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Title: AN HISTOCHEMICAL STUDY OF THE CHANGING PATTERNS OF GLYCOGEN DISTRIBUTION IN THE UTERUS OF THE EARLY PREGNANT AND LACTATING GOLDEN HAMSTER (*Mesocricetus auratus* Waterhouse).

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This study deals with the histochemical identification of the changing patterns of glycogen in the uterus of the early pregnant and lactating golden hamster (*Mesocricetus auratus* Waterhouse). The uterus of the hamster representing days one through five of gestation, and one through ten, twelve, and fourteen of lactation, provided the data for this study. Diastase digestion was used on alternate sections of the uterus prior to staining with periodic acid-Schiff, and these sections were compared with adjacent stained sections (untreated with diastase) for assessing the glycogen pattern. The presence and amounts of glycogen in the uterine tissues of both the pregnant and lactating animals are inconsistent. The outer longitudinal myometrium of the pregnancy uteri generally contains more

glycogen than the inner circular myometrium, whereas these two layers in the lactation uteri usually contain equal amounts. Granular glycogen in the endometrium was seen only during gestation and solely on the fifth day, on which it is restricted to the locular level of the uterus and in very dense deposits. The uterine epithelium proper contains granular glycogen in small amounts on some days of gestation, but none occurs in the lactation uteri. Although the associated basement membrane never contains granular glycogen, the diffuse form is present but rather inconsistently. The epithelial border of the pregnancy uterus contains patches of glycogen granules with greater frequency than does that of the lactation uterus. Seldom does glycogen occur in the uterine lumen but when it does, it is always in patches. The epithelium and lumen of the uterine glands contain small amounts of granular glycogen on some days of pregnancy whereas, with one exception, neither of these areas in the lactation uteri have granules. Diffuse glycogen in varying amounts commonly occurs in the basement membrane associated with the uterine glands. Whereas the tunic of the veins during pregnancy contains more granular glycogen than does the tunica media of the arteries, only diffuse glycogen was noted in these tunics of the lactation uteri and in equal amounts. A comparison is made between current observations on the hamster and those made by others not only in the hamster but also on other species.

An Histochemical Study of the Changing Patterns of Glycogen Distribution in the Uterus of the Early Pregnant and Lactating Golden Hamster (*Mesocricetus auratus* Waterhouse)

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I. INTRODUCTION

A number of papers have appeared on various aspects of development of the golden hamster. Graves (1945) described the development of both the embryo and fetal membranes, and the associated changes in the uterine tissues during the first nine days of gestation; Venable (1946) concerned himself primarily with preimplantation stages; Ward (1948) studied in detail the development, implantation, and associated endometrial changes during the first five days of pregnancy. The morphogenesis of the vitelline and allantoic placentae was investigated by Adams and Hillemann (1950). Orsini has several papers, including those dealing with the trophoblastic giant cells and the endovascular cells associated with pregnancy (1954), the apposition and fusion of placentae (1956), the vascular knot of the uterus (1957), and with ovo-implantation in the cleared uterine tracts of several species (1962). In 1965, Enders, by means of electron microscopy, determined the hamster placenta to be hemotrichorial. Developing connective tissue fibers in the hamster were studied by Hillemann and Ritschard (1963, 1967), and by Ritschard and Hillemann (1967).

The only previous work concerned with glycogen in the hamster uterus, placenta, and extraembryonic membranes is that of York and Hillemann (1968). Their study was restricted to a period extending from day six to sixteen (term), and included a two and a four-hour post partum animal as sample comparisons. Their study did not include days one through five of pregnancy. However, a number of persons have investigated at least uterine glycogen in other animal forms in both the pregnant and post partum condition; the more pertinent findings are related below.

In 1931, Loveland, Maurer and Snyder noted a marked diminution of glycogen in the rabbit uterus during the last third of pregnancy. The preimplantation changes in the uterine mucosa of the cat were studied by Dawson and Kosters (1944) who saw glycogen at a maximum level on day seven of gestation. Wislocki and Dempsey (1945) described glycogen in both the lining epithelium and distal parts of the uterine glands of this animal. In this same paper these authors reported the absence of glycogen in the endometrium of the sow both before and after implantation; as for man and rodents during the first half of gestation, there were great quantities of glycogen in the decidually transformed tissues at the placental sites. In a study of human myometrial glycogen, Brody (1958) recorded a continuous increase during later pregnancy along with a sharp decline immediately following parturition.

According to Morris (1957), glycogen in the hedgehog uterus is located mainly in a circular zone of decidual tissue around the blastocyst, as well as in the outer trophospongium. He specifically commented on the absence of glycogen from both the decidua capsularis and the epithelial elements of the deeper coiled portions of the uterine glands.

A histochemical study concerned with glycogen metabolism in the myometrium of pregnant and parturient mice by Hall (1966), demonstrated that glycogen remained near its histochemically detectable limit in both the circular and longitudinal myometrium until the end of the second week of pregnancy. But in the third week, there was a progressive accumulation of glycogen terminating in heavy deposits in both myometrial layers. During parturition, heavy glycogen deposits were common in the circular myometrium, but in the longitudinal layer glycogen was either absent or at best represented by a patchy distribution.

The glycogen of the pregnant and post partum rat uterus has received considerable attention. For example, Krehbiel (1937) found glycogen confined to the antimesometrial portion of the primary decidual zone in the six-day pregnant rat uterus; the rest of the stroma and the entire uterine epithelium remained free of glycogen. During the seventh day glycogen appeared in the new decidual cells adjacent to the epithelium, but by the eighth day it had disappeared from all the areas where it had hitherto been found. An increase mainly in rat myometrial glycogen during pregnancy was noted by both Walaas (1952) and Kostyo (1957). Sharov (1958) found a gradual rise in the glycogen content of the decidua basalis during pregnancy in the rat. In 1962 Connolly et al., working on the rat, noted a progressive increase in uterine glycogen as a whole during pregnancy. At the completion of implantation on the eighth day, Padykula and Richardson (1963) found the rat decidua to be rich in glycogen, especially in its lateral mesometrial portions. The decidua basalis on the thirteenth day of gestation was a thick layer rich in glycogen, but this store decreased steadily as gestation advanced, whereas the glycogen content of the uterine smooth muscle increased steadily between thirteen and 21 days of pregnancy. Christie's observations of 1966 on the rat decidua, were similar to those of Krehbiel (1937), and Padykula and Richardson (1963). However Christie, in contrast to Padykula and Richardson, found moderate amounts of glycogen with little change in the smooth muscle layers during gestation. In another paper on the rat, Bo and Smith (1966) reported a marked drop in the myometrial glycogen content in animals from one to five days post partum.

In their study on the hamster, York and Hillemann (1968) found glycogen present in consistently large amounts in the myometrium, the uterine glands, and the tunica media of blood vessels. The endometrial glycogen content diminished progressively during gestation as the endometrial cells became compressed. There was also less glycogen in the interlocular than in the locular level.

This thesis as presented below, deals with the changing patterns of glycogen distribution in the uterus of both the early pregnant (days one through five), and the lactating hamster. Specific attention was directed to both the time of appearance of glycogen and to its levels as visually assessed.

II. MATERIALS AND METHODS

The data in this study were provided by five hamsters representing days of gestation one through five, and by twelve in lactation, representing days one through ten, twelve, and fourteen.

The pregnancy uteri were dated from the time of observed coitus. The young sexually mature males and females were allowed to remain together for an interval varying from 30 to 45 minutes during which period copulation occurred repeatedly.

Although complete serial sections were not made of the uteri, and the blastocyst therefore, which enters the uterus during the third day and implants during the fifth day (Graves, 1945; Venable, 1946; Ward, 1948), was not located in those sections selected and stained, it was assumed nevertheless, that the hamsters were pregnant. This assumption was based in each case on three different criteria as follows: 1) observation of repeated copulation; 2) presence of corpora lutea at sacrifice, and 3) comparison of the uterine histology with that given by Graves (1945) for the hamster uterus during the first five days of gestation.

The lactation uteri were dated from the time of termination of parturition.

All females were killed by cranial concussion, and the uteri promptly removed for fixation in Carnoy's fluid (absolute alcoholacetic acid) for eight hours at 0-5°C. The uteri were then washed in 95% alcohol and stored in 80% alcohol. Later they were dehydrated in a graded series of alcohols, cleared in xylene, and embedded in paraffin (56-58°C); infiltration was facilitated with a vacuum pump. Either longitudinal or transverse sections were cut at eight or ten microns.

Sections used as controls were subjected to a 1% malt diastase digestion for 30 minutes at 37°C. These along with those adjacent sections not subjected to diastase, were stained with either periodic acid-Schiff (PAS), or Best's carmine (McManus and Mowry, 1960). A comparison was then made of treated and untreated sections. The identification of glycogen is said to be established by the failure of either periodic acid-Schiff, or Best's carmine to stain the diastase treated sections. Although diastase does indeed remove glycogen, it may also eliminate other polysaccharides according to Wolman and Feingold (1953).

Since Best's carmine technique repeatedly gave very poor results, it was discontinued, and all values given for the amounts of glycogen were based solely upon the periodic acid-Schiff technique.

There is disagreement in the literature on the value of Best's carmine as a stain for glycogen. According to Lillie (1948, p. 145) the PAS method "appears to demonstrate heavier glycogen infiltration than either the Bauer or Best's technique, and has shown the presence of glycogen in material on which the other two methods have failed." McManus and Mowry (1960, p. 129) state concerning Best's carmine: "This is a capricious stain, failing in our hands many times but producing extremely attractive appearances when successful." However, Kugler and Wilkinson (1959) had claimed that the PAS technique is less sensitive for glycogen than either Gomori's silver technique or Best's carmine.

Pertinent to this study is the suggestion of Kugler and Wilkinson (1960) that only the trichloroacetic acid-soluble fraction of glycogen is demonstrated by histochemical methods, the acid-insoluble fraction remaining unstained.

The sections were studied under both low power and oil immersion, and the intensities of the staining reaction in the diastase treated and untreated sections were compared. The amounts of glycogen were visually assessed, but since glycogen occurs in both granular and diffuse form, the amounts present were necessarily judged using two different methods. The granular glycogen was assigned values ranging from zero through five as explained in the key to Tables I and II. The diffuse glycogen is uniform in distribution, and the amount present is considered to be related directly to the intensity of the staining reaction. Thus, a light intensity stain indicates a small amount of diffuse glycogen, and a dark intensity stain indicates a large amount.

III. OBSERVATIONS

Since the uterine loculus as such is not distinctly developed until day five of pregnancy, the discussion below provides separate sections, one dealing with uterine glycogen during the first four days of gestation, and a second concerning itself with glycogen on day five in both the locular and interlocular uterine levels. A third section considers glycogen in the lactation uterus.

Glycogen in the Pregnancy Uterus (days one through four)

Although the perimetrium of the pregnancy uterus presents no glycogen in granular form at any time, there is at least some glycogen in diffuse form during the first three days of pregnancy.

The outer longitudinal myometrium contains abundant granules on days three and four, whereas on day one this tissue shows a sparse number of granules having a patchy distribution, and on day two it contains none. On days two and three, the inner circular myometrium contains an abundance of granular glycogen with a patchy distribution, while on day four this tissue contains only a sparse amount, with none on day one.

None of the pregnancy uteri contain glycogen granules in the endometrial stroma cells, but the diffuse form does occur in varying amounts (Tables I and II).

The uterine epithelium proper contains, on days one and two, both sparse granular and lightly staining diffuse glycogen, whereas on days three and four there is neither form of glycogen. Its adjacent basement membrane contains only diffuse glycogen, staining with a medium intensity on days one and two, and with a light intensity on days three and four. The border of the uterine epithelium proper shows a gradual transition from a content of patchy dense granules of glycogen (not masking the cytoplasm) on day one, to a moderate amount which likewise is distributed in patches on day four. The uterine lumen contains patches of dense granules on day one only (there are none on the other three days of pregnancy).

The epithelium and lumen of the uterine glands on some days show granular glycogen in sparse amounts, and the associated basement membrane contains glycogen on all four days but in diffuse form only.

The arterial tunica media, composed of circular smooth muscles, and easily distinguished from the other tunics, contains glycogen inconsistently during the early days of pregnancy. Whereas granular glycogen occurs in the tunic on day three only (in moderate amounts and in patches), darkly staining diffuse glycogen is present on day one, but on day four the diffuse glycogen stains lightly. In the tunic of the vein, granular glycogen is present in moderate, sparse, and abundant amounts on days one, two and three respectively, whereas on day four there is none. Its presence was inconsistent, that is to say, it was present in some veins as seen in section, and not in others. It is interesting to note that the occurrence of granular glycogen was found in more veins, on more days, and in larger amounts than in the arteries.

Glycogen in the Locular and Interlocular Uterus (day five)

Neither granular nor diffuse glycogen was noted in the perimetrium of the five-day uterus at either the locular or interlocular level.

In the locular area the outer longitudinal myometrium contains a very dense accumulation of glycogen which masks most or all of the cytoplasm, whereas the interlocular concentration is not so dense. The inner circular myometrium however, contains only trace amounts of glycogen in the locular, and none in the interlocular area.

The amount of glycogen in the several endometrial regions varies greatly. Peripherally, no granules are found at either locular or interlocular level, but in the middle and luminal areas of the loculus very dense accumulations occur and mask most or all of the cytoplasm, while these same areas have no granules between loculi.

With respect to the uterine epithelium, the loculus contains a moderate number of granules restricted mostly to the apical portions of cells. Granules are completely lacking in the uterine epithelium at the interlocular level. Although the associated basement membrane at both locular and interlocular levels contains no granules, the epithelial border does contain them and in large numbers, but at the locular level only.

At both the locular and interlocular levels, both the epithelium and basement membrane of the uterine glands contain only lightly staining diffuse glycogen, but the lumen of the glands contains a small quantity of granular glycogen.

Neither the tunica media of the arteries, nor the tunic of the veins, contains any granular glycogen in either the locular or interlocular levels.

Glycogen in the Lactation Uterus

During the entire lactation period studied (days one through ten, twelve, and fourteen), the perimetrium never contained granular glycogen, although lightly staining diffuse glycogen was noted on some days but not on others.

The outer longitudinal myometrium contains the most glycogen on the three days following parturition, and again on day nine; the same finding applies to the inner circular myometrium.

As in the pregnancy uteri (except at the locular level on day five), no granular glycogen is present at any time in the several endometrial areas, although on certain days there is some diffuse glycogen, almost invariably staining lightly.

The uterine epithelium proper and its associated basement membrane, do not contain granular glycogen in any of the lactation uteri. But there are varying amounts of diffuse glycogen on some days, while on others it is entirely absent. The uterine epithelial border on days four and seven contains large amounts of granular glycogen, whereas on all other days there is none. Except for days one, four, and seven, the uterine lumen contains no granular glycogen.

With respect to the epithelium and basement membrane of the uterine glands, it is only on day nine that granules appear, but the diffuse form occurs randomly in these sites throughout the days of lactation studied. Neither granular nor diffuse glycogen is present in the lumen of the glands at any time.

The tunica media of the arteries and the tunic of the veins contain diffuse glycogen on some days of lactation but never any granules.

The observations above are summarized in Tables I and II below, along with other pertinent details.

Table I. Glycogen distribution in the pregnancy uterus

Uterine Tissue	Days of Pregnancy 1	2	3	4	5 Interloc.	5 Loc.
Perimetrium	L	L	L	0	0	0
Outer longitudinal myometrium	1PD	L	3D	4	3	5
Inner circular myometrium	M	3PL	3PD	1D	0	1L
Endometrium (peripheral)	M	L	M	M	0	L
Endometrium (middle)	M	M	L	M	0	1M* & 5*
Endometrium (luminal)	D	L	L	M	L	5
Uterine epithelium proper	1L	1L	0	0	0	2L
Basement membrane of epithelium	M	M	L	L	0	L
Uterine epithelial border	4P	4P	3P	2PL	0	3
Uterine lumen	4P	0	0	0	0	0
Epithelium of uterine glands	L	1	0	0	0	L
Basement membrane of uterine glands	M	L	L	L	L	L
Lumen of uterine glands	0	0	0	1	1	1
Tunica media of arteries	D	0	2P	L	0	L
Tunic of veins	2PM	1P	3PD	L	0	L

*Sparse amounts near peripheral endometrium but very dense near luminal endometrium

Table II. Glycogen distribution in the lactation uterus.

Uterine Tissue	Days of Lactation											
	1	2	3	4	5	6	7	8	9	10	12	14
Perimetrium	0	L	0	0	0	L	L	L	0	0	0	L
Outer longitudinal myometrium	3M	1 PD & 2 PD	2L	1L	L	M	1L	L	3	0	0	L
Inner circular myometrium	2M	2PL	3P & 4P	L	L	M	1L	L	3	0	0	L
Endometrium (peripheral)	0	L	L	0	n	0	L	0	0	0	0	L
Endometrium (middle)	0	L	L	n	L	L	0	L	L	0	0	L
Endometrium (luminal)	L	M	L	0	L	L	L	L	0	0	0	L
Uterine epithelium proper	0	L	L	0	0	L	0	0	0	0	0	0
Basement membrane of epithelium	0	0	0	M	M	M	M	L	M	0	0	L
Uterine epithelial border	L	0	0	4P	M	L	3P	M	L	0	0	L
Uterine lumen	3P	0	0	2P	0	0	2P	0	0	0	0	0
Epithelium of uterine glands	L	L	0	0	L	L	0	0	3	0	0	L
Basement membrane of uterine glands	L	0	L	0	M	M	L	L	2L	0	0	M
Lumen of uterine glands	0	0	0	0	0	0	0	0	0	0	0	0
Tunica media of arteries	0	L	L	0	D	L	0	L	0	0	0	0
Tunic of veins	0	L	L	0	D	L	0	L	0	0	0	0

Key to Tables I and II. . Relative value judgments on glycogen granule density on a scale of 0 through 5 and, as light, medium and dark diffuse glycogen.

0 = Neither granular nor diffuse glycogen present
1 = Glycogen granules sparse
2 = Glycogen granules moderate in number
3 = Glycogen granules abundant and distinct
4 = Glycogen granules dense but not masking most of cytoplasm
5 = Glycogen granules very dense and masking most or all of cytoplasm

P = Granular glycogen has patchy distribution, occurring in some cells but not in others

L = Glycogen diffuse - light intensity staining
M = Glycogen diffuse - medium intensity staining
D = Glycogen diffuse - dark intensity staining

Loc. = Uterine section taken at level of loculus
Interloc. = Uterine section taken at level between loculi
n = Not studied

IV. DISCUSSION

Glycogen in the Pregnancy Uterus

Most of the many students of uterine glycogen have been concerned with either total uterine glycogen, or with the glycogen content of only one or a few specific uterine tissues. With the exception of York and Hillemann (1968) and Sype (1968), very few have paid attention to glycogen in the individual tissue components of the uterus.

For example in the rat, Connolly et al. (1962) found a progressive increase in total uterine glycogen during pregnancy, an observation similar to that made by Usuelli in the guinea pig (cited by Connolly).

It is perhaps by reason of the prominence of glycogen in the myometrium that this tissue has been given preferential treatment. In the rat, glycogen deposition was hormonally induced by Bo and Atkinson (1952) who found a difference (without specifying) between the longitudinal and circular myometrium in their capacity to store glycogen. According to Bo and Smith (1966), there is more granular glycogen in the outer longitudinal than in the inner circular layer in both pseudopregnant and pregnant rats.

Although an increase in myometrial glycogen during pregnancy has been recorded by several investigators (in several species), there exists some discrepancy. Thus, an increase was noted by Kostyo

(1957) and by Padykula and Richardson (1963) in the myometrium of the pregnant rat, whereas Christie (1966) saw little change, with the content of glycogen remaining moderate over the period of pregnancy.

An accumulation similar to that in the rat, was found in both of the two myometrial layers of the mouse (Hall, 1966), of the guinea pig (DuBois and Ducommun, 1955), of the rabbit (Vasilevskaya, 1954), and of man (Brody, 1958).

In the hamster however, no significant increase in myometrial glycogen was detected from day six through term on day sixteen (York and Hillemann, 1968).

In the hamster, there is an increase in glycogen in the outer longitudinal myometrium leading to a particularly dense deposition in the locular level on day five; by comparison the inner circular myometrium does not present such a marked increase. Pertinent here is the assumption made by many investigators to the effect that this glycogen is a source of energy for muscular contraction during parturition.

Endometrial glycogen has been reported by a number of investigators, most of whom worked on the rat. In 1937, Krehbiel found glycogen in the six-day pregnant rat in approximately one-half of the cells in the antimesometrially located primary decidual zone, but with the remainder of the stroma free of glycogen. Boettiger (1946) found that the glycogen content of the rat endometrium was low when the uterus is under preparation for implantation. A gradual rise in glycogen was recorded for the decidua basalis of the rat during pregnancy (Sharov, 1958). Glycogen was observed to be especially concentrated in the lateral mesometrial decidua on the eighth day of gestation (Padykula and Richardson, 1963). In 1966 Christie found glycogen in the stroma of the rat to be antimesometrial and lateral to the embryo, and that it accumulated at six to six and one-half days of gestation, beyond which it was gradually compressed laterally by the expanding decidua.

In several animals studied, Wislocki and Dempsey (1945), found glycogen to be absent from the endometrium of the sow both before and after implantation, but that in both rodents and man it occurs in great quantities during the first half of pregnancy in the decidually transformed tissues at the placental sites. These same investigators (Dempsey and Wislocki, 1944) had found that the decidual cells of the human varied considerably in their glycogen content. The hedgehog according to Morris (1957), contained glycogen in the decidual tissue adjacent to the trophospongium.

Beginning with the six-day pregnant hamster, the endometrial cells in the middle and luminal areas contain dense granular glycogen, but lose it as gestation advances (York and Hillemann, 1968). In earlier gestation (days one through four) however, only diffuse glycogen is present in the endometrial areas. On the fifth day very dense granular glycogen deposits appear in both the middle and luminal areas at the locular level of the uterus.

Many investigators have assumed the endometrial glycogen to be a potential source of nourishment for the implanting blastocyst. The rapid accumulation of large amounts of glycogen in the hamster on day five (the day of implantation) provides support for their view.

Krehbiel (1937) found it impossible to demonstrate glycogen in the uterine epithelium of the rat at any stage, but Christie (1966) not only found glycogen here, but also observed variations in its content. Christie considered the glycogen content of the uterine epithelium to mirror that of the underlying stroma. He also noted that the uterine secretion contained some glycogen. Related to this is the observation (Wislocki, Deane and Dempsey, 1946) of glycogen in the secretions of the mouse uterine lumen. Dawson and Kosters (1944) found basal deposits of glycogen in the cat uterine epithelium on the fourth day after ovulation; Wislocki and Dempsey (1945) made similar observations for early pregnancy.

According to York and Hillemann (1968), glycogen is very scant in the hamster uterine epithelium, in its associated basement membrane, and in the uterine epithelial border. It occurs in diffuse form only in the latter two sites beyond mid-term. In the present study also on the hamster, glycogen is very sparse in the uterine epithelium, and diffuse glycogen alone occurs in its associated basement membrane. However, with the exception of the five-day uterus at the interlocular level, the uterine epithelial border presents a large number of granules with a spotty distribution. The uterine lumen contains dense patches of glycogen on day one of gestation only.

In man, Spyker and Fidler (1942) found that although glycogen occurs chiefly in the epithelial cells of the glands, some may appear also in the lumen of the glands as well as in the uterine cavity. The glandular epithelium of man is rich in glycogen in the first months of pregnancy (Dempsey and Wislocki, 1944; Wislocki and Dempsey, 1945, 1948; Villee, 1960). Interestingly, Wislocki and Dempsey (1948) found a secretion in the lumen of the endometrial glands which proved to be saliva resistant; the same applies to the hamster.

Wislocki and Dempsey (1946), while working on the sow, found no glycogen in the uterine glands during pregnancy, and neither did Morris (1957) find it in the uterine glands of the hedgehog. Glycogen was found in the cat in small deposits in the glandular epithelium at the end of the second day of gestation (Dawson and Kosters, 1944) and in the outer parts of the uterine glands in early gestation (Wislocki and Dempsey, 1945).

York and Hillemann (1968) demonstrated the occurrence of scattered glycogen granules in the uterine glands of the hamster throughout pregnancy. In the present study sparse granules are seen in the glandular epithelium on day one only; the basement membrane contains only diffuse glycogen; the gland lumen contains sparse granules on day four, and also in both the locular and interlocular levels on day five.

Few investigators have been concerned with glycogen in the uterine blood vessel walls of early pregnant animals. In the human, Boyd (1957) identified glycogen in the uterine veins. York and Hillemann (1968) consistently found dense accumulations of glycogen granules in the tunica media of blood vessels in the hamster, but these accumulations occurred in only a small percentage of the cells. In the present study, no such dense accumulations of glycogen were found in the blood vessel walls, but although there was some glycogen present, it had a special pattern as previously given in the Observation section above.

Glycogen in the Post Partum Uterus

Among the investigators concerned with the glycogen content of the perimetrium, York and Hillemann (1968) found none in the hamster, and neither did Sype (1968), but in this study on the hamster, only lightly staining diffuse glycogen could be identified, and on certain days only.

In the rat, a marked drop in the myometrial glycogen content was observed in the one to five-day post partum animals (Bo and Smith, 1966). In the same year, Hall found that during parturition in mice the most common pattern was one of heavy deposits of glycogen in the circular myometrium, but with an absence or at best a very patchy distribution of it in the longitudinal myometrium. In studying the human myometrium, Brody (1958) found a sharp decline in glycogen immediately after parturition. With reference to the hamster, York and Hillemann (1968) found in both a two and a four-hour lactation animal, a decrease in the myometrial glycogen. In the present study, results were similar to those for the rat, as found by Bo and Smith (1966). There is a decrease from day one to day five post partum, but day nine shows an abundant amount of glycogen granules which are again absent on the later days of post partum.

In comparing the unpublished data of Sype (1968), dealing with post partum non-lactating hamsters, with those on post partum lactating animals, one notes differences in the amounts of glycogen for the same days. On the first day after parturition both myometrial layers contain slightly more glycogen in the non-lactation uterus than in the lactation uterus; however on day two the amounts are equal but more evenly distributed in the non-lactation uterus. In contrast, on day three the lactation uterus contained markedly more glycogen in its outer longitudinal myometrium and only slightly more in its inner circular myometrium, than did the non-lactation uterus. On the remaining days studied, the non-lactation uteri contained generally more glycogen than did the lactation uteri.

As noted from the literature, the remaining tissues of the post partum uterus have received very little attention insofar as glycogen content is concerned. Therefore the discussion which follows, is of necessity restricted to the recent studies on the hamster by York and Hillemann (1968), Sype (1968), and the present author.

In the hamster paper by York and Hillemann (1968), glycogen was stated to be absent from the peripheral endometrium in both the two and four-hour lactation uteri, but the inner compact endometrium contained large, dense granules in the four-hour lactation uterus with none in the two-hour lactation uterus. In the present study, no granular glycogen was seen in the endometrial areas on any day, whereas in the non-lactation uteri studied by Sype (1968), a few granules occurred on some days and not on others.

The uterine epithelium proper contained abundant granules in the two and four-hour lactation uteri, but none in either the associated basement membrane, or in the border of the uterine epithelium (York and Hillemann, 1968). According to present observations, the uterine epithelium proper and its associated basement membrane never possess granular glycogen, but the epithelial border on days four and seven does contain it and in large amounts. The uterine lumen contains glycogen on some days and not on others, but when present it is always in patches and in moderate to abundant amounts.

In comparison, the non-lactation series studied by Sype (1968) contained few granules in the uterine epithelium proper, on approximately half of the days studied. His results were similar to those based on the lactation series as far as the associated basement membrane of the uterine epithelium is concerned; but although he never found granular glycogen on the uterine epithelial border, it did contain it in some of the lactation stages. The uterine lumen contained granular glycogen on more days and generally in larger amounts in the non-lactation post partum uteri than in the lactation uteri.

York and Hillemann (1968) found scattered dense glycogen granules in the uterine glands in both the two and four-hour lactation uteri. With the exception of the nine-day lactation uterus, which contains an abundant amount of granules in the glandular epithelium and a moderate amount in the adjacent basement membrane, the lactation uteri do not contain granules in these two tissues. However, diffuse glycogen is present in these two tissues on some days only. The lumen of the glands contains neither granular nor diffuse glycogen on any day. Sype (1968) likewise found no glycogen in the gland lumen or basement membrane on any day in the non-lactation post partum uteri, but did find small amounts of granular glycogen in the glandular epithelium on most days.

The tunica media of the uterine blood vessels contained scattered dense granules in the two and four-hour lactation uteri as reported by York and Hillemann (1968). In contrast, as seen in the present study, the later lactation uteri never contain granules in either the tunica media of the arteries or the tunic of the veins, although diffuse glycogen is present on some days. In the non-lactation series studied by Sype (1968), the blood vessel tunics consistently contained granular glycogen throughout the period, but in generally larger amounts on the first seven days following parturition.

V. SUMMARY

1. The uterus of the golden hamster representing days one through five of gestation, and one through ten, twelve, and fourteen of lactation, provided the data in this study. Diastase digestion was used on alternate sections of the uterus prior to staining with periodic acid-Schiff, and these sections were compared with adjacent stained sections (untreated with diastase) for assessing the glycogen pattern.

2. The outer longitudinal myometrium of the pregnancy uteri generally contains more glycogen than the inner circular myometrium. The glycogen content increases in the longitudinal layer as gestation proceeds, whereas no such increase is noted in the circular myometrium. These two layers in the lactation uteri contain equal amounts of glycogen on the same days, but the presence of glycogen is very inconsistent.

3. Granular glycogen in the endometrium was seen only during gestation and solely on the fifth day, on which it is restricted to the locular level of the uterus and in very dense deposits.

4. The uterine epithelium proper contains granular glycogen in small amounts on some days of gestation, but none occurs in the lactation uteri. The associated basement membrane never contains granular glycogen , and diffuse glycogen is present inconsistently. In the pregnancy uteri, the border of the uterine epithelium generally

has patches of glycogen granules in varying amounts on different days. In contrast, only three of the twelve lactation uteri presented granules in the epithelial border. Seldom does glycogen occur in the uterine lumen, but when it does it is always in patches.

5. The epithelium and lumen of the glands contain small amounts of granular glycogen on some days of pregnancy, whereas with one exception, neither of these areas in the lactation uteri have granules. Diffuse glycogen in varying amounts commonly occurs in the basement membrane associated with the uterine glands.

6. The tunic of the veins contains more granular glycogen than the tunica media of the arteries during early pregnancy, whereas only diffuse glycogen in the lactation uteri was noted, and then in equal amounts on the same days in the two tissues.

7. Observations dealing with uterine glycogen in the hamster and in other animals are compared with those presented in this study.

8. The glycogen patterns in the hamster uterus during early pregnancy, and during lactation, are given in detail in Tables I and II.

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