. No glycogen has been found in the parietal yolk sac of the mouse. Wislocki, Deane, and Dempsey (34) noted that glycogen is in the yolk sac entoderm, and in the columnar epithelial cells of the placenta of the guinea pig, and Dubois and Ducommun, as cited by Padykula and Richardson (24), in 1955 found that placental glycogen decreases, while fetal glycogen increases in amount.

Lockhead and Cramer (15, p. 263-384) in 1908 reported only traces of glycogen in the maternal and fetal cotyledons of the sheep placenta.

In 1945 Wislocki and Dempsey (36) found glycogen in the surface epithelium and outer parts of glands of cat uteri of early gestation and in the endometrium, necks of uterine glands, and surface epithelium between placental

sites, but no glycogen in maternal elements of the labyrinth in uteri of late gestation.

Fitch (12, p. 331-343) in 1964 studied the variation in glycogen content of the endometrial stroma and glands of dogs and of the myometrium during the estrous cycle.

Glycogen was found only in the maternal portion of the rabbit placenta by Chipman (6, p. 1-261) in 1902, Driessen, as cited by Dempsey and Wislocki (10) in 1908, Loveland, Maurer, and Snyder (16, p. 265-274) in 1931, and Lockhead and Cramer (15) in 1908. Bernard, as cited by Bridgman (3, p. 195-223), in 1859, Lockhead and Cramer (15) in 1908, and Tuchman - Duplessis, as cited by Vिलlee (30, p. 258), in 1954 stated that the placental glycogen of rabbits decreases as the glycogen in fetal tissues increases. Lockhead and Cramer (15) in 1908 and Davies (9, p. 135-142) in 1956 reported that glycogen appears in decidual stroma of the maternal placenta by day 12 and reaches a maximum by day 18, after which it decreases in amount.

Dempsey and Wislocki (10, p. 409-429) in 1944 reported glycogen to be present in man in the fetal elements of the cytotrophoblastic shell and cell columns, in uterine glands and in decidual cells, but not in the syncytium or in Langhans' cells. The glycogen was seen to diminish in the decidual cells as gestation advanced. Vилlee (29, p. 437-444) stated in 1953 that after eight

weeks the maximum concentration of placental glycogen is reached, and that the amount then decreases until 18-20 weeks. Thereafter the concentration remains constant until term. He observed that the placental glycogen concentration decreases as the fetal tissue concentration increases. In 1858 Brody (4, p. 377-384) noted a myometrial glycogen increase in man, and Sharov (27, p. 969-971) in 1960 noted glycogen in amniotic ectoderm and chorionic mesenchymal cells from week four to forty and in the entire cross section of the umbilical cord up to the sixteenth week. Sharov noticed an increase in the stroma of larger and stem villi especially during the last half of pregnancy. Boyd (2, p. 605) in 1957 noticed glycogen in placental blood vessels.

The glycogen of the rat placenta and non-pregnant uterus has been extensively investigated. Selye and McKeown (25, p. 1-30) in 1935, Bridgman (3) in 1948, and Connolly et al. (7, p. 717-719) in 1962 noted a five fold increase in glycogen from day ten to day 21. In 1952 Walaas (31) noted an increase in myometrial glycogen during pregnancy, and in 1958 Sharov (26, p. 1008-1011) noted glycogen in the decidua basalis, in the syncytial trophoblast of the labyrinth, and in the giant cells of trophoblast. Bulmer and Dickson (5, p. 46-58) in 1960 saw no glycogen in the rat uterine epithelium and noted its rarity in endovascular plasmodial cells. They found glycogen in the

trophospongial cells, giant cells (especially next to the decidua), in the metrial gland cells, and in the labyrinth. Padykula and Richardson (23, p. 261 and 24) in 1961 and 1963 noted glycogen in the decidua, binucleate cells of trophoblast, giant cells, labyrinth cells, yolk sac entoderm and mesenchymal cells. They observed a steady decrease of glycogen in the decidua, a maximum in concentration of glycogen in the trophospongium on day 15 with a decrease toward term, no change in glycogen concentration in the giant cells, a labyrinth glycogen storage beginning on day 14, and then a maximum in concentration on day 18 followed by a reduction, and a glycogen storage beginning in the yolk sac on day 14, reaching a maximum at 18 days , and then decreasing until term.

The present study deals with the presence and patterns of glycogen distribution in the uterus and extra-embryonic membranes of the hamster from day six to day 16 (term).

MATERIALS AND METHODS

Eleven pregnant hamsters representing days of gestation six through 16 (term) were sacrificed and their uteri removed intact with placentae and embryos.

The uteri were fixed in Carnoy's fluid for eight to 12 hours, dehydrated in a graded series of alcohols, cleared in xylene, embedded in paraffin (56 - 58° C), and sectioned at seven to ten microns. A vacuum pump was used to facilitate paraffin infiltration.

Diastase was used on sections prior to staining with periodic acid - Schiff and Best's carmine. These sections were compared with those stained with either periodic acid - Schiff or Best's carmine (untreated with diastase). Diastase is very specific for glycogen but might remove also other polysaccharides according to Wolman and Feingold in 1953, as cited by Bulmer and Dickson (5).

If sections are left in diastase (one percent) for more than 30 minutes, they disintegrate. Best's carmine sections should be left in Best's aqueous differentiator for no longer than two dips and then transferred to 80 percent alcohol. No other step is as critical as this.

The Best's carmine technique seems to be more specific for glycogen but not so reliable as the periodic acid - Schiff technique. They therefore serve to check on one another.

As a first approach, the amounts of glycogen in the cells were measured qualitatively, and in lieu of necessarily arduous quantitative measures. "Quantity" judgments from one to six are explained in the key to Table I.

OBSERVATIONS

Glycogen in the Fetal Membranes and their Derivatives

The glycogen granules of Träger cells stain with great intensity from day six to day ten. Thereafter these cells begin to diversify into trophospongial, labyrinth, and giant cells. On day six the granules in these cells are dense, and may or may not form solid masses in the cytoplasm. From day six to day ten, the glycogen granules often run together and completely pack the cytoplasm of the cells (Figure 1). The trophospongial cells stain for glycogen with great intensity from their first appearance on approximately day 11 to term, but the glycogen granules are less dense on days 14, 15, and 16.

The giant cells contain glycogen granules which stain deeply. Obplacental giant cells always stain with great intensity (Figure 2), but the density of the glycogen granules is slightly less from day seven to day ten than from day ten to term. Giant cells of the trophospongium (Figure 3) and trophoblast cells of the labyrinth (Figure 4) stain for glycogen with great intensity, and their granules are very dense, often running together to form very large granules.

The visceral and parietal yolk sac and the splanchnic mesoderm of the visceral yolk sac contain scattered glycogen granules from day seven to ten, especially in the

region next to the placental disc. From day 11 to term the visceral yolk sac entoderm cells stain intensely for glycogen, but the density of granules on day 11 is less than that of later gestation (Figure 5). On day 11 not all of the cells stain, and on days 11 and 12 the granules are in the basal region of the cells. The parietal yolk sac entoderm cells stain deeply on day 12. The visceral yolk sac splanchnic mesoderm possesses sparse granules in some of its cells during days 11 to 14 (Figure 6). On day 15 its borders stain intensely.

On the only day the umbilical cord was investigated (day eight) it possessed sparse glycogen granules (in Wharton's jelly).

The amniotic ectoderm and adherent somatic mesoderm, the labyrinth mesoderm, and Reichert's membrane possess no glycogen at any time.

Glycogen in the Locular Uterus

Cells of the myometrium at the level of the loculus possess dense granules which stain intensely and uniformly throughout gestation (Figure 7).

Endometrial cells do not always stain uniformly. On days six, seven, nine and eleven their glycogen granules stain intensely and they are more dense in the luminal than in the peripheral region (Figure 8). The endometrial cells stain uniformly on days eight, ten, and

twelve. A reduction in glycogen granules is noted on days 13 and 14, and the endometrial stroma regresses on days 15 and 16.

Glycogen granules of uterine gland cells are moderately dense, and stain intensely (Figure 9).

The tunica media cells of blood vessel walls contain an appreciable amount of dark-staining glycogen granules (Figure 10).

The basement membrane, uterine epithelium, and perimetrium contain very little or no glycogen.

Glycogen in the Interlocular and Non-Pregnant Uterus

The myometrium at levels between loculi stains intensely, but the glycogen granules of its component cells are not so dense as those of the locular myometrium.

Endometrial stroma cells contain a few granules on days six, seven, eleven, fourteen, and fifteen, and uterine epithelial cells contain some granules on days eight, nine, and ten.

Uterine gland cells stain intensely throughout gestation and contain dense granules.

The perimetrium and basement membrane do not contain glycogen.

All of the non-pregnant and post-partum uteri used for controls show scattered, dense glycogen granules in the cells of the epithelium and uterine glands. The

endometrial stroma and myometrium have dense, deeply staining granules in one non-pregnant animal but not in another. The four-hour post-partum animal possessed less myometrial and epithelial glycogen than the two-hour post-partum animal.

Summarizing Statement

Glycogen is a cytoplasmic inclusion. The distribution of glycogen in the uterus and extra-embryonic membranes of the hamster is summarized in detail in Table I.

Table 1. Glycogen in the Developing Hamster Placenta and Uterus.

	6P	7P	8P	9P	10P	11P	12P	13P	14P	15P	16P	NP	NP	2hrPP	4hrPP
Locular															
1.	4DI & 5DI	6DC	6DC	5DC	5DC										
2.						6DC	6DC	5DC	5DC	5DC	5DC & 6DC				
3.		4DC	4DC	4DI	4DC	5DC	5DC	6DC	5DC	5DC	5DC				
4.		5DC	5DC	5DC	5DC	5DC	5DC	5DC	5DC	5DC	6DC				
5.				Ns	Ns	Ns	5DC	5DC	5DC	5DC	5DC				
6.		0	0	Ns	Ns	0	Ns	0	Ns	0	0				
7.		0	0	Ns	Ns	0	Ns	0	Ns	0	0				
8.			0	0	0	0	0	0	0	0	0				
9.			0	0	0	0	0	0	0	0	0				
10.		2DI*	0	2DI*	0	B4DCBa & 3DI*	B4DCBa & 5DC**w	5DC*	5DC	5DC	Ns				
11.		2DI*	0	0	0	0	5DC				Ns				
12.		2DI*	0	0	0	2DS	3DI	2DS	3DS	B4DC	Ns				
13.		0	0	0	0	0	0	0	0	0	0				
14.		Ns	3DC	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns				
15.	0	0	0	0	0	0	0	0	0	0	0				
16.	4DC	4DC	5DC	5DC	4DC+	4DS	5DC	4DI	5DC	5DC	5DC				
17.	4DC	4DC	4DC	5DC	4DC+	4DS	4DC	4DI	5DC	5DC	5DC				
18.	6DC	5DC	5DC	5DC	4DS	4DI	2DC	0	4DI	Ns	Ns				
19.	5DC	4DC	5DC & 6DC	4DC	4DS	0	2DC	0	4DI	Ns	Ns				
20.	1DC	1DC	6DC	0	4DS	0	3DC	0	4DI	Ns	Ns				
21.a		5DC	Ns	0	0	4DC	0	0	Ns	Ns	0				
b		4DC	Ns	0	0	4DC	4DI	0	Ns	Ns	0				
c		1DC	Ns	0	0	4DC	0	0	Ns	Ns	0				
22.	6DC	4DI	0	0	0	0	0	0	0	0	0				
23.	B4DCA	3DI	B5DIF	4DS	B4DSF	5DC	3DC	4DI	5DC	BD5F	5DI				
24.					0	0	0	0	0	4DDiC	0				
25.	4DI	4DS	4DS	4DI	5DS	5DS	5DI	4DS	5DS	4DI	4DC				
26.					LiDiC ^x	LiDiC ^x	LiDiC ^x	0	0	4DDiC	0				
27.	Ns	Ns	Ns	4DC ^x	4DI ^x	Ns	Ns	Ns	Ns	Ns	Ns				
28.					MDi ^x	MDi ^x	LiDi ^x			0	0				
Interlocular															
1.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.	4DS	5DC	4DC	4DC	4DC	⊗ 4DS	4DC	4DC	4DC	5DC	5DS	0	5DC	5DC	5DI
3.	4DS	5DC & 4DI	4DC	4DC	4DC	4DC	4DC	4DC	4DC	5DC	5DS	0	5DC	5DC	5DI
4.	0	0	0	0	0	4DC	0	0	4DI	5LiI	0	0	3DC	0	0
5.	4DS	4DS	0	0	4DS	0	0	0	0	0	0	5DS	0	0	4DS
6.	0	0	4DS	4DI	B4DSF	0	0	0	0	0	0	B5DSBa	3DS	4DS	3DS
7.	0	0	0	0	LiDi ^x	0	LiDi ^x	0	0	0	0	0	0	0	0
8.	B4DIF	4DIF	4DIF	4DS	4DC	5DC	5DC	B4DCF	5DC	4DC	5DI	B4DCF	4DS	5DI	4DI
9.	0	0	0	LiDi ^x	DDi ^x	MDi ^x	MDi ^x	LiDi ^x	LiDi ^x	0	0	0	0	0	MDI
10.	4DS	4DS	4DS	4DS	1DS	5DS	5DS	4DI	4DI	4DI	5DS	4DI	4DI	4DS	4MS

* Near placental disc
+ Middle region o
* Information from Best
x No evidence from Best
⊗ Best shows a reverse
w Less near placental disc

KEY

Cell Area ... B - Adjacent to cell border (within the cell).

Granule Density

6 - Glycogen granules dense and running together, often masking all of cytoplasm.

5 - Glycogen granules dense, running together in some, but not in all areas.

4 - Glycogen granules dense and large, not running together.

3 - Glycogen granules large, approximately 6-10/cell in section.

2 - Glycogen granules large, approximately 2-5/cell in section.

1 - Glycogen granules fine, abundant.

0 - No granules or at most one.

Intensity ...

D - Dark (heavy intensity).

M - Medium (medium intensity).

Li - Light (light intensity).

C - Occur in most or all cells.

I - Occur commonly in about half of the cells.

S - Occur uncommonly in cells (in about one per 15-20 cells).

Di - Diffuse.

- Not applicable.

Ns - Not studied.

P - Days of pregnancy.

Ba - Basal region.

NP - Non-pregnant.

F - Apical region.

PP - Post partum.

Locular

- Träger
- Glycogen cells of trophospongium
- Obplacental giant cells
- Giant cells of trophospongium
- Residual trophoblast of labyrinth
- Amniotic ectoderm
- Amniotic somatic mesoderm
- Labyrinth mesoderm
- Labyrinth endothelium
- Visceral yolk sac entoderm
- Parietal yolk sac entoderm
- Visceral yolk sac splanchnic mesoderm
- Reichert's membrane
- Umbilical cord
- Perimetrium
- Outer myometrium
- Inner myometrium
- Luminal endometrial stroma (antimesometrial)
- Middle endometrial stroma (antimesometrial)
- Peripheral endometrial stroma (antimesometrial)
- Endometrial stroma of decidua capsularis (mesometrial)
 - Luminal
 - Middle
 - Peripheral

- Stratum compactum of endometrium
- Uterine glands
- Uterine epithelium
- Tunica media of blood vessels
- Uterine epithelial border
- Endovascular plasmodial cells
- Basement membrane

Interlocular

- Perimetrium
- Outer myometrium
- Inner myometrium
- Endometrial stroma
- Stratum compactum of endometrium
- Uterine epithelium
- Uterine epithelial border
- Uterine glands
- Basement membrane
- Tunica media of blood vessels

DISCUSSION

The Bauer-Feulgen, periodic acid - Schiff, Best's carmine, and Mitchell's ammoniacal silver nitrate methods (17, p. 261-266) are those commonly used for the detection of polysaccharides, some being more specific techniques than others. The benefits of using more than one of these stains have already been discussed.

Glycogen in the chorionic trophoblast cells of the bat has been reported by Wimsatt (33), in the trophospongial cells of the rat by Padykula and Richardson (24), Bulmer and Dickson (5), and Sharov (26), of the hedgehog by Morris (18), and of the rabbit by Lockhead and Cramer (15). It has been reported in cells of the human cytotrophoblast by Dempsey and Wislocki (10). Diminution of glycogen later in gestation in these areas was reported by Wimsatt (33), and Padykula and Richardson (24). An overall glycogen decrease during pregnancy was observed by Lockhead and Cramer (15). No marked decrease in trophospongial glycogen was observed by Morris (18) in the hedgehog. The same observation applies to the hamster.

That giant cells of the rat placenta contain glycogen has been shown by Padykula and Richardson (24) and by Bulmer and Dickson (5). The glycogen concentration was relatively constant throughout gestation in these animals.

Loveland, Maurer, and Snyder (16) reported no glycogen in rabbit obplacental giant cells. The glycogen concentration of the hamster giant cells does not fluctuate to any great degree throughout gestation.

Certain "mesenchyme" cells of the bat labyrinth were reported by Wimsatt (33) to contain glycogen, and also mesenchymal connective tissue in the placenta near the junction of the umbilical cord was reported by Dempsey and Wislocki (10) to contain glycogen. Glycogen storage begins in rat labyrinth cells on about 14 days, reaches a maximum on day 18, and then decreases until term according to Padykula and Richardson (24). Dense glycogen granules appear in the hamster labyrinth trophoblast on day 12 and stain intensely until term.

Wimsatt (33) reported that glycogen appears early in the bat yolk sac and then decreases, and that glycogen may be found in the mesenchyme between its layers. Glycogen in the hedgehog yolk sac entoderm and splanchnic mesoderm was reported by Morris (18), and Wislocki, Deane, and Dempsey (34) stated that glycogen increases to a maximum by day 15 in the yolk sac of the mouse and remains in undiminished quantities until term. They also reported glycogen in the guinea pig yolk sac. Padykula and Richardson (24) reported an increase in yolk sac glycogen from day 14 (its first appearance) to day 18 after which there is a loss of glycogen. In addition, they reported

glycogen in the mesenchyme between the entoderm and vitelline vessels. Glycogen appears on day 11 and remains in great quantities until term in the hamster visceral yolk sac. Often glycogen granules may appear in the visceral yolk sac splanchnic mesoderm. Glycogen was often found to be basal in the visceral yolk sac cells of the hamster, a condition also found in the rat by Padykula and Richardson (24) and in the bat by Wimsatt (33). Morris (18) found glycogen to be distal in yolk sac cells of the hedgehog. Wislocki, Deane, and Dempsey (34) reported no glycogen in the parietal yolk sac of the mouse, and it rarely appears in this tissue in the hamster.

Glycogen has been reported in the umbilical cord of man by Sharov (27) (up to week 16), and by Boyd (2). Glycogen can be shown to be present on day eight in the hamster umbilical cord. No tissue from other days of gestation was investigated.

Glycogen has been reported in the amniotic ectoderm of man by Sharov (27) and of the bat by Wimsatt (33) (mid and late gestation). No glycogen appears in the amnion or in Reichert's membrane of the hamster. No glycogen was found by Wislocki and Padykula (39, p. 117-152) in Reichert's membrane.

An increase in myometrial glycogen has been observed in the rat by Walaas (31) and by Padykula and Richardson (24), in the rabbit by Vasilevskaya, as cited by Connolly

(7), and in man by Brody (4). No significant increase in myometrial glycogen of the hamster could be detected.

Glycogen was observed to be especially concentrated in the lateral mesometrial decidua of the rat by Padykula and Richardson (24), in decidual tissue adjacent to the trophospongium of the hedgehog by Morris (18), in the major decidual tissue of the mouse by Wislocki, Deane, and Dempsey (34), in the guinea pig by Wislocki, Deane, and Dempsey (34), in man by Dempsey and Wislocki (10), in the rabbit by Lockhead and Cramer (15), and in the paraplacental decidua of the bat by Wimsatt (33). A decrease in decidual glycogen was noted in the rat by Padykula and Richardson (24), in the cat by Wislocki and Dempsey (36), in man by Dempsey and Wislocki (10), and in the rabbit by Lockhead and Cramer (15). The cells of the hamster decidua appear to lose glycogen in later gestation.

Uterine epithelial cells of the hedgehog have been found by Morris (18) to stain for glycogen, and Wislocki and Dempsey (36) found glycogen in the epithelium, decidua, and glands between placental sites in the cat. Glycogen is very scant in hamster uterine epithelium.

Glycogen in uterine glands of the bat has been demonstrated by Wimsatt (33), and of man by Dempsey and Wislocki (10). In the hamster, scattered glycogen granules occur in the uterine glands throughout gestation.

Glycogen in blood vessel walls of the chorio-allantoic membrane of the sow was demonstrated by Wislocki and Dempsey (36 and 38, p. 181-225). Blood vessel walls of the human placenta containing glycogen were described by Boyd (2). Practically all blood vessel tunica media cells of the hamster placenta contain glycogen. In the hamster, the basement membrane and perimetrium do not contain glycogen.

There is a large reserve of glycogen built up in the hamster placenta, most of which does not appear to be mobilized. The elucidation of the enigma of how glycogen is stored and transferred in the placenta awaits further study. Wislocki and Dempsey (35, p. 1-41; 36; 37, p. 1-46) maintain that glycogen is present in those areas of the placenta with sluggish oxidative metabolism and that this glycogen deposition is related to altered circulation. They say that the pig and cat have relatively unaltered circulation in the maternal decidua compared to the altered circulatory condition of human and rat placentae. This assertion automatically involves a discussion of why glycogen is found in muscle and liver tissues. Wislocki and Dempsey (35) state that there may be some anaerobic mechanism in muscle, and that the blood supply to the liver from the hepatic portal system has low oxygen saturation. That glycogen storage in liver and muscle is under hormonal control, and that it is determined by

rates of delivery and utilization of glucose, might be an alternative explanation.

Free glycogen in the maternal blood of the placenta has been reported by Goldmann, as cited by Padykula and Richardson (24), and Villee (29). Bridgeman (3) states that this glycogen may be ingested directly by cells of the syncytium, or that metrial gland cells and decidual cells may absorb glucose from the maternal blood and store it as glycogen. This store would later be "ingested" by the syncytial cells of the junctional zone, or by the giant cells, which may break it down and pass it in turn to the glycogen cells of the junctional zone as glucose, or hand it over unchanged to the yolk sac cells. The yolk sac cells of the hamster definitely store more glycogen later in gestation.

The quantitative variations of glycogen in non-pregnant and post-partum animals can perhaps be explained as cyclic fluctuations timed by and correlated with the estrous cycle.

SUMMARY

1. The uterus and extra-embryonic membranes of the golden hamster representing days of gestation from six to term were stained with periodic acid - Schiff and Best's carmine. Diastase was used to check the glycogen-staining efficiency of these two procedures. Non-pregnant and post-partum uteri (day one) were also stained and checked with diastase for comparison.
2. Glycogen is present in great quantities in the Träger, trophospongium, myometrium, uterine glands, and blood vessel tunica media. No observable increase or decrease in glycogen content was observed in these tissues.
3. Glycogen appears in the visceral yolk sac on day 11 and remains in great quantities until term. Glycogen may be observed in small quantities in the parietal yolk sac entoderm near the visceral yolk sac, and in the visceral yolk sac splanchnic mesoderm, from day 11 to term.
4. Endometrial glycogen concentration appears to decrease as gestation proceeds, and as the endometrial cells become compressed.
5. There appears to be slightly less glycogen in inter-ocular than in locular uterine regions.
6. The residual trophoblast cells in the labyrinth stain for glycogen throughout gestation.

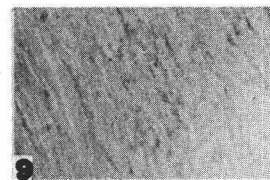
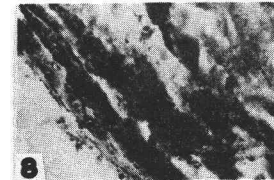
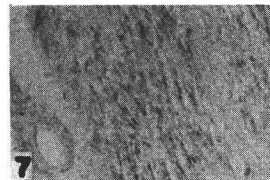
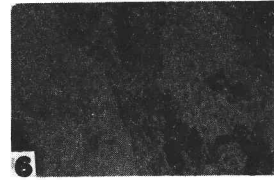
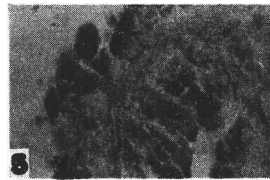
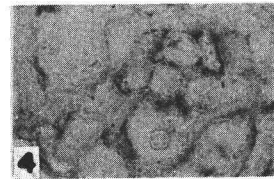
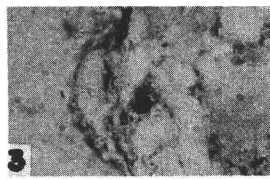
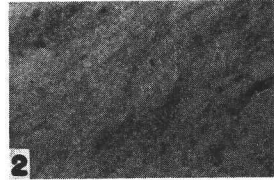
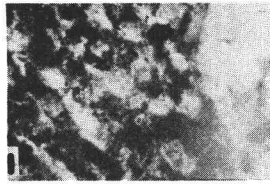
7. Glycogen may occasionally appear in uterine epithelium.
8. Some theories on glycogen storage and transport are reviewed.
9. The distribution of glycogen in the hamster uterus and fetal membranes from day six to 16 is given in detail in Table I.

EXPLANATION OF FIGURES

Plate I

1. Glycogen granules in cells of Träger of a seven day placenta showing the 6DC condition. X 1000.
2. Obplacental giant cells of an eight day placenta showing glycogen granules representing condition 4DC and 5DC. X 1000.
3. Condition 5DC shown by glycogen granules of trophos-
pongial giant cells of a 14 day placenta. X 1000.
4. Residual trophoblast of the labyrinth of a 14 day placenta showing glycogen granules of condition 5DC. X 1000.
5. Visceral yolk sac cells of the same area as presented in Figure 4 showing showing glycogen granules representing condition 5DC. X 1000.
6. Yolk sac splanchnic mesoderm showing condition 2DS (11-day placenta). X 1000.
7. Myometrial cells of a seven day locular uterus with glycogen granules representing condition 4DC. X 1000.
8. Endometrial stroma of a six day placenta showing condition 6DC. X 1000.
9. Glandular cells of the uterus of an animal of seven days gestation showing condition 3DI. X 1000.
10. Tunica media of a blood vessel of a nine day locular uterus at level of loculus. X 1000.

PLATE I.



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