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Title: AN HISTOCHEMICAL STUDY OF THE CHANGING PATTERNS OF GLYCOGEN DISTRIBUTION IN THE ESTRUS CYCLE AND POST PARTUM (NON-LACTATION) UTERUS OF THE GOLDEN HAMSTER (MESOCRICETUS AURATUS WATERHOUSE)

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This histochemical study deals with the changing patterns of glycogen in the uterus of the immature virgin, cycling virgin, and post partum (non-lactating) golden hamster (Mesocricetus auratus Waterhouse). The periodic acid-Schiff technique was used for the histochemical localization of glycogen in the uterus of the immature, cycling and non-lactating post partum (days 1 through 10, 12, and 14) hamster. Diastase controls were employed to determine the specific sites of glycogen throughout this organ. During the sexual cycle, the total uterine glycogen content is low in both metestrus and diestrus uteri, slightly higher in the proestrus uterus, and maximal in the estrus uterus. The glycogen content in every phase of the cycle exceeds that found in the immature hamster uterus. In proestrus,

glycogen is present in the outer myometrium and blood vessel tunic in moderate amounts, and in minimal amounts in the inner myometrium, endometrial glands, and uterine lumen. The uterine epithelium stains lightly for diffuse glycogen at this time. During estrus, glycogen increases to large amounts in both the endometrial glands and uterine lumen, but to small amounts at most in both the inner myometrium and uterine epithelium. Glycogen remains in moderate amounts in the outer myometrium and blood vessel tunic. Decreases in glycogen content are seen in all areas of the uterus during metestrus: the outer myometrium contains small amounts of glycogen; the endometrial glands, vascular tunic and uterine lumen all demonstrate minimum amounts of glycogen; and both the inner myometrium and uterine epithelium stain lightly for diffuse glycogen. During diestrus, glycogen content increases slightly in both the inner myometrium and uterine lumen, but remains unchanged in the outer myometrium, blood vessel tunic, and uterine epithelium. The endometrial glands contain no glycogen at this time. Throughout the estrus cycle, glycogen is either absent, or present in varying amounts of the diffuse form in both the endometrial stroma and basement membrane of the uterine epithelium. No glycogen is present in the perimetrium during the estrus cycle.

During the first six days after parturition, there is a rise in the amounts of myometrial glycogen above those found in the myometrium of the uteri of both cycling and immature hamsters. Throughout the

rest of the post partum period however, myometrial glycogen deposits return to estrus cycle values. The vascular tunic contains markedly more glycogen during the first seven days post partum than in the last half of the period studied, while glycogen content in the uterine lumen fluctuates greatly during the post partum period studied. Relatively insignificant amounts of glycogen occur in the endometrial stroma, endometrial glands, and the uterine epithelium and associated basement membrane, throughout this period. Glycogen is absent from the perimetrium during this post partum period. In comparison with the amount of uterine glycogen at term as given by York and Hillemann (1968), the postparturient content is relatively lower; however, during the first seven days after parturition, the glycogen levels are still above those found in the uteri of all cycling hamsters, except those in estrus. Throughout the post partum period studied, total uterine glycogen is slightly higher in amount in non-lactating (post partum) animals, than in the lactating post partum animals studied by Wicklund (1968). The suggested relationship of glycogen and uterine energy requirements is discussed.

An Histochemical Study of the Changing Patterns of Glycogen Distribution in the Estrus Cycle and Post Partum (Non-lactation) Uterus of the Golden Hamster (Mesocricetus auratus Waterhouse)

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TABLE OF CONTENTS

LIST OF TABLES

AN HISTOCHEMICAL STUDY OF THE CHANGING PATTERNS OF GLYCOGEN DISTRIBUTION IN THE ESTRUS CYCLE AND POST PARTUM (NON-LACTATION) UTERUS OF THE GOLDEN HAMSTER (MESOCRICETUS AURATUS WATERHOUSE)

INTRODUCTION

A number of publications have dealt with the histology and physiology of the hamster uterus during the estrus cycle. Deansely (1938) gave the first detailed account of the reproductive cycle of the golden hamster, and Klein (1938) summarized the main features of the cycle, including some special facts in connection with the vaginal smear. Peczenik (1942), by using the vaginal smear technique, studied the effects of gonadotrophins and estrogens on the estrus cycle. Other workers (Graves, 1945; Ward, 1946) have since discussed the value of the vaginal smear in recognizing the various stages of the cycle. Ward (1948) examined in detail, the histological changes of the cycling non-gravid uterus with special reference to the endometrium. Asdell (1964) reviewed all previous research pertaining to both the histological and physiological aspects of the estrus cycle.

Studies on the location and distribution of glycogen in non-pregnant and post partum animals other than in the hamster are numerous. Corner (1921), utilizing Best's carmine and Bauer's method, was unable to demonstrate glycogen in the endometrium of the sow at any stage of the cycle.

The hedgehog was investigated by Morris (1957) who found no stainable glycogen in the endometrium of the non-pregnant cycling uterus.

Hall (1965) reported that little histochemically detectable glycogen is present in the myometrial muscle (and none in the endometrium) of the mouse uterus at any stage of the cycle. Later this same author (Hall, 1966) detected heavy deposits of glycogen in the whole of the myometrium of the mouse before parturition, and heavy deposits of this material in the inner circular muscle fibers during parturition, with little if any in the outer longitudinal layer. One day after normal parturition (lactating) according to Hall, there remain moderate amounts of glycogen in the inner myometrium, but on the second day there is none.

Buchanan (1966) found moderate amounts of glycogen in the luminal epithelium and in the cells of the gland necks in the the ferret uterus at estrus, with no glycogen in the cells of the gland fundi. In addition to granules, the cytoplasm of the cells in both regions gives a diffuse PAS-positive reaction.

In a limited study on the cat, Dawson and Kosters (1944) were unable to detect uterine glycogen in either the surface or glandular epithelium during proestrus, estrus, or anestrus.

In an extensive account on the dog, Fitch (1963) found abundant glycogen in the myometrium along with moderate amounts in the

stroma and glands during proestrus. At estrus however, glycogen is less evident in the stroma, myometrium, and gland necks, but more abundant in the gland fundi. At metestrus, glycogen reappears in moderate amounts in the necks of the glands and is very abundant in their deeper secretory portions. At this same time, while glycogen is only faintly evident in the stroma, there are some increases in its amount in the myometrium. During anestrus, glycogen reappears in the gland necks along with heavier deposits in both the stroma and myometrium. There is no glycogen in the uterine surface epithelium at any stage of the estrus cycle.

Zondek and Stein (1940) reported that although the immature rabbit uterus contains no measurable amount of glycogen, it is present in a stable amount during the proliferative and luteal phases. Godlewski (1962) stated that there is very little histochemically detectable glycogen in the tunica muscularis of the rabbit uterus on day 10 of pregnancy and none on day 20. On the first day after parturition however, the muscle fibers of both layers contain glycogen in abundance; two days later glycogen is again lost from this tissue.

Burgos and Wislocki (1956), working on the guinea pig uterus, noted traces of cytoplasmic glycogen in both the surface epithelial and periglandular stromal cells during estrus and metestrus. Glycogen-bearing leukocytes accumulated during diestrus.

Using chemical methods, van Dyke and Ch'en (1936) stated that

although the glycogen content of the myometrium of the intact monkey is approximately 40-50% greater than that of the endometrium, the fluctuation in the amounts of glycogen during the menstrual cycle is less pronounced in the myometrium than in the endometrium. According to these workers, the glycogen content of the uterus is high during the luteal phase but low during the follicular maturation phase.

Insignificant amounts of glycogen occur in the human endometrium during the follicular maturation phase, but in the second generative phase (corpus luteum phase) the amounts increase rapidly (Zondek and Stein, 1940).

Brody (1958) used quantitative methods to determine the glycogen content of the adult human myometrium during the menstrual cycle, at different stages of pregnancy, during the first four days postparturient, and in the postmenopausal period. During the menstrual cycle the uterus stores much more glycogen than after menopause. Although no marked changes occur in glycogen levels between the follicular and luteal phases of the cycle, there is a marked decrease during menses. In pregnancy there is a progressive increase in myometrial glycogen content to an amount at term which is 50 times that of the postmenopausal myometrium. At parturition there is a sharp fall in glycogen content, but four days thereafter its concentration is still well above that found in the non-pregnant uterus.

The changing patterns of glycogen distribution in the cycling rat

uterus have been extensively studied by such individuals as Boettiger (1946), who showed by chemical analysis that glycogen exists in both the myometrium and endometrium, and that it is maximal during proestrus, and minimal during diestrus. On the other hand, Bo and Atkinson (1952) found that glycogen deposition in the rat uterus is limited to the myometrium, and that while it is abundant throughout the longitudinal layer in proestrus, it has a discontinuous distribution in the circular layer. In diestrus the glycogen content of the longitudinal muscle is greatly reduced and that of the circular layer disappears entirely. Walaas (1952) detected low but constant levels of glycogen in the endometrium of the cycling rat, while those in the myometrium exhibit the same fluctuations as reported by Boettiger (1946). Walaas (1952) has also shown that the glycogen content of the immature rat uterus is less than that of the proestrus animal, equal to that of the estrus animal, and greater than that found during either metestrus or diestrus. Rosenbaum and Goolsby (1957) found glycogen-bearing cells in varying numbers within the endometrium of the rat throughout the estrus cycle. These cells are associated primarily with the subepithelial stroma, periglandular stroma and endometrial glands.

York and Hillemann (1968) studied the glycogen content of both the uterus and fetal membranes in the hamster from day six to term, as well as the uteri of non-pregnant and post partum animals.

Throughout pregnancy glycogen is present in great quantities with little variation in the myometrium, uterine glands, and blood vessel walls. Endometrial glycogen concentration decreases as gestation proceeds. Glycogen was occasionally found also in the uterine epithelium.

The present study deals with the changing patterns of glycogen distribution in the uterus of the immature virgin, the cycling virgin, and the post partum (non-lactating) hamster.

MATERIALS AND METHODS

The 17 female hamsters used in this experiment included one sexually immature non-cycling animal (age 36 days, weight 52 grams), four sexually mature virgins representing proestrus, estrus, metestrus and diestrus, and 12 non-lactating post partum animals (days 1 through 10 and 12, 14). The stage of the estrus cycle was identified by stained vaginal smears (Ward, 1946; Asdell, 1964). The non-lactating condition was assured through the prompt destruction of all newborn.

At appropriate times, individual hamsters were killed by a blow on the head. The uteri were quickly removed and transferred for a period of eight hours to Carnoy's fluid (alcohol-acetic acid) at 0° C. Fixation at low temperature both reduces intracellular polarization of glycogen and diminishes its loss from post-mortem glycolysis (Barka and Anderson, 1963).

Following dehydration (alcohol series) and embedding in paraffin (56° C. - 58° C.), longitudinal sections were cut at 10μ. Deparaffinized mounted sections were treated with 0.5% aqueous periodic acid (pH 3.0) and stained with Schiff's leucofuchsin (McManus and Mowry, 1960). Jackson (1944) pointed out that only at a pH lower than 6.0 will periodic acid be highly specific for 1, 2 - glycols and terminate their oxidation after the formation of aldehydes. Since both glycogen and mucopolysaccharides are stained reddish-purple by PAS,

suitable digestion controls were applied. Accordingly, adjacent sections of tissue were incubated for 30 minutes in a solution of 1% malt diastase in phosphate buffer (pH 6.0) at 37° C to remove glycogen before applying the periodic acid-leucofuchsin procedure. The non-digest sections were placed in phosphate buffer (pH 6.0) without diastase at 37° C for 30 minutes. For most sections, no counterstain was used because the smaller amounts of glycogen were often obscured. The Best carmine stain for glycogen, although used on some sections, was found to be unreliable. Both McManus and Mowry (1960), and Lillie (1948) have reported that the PAS method is preferable to Best's carmine for the histochemical detection of glycogen because of its consistent staining ability.

Glycogen deposits were identified by their positive reaction to PAS and concomitant removal by diastase.

The quantity of tissue glycogen was assessed visually (under oil immersion), and values were assigned on an arbitrary scale as explained in Table 1.

OBSERVATIONS

Glycogen is a cytoplasmic inclusion which appears in both granular and diffuse form in the uteri of immature, cycling, and post partum (non-lactating) hamsters. In the following discussion, glycogen is assumed to be granular whether specified as granular or not, and diffuse when specifically stated as such.

Glycogen in the Immature Hamster Uterus

Glycogen deposition in the immature hamster uterus is associated principally with the outer myometrium, although the basement membrane of the uterine epithelium does show a trace of glycogen in diffuse form. All other areas of the uterus fail to react with the PAS stain.

Glycogen in the Cycling Hamster Uterus

Throughout the estrus cycle the greatest amount of uterine glycogen is in the myometrium. This polysaccharide is most abundant during both proestrus and estrus, and in lesser amounts during both metestrus and diestrus. Invariably, there is more glycogen in the outer longitudinal myometrium than in the inner circular layer. The intensely-staining glycogen granules in the longitudinal muscle cells appear moniliform while those in the cells of the circular layer are dispersed.

At no time during the estrus cycle do significant amounts of glycogen accumulate in the endometrial stroma. Glycogen granules as such are never seen, but diffuse sheets of the PAS-positive material are occasionally found, especially in the stratum compactum at estrus. The stromal reaction to PAS is only partially prevented by prior digestion with diastase. Throughout the estrus cycle, there appears to be somewhat more glycogen in the inner than in the outer zone of the endometrium. But this appearance may not represent a real difference in actual glycogen content since the stromal cells of the stratum compactum are much more compressed than those in the stratum spongiosum.

The amount of uterine glycogen associated with the endometrial glands fluctuates markedly during the estrus cycle. Glycogen granules in the glandular epithelium are rare during both proestrus and metestrus, and at diestrus there are none. But in estrus the number of glycogen granules is very high especially in the apical cytoplasm. In addition, the thin apical cap of the glandular epithelial cell stains with PAS even though the tissue sections have previously been treated with diastase. Although no glycogen is present in the glandular lumen, the cavity is filled with masses of a homogeneous, non-granular substance which is not removed by prior diastase treatment. The size of this luminal plug of material is correlated with the degree of dilation of the glands, being maximum at both estrus and metestrus when the ducts

are distended, and minimal when the ducts are closed during diestrus.

The uterine epithelium proper exhibits low but constant levels of diffuse, apically located glycogen on all days of the estrus cycle, except at estrus when small amounts of glycogen in granular form appear as columns along the cell walls. At this time large numbers of heavily PAS-positive leukocytes suddenly appear among the cells of both the epithelium and subepithelial stroma. Such cells are found to a lesser extent during metestrus, but are absent at all other stages of the estrus cycle. A coating of diastase resistant material adheres to the free border of the uterine epithelium proper. This apical cap is very thin at both metestrus and diestrus, but becomes progressively thicker during both proestrus and estrus. It is in the estrus phase of the cycle that a large amount of irregularly distributed granular PAS-positive material (partially digestible) is seen either free within the uterine lumen itself, or adherent to the free border of the epithelium. This substance is found in lesser amounts at all other stages of the cycle.

The basement membrane of the uterine epithelium is a thick, wavy band, staining intensely for diffuse glycogen at estrus; but at both metestrus and diestrus this band of diffuse glycogen (partially digestible) is thin, smooth and lightly-staining.

Moderate amounts of glycogen appear in the walls of the uterine blood vessels during both proestrus and estrus, with only traces of the

material found at both metestrus and diestrus. And while more glycogen accumulates in myometrial than in endometrial vessels, there are more sections of veins than of arteries which show glycogen.

No glycogen, either diffuse or granular, is detectable in the perimetrium at any stage of the cycle.

No significant differences can be found in the glycogen pattern in the anterior, middle, and posterior segments of the uterus, either mesometrially or antimesometrially.

Glycogen in the Post Partum (Non-lactation) Hamster Uterus

During the first six days after parturition, there is a rise in the amount of myometrial glycogen above that found in the uterine myometrium of both cycling and immature hamsters. During the first four days post partum this increase is due primarily to the consistently heavier deposits of glycogen in the inner myometrium in contrast with those of the outer layer. This pattern is especially noticeable on days one and two post partum, at which times the intensely staining glycogen granules display "glycogen flight" within the cytoplasm of the cells of the circular muscle layer. On days five and six post partum, the slight additional increases in glycogen of the outer myometrium account for the total high glycogen levels of the entire myometrium. Throughout this six day post partum period, glycogen deposits in the inner myometrium are always greater than those in the same tissue of

immature or cycling animals. However, the glycogen content in the outer myometrium (post partum days one through six) never exceeds the values reported for this zone in the other uteri (in immature and cycling animals), except for the slight increase on day six post partum. In both layers of muscle, glycogen distribution is occasionally patchy. From days 7 through 10, and 12, and 14 post partum, the myometrial glycogen content returns to levels similar to those in the uteri of cycling animals.

Throughout the post partum period, glycogen in the endometrial connective tissue is either entirely absent or present in so small an amount as to be near the limit of histochemical detection. Total glycogen content throughout this period is slightly higher in the stratum compactum than in the spongiosum. While only traces of diffuse glycogen are occasionally noted in the outer endometrial zone, insignificant amounts of granular glycogen mixed with the diffuse form appear irregularly in the stratum compactum. No correlation is evident between any particular stage of postparturition, and the amount of glycogen present in the endometrium.

During the entire post partum period the endometrial glands contain very little glycogen, while the gland lumina, which are open to a variable degree throughout this time, contain PAS-positive plugs of diastase-resistent material. Additionally, a diastase-resistant cap can be detected on the free borders of the glandular epithelial cells.

The appearance and distribution of glycogen granules within these glands is similar to that described for the glands of the estrus cycle.

Glycogen is occasionally found, but only in minimal amounts, in the apical portions of the uterine epithelial cells throughout the fourteen days studied. Situated on the free borders of these cells is a diastase-resistant but PAS-positive material which varies irregularly in thickness at different stages post partum.

The basement membrane, which is wavy and thick at all times, contains only insignificant amounts of diffuse glycogen.

Patches of diffuse glycogen, which are either completely or partially digested by diastase, appear in the uterine lumen in varying amounts throughout the post partum period studied, except for days 5, 6, 8, 9, and 14, on which glycogen is absent.

Erythroplasts appear in large numbers in the lumen of the uterus of the four day post partum animal, and whatever the composition of the PAS-staining material present within the individual plast may be, it is not digested by diastase.

Extremely large deposits of glycogen occur in the tunic of the blood vessels at 24 hours after parturition. Six days later, significant amounts of this substance can still be detected, but little glycogen is seen for the duration of the post partum days studied. These blood vessels show a pattern of glycogen distribution similar to that found in the cycling animal.

The perimetrium demonstrates no glycogen of either type throughout the period, and all regions of the uteri display similar patterns of glycogen deposition.

The observations presented above are given in detail in Table 1.

Table 1. Glycogen distribution in the uterus of the immature virgin, cycling virgin, and post partum (non-lactating) hamster.

		Cycling Virgins				Days Post Partum											
	I. V.	P	E	M	Di	1	2	3	4	5	6	7	8	9	10	12	14
Perimetrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Outer myometrium	2L	3D	3D	2L	2D	2*	2	2L*	2L*	3M	4*	2L	1L	2D*	3L	1	2
Inner myometrium	0	1M	2M*	L	1M	5D	5*	4*	4L	3L	3	1L	1M	2D	2	2D	2
Endometrial (outer) stroma	0	0	L	0	0	L	0	L	0	L	L	M	0	0	0	0	L
Endometrial (inner) stroma	0	L	D	L	L	1D	1M	L	L	1M	1M	M	M	D	0	1M	M
Blood vessel tunic	0	3M	3	1L	1L	5M	3M	2L	3M	2L	2L	3D	M	1L	1M	1D	L
Endometrial glands	0	1M	4	1	0	1	2L	1L	1L	1M	1L	1L	M	L	L	L	1L
Basement membrane of uterine epithelium	L	0	D	L	L	M	L	M	M	M	M	L	L	M	L	M	L
Uterine epithelium	0	L	2D	L	L	1	1L	0	1M	L	M	1L	0	1M	0	L	M
Uterine lumen	0	1*	4*	1*	2	3	3*	5*	1*	0	0	3*	0	0	3*	1*	0

Key:

Granular glycogen

0 - No histochemically detectable glycogen
1 - Minimum amount of glycogen
2 - Small amount of glycogen
3 - Moderate amount of glycogen
4 - Large amount of glycogen
5 - Maximum amount of glycogen

Diffuse glycogen

D - Dark (heavy intensity stain)
M - Medium (medium intensity stain)
L - Light (light intensity stain)

Miscellaneous

I. V. - Immature virgin
P - Proestrus virgin
E - Estrus virgin
M - Metestrus virgin
Di - Diestrus virgin
* - Glycogen present in well-defined areas

DISCUSSION

Although the value of the periodic acid-Schiff method for the detection of glycogen was mentioned above, yet this technique is not without fault. For instance, it has been shown that a certain minimal quantity of glycogen must be present in tissues before the substance can be demonstrated histochemically (Kugler and Wilkinson, 1960). Thus, a discrepancy must exist between the actual total tissue glycogen and that which is histochemically demonstrable. In a visual assessment of glycogen, one cannot be certain that the amounts are roughly equal, when in a given tissue, both the granular and the diffuse forms stain with equal intensity. Also, both forms may occur at the same site within the cytoplasm.

Glycogen in the Immature Hamster Uterus

The limited information on glycogen in the uteri of various immature animals is introduced below, and comparisons are made with the hamster where applicable. Zondek and Stein (1940) reported the uterus of the immature rabbit as containing no measureable amount of glycogen. On the other hand, Walaas (1952), working with the immature rat, showed that its uterus does contain glycogen, and in an amount equal to that found in the sexually mature estrus animal. The glycogen content in the immature hamster uterus is very small, always less than that found in the uteri of cycling animals.

Glycogen in the Cycling Hamster Uterus

The fluctuations in the amounts of total uterine glycogen throughout the estrus cycle have been described for several animals. In both the human (Zondek and Stein, 1940) and the monkey (van Dyke and Ch'en, 1936) the glycogen content of the uterus is high during the luteal phase and low during follicular maturation. Fitch (1963) found the total glycogen content in the dog uterus to be highest during both late metestrus and anestrus, and lowest in both proestrus and estrus. The findings of Boettiger (1946), Bo and Atkinson (1952), Walaas (1952), and Rosenbaum and Goolsby (1957) have all confirmed that the total glycogen content of the rat uterus is maximal during proestrus and minimal during diestrus. Fluctuations in total glycogen content are also seen in the uterus of the hamster, in which glycogen deposition is low in both metestrus and diestrus uteri, is slightly higher during proestrus, and is maximal at estrus.

In many animals, the greatest quantities of uterine glycogen are found in the myometrium and in varying amounts throughout all or part of the estrus cycle. Fitch (1963) reported that myometrial glycogen exceeds the endometrial during both proestrus and estrus in the dog, and that these two zones store equal quantities of the substance during both metestrus and anestrus. The dog myometrium itself contains greater deposits of glycogen during anestrus than at any other time in the cycle. Hall (1965) found no endometrial glycogen, and small but

constant amounts of this polysaccharide in the myometrium of the mouse throughout the cycle. In the monkey, van Dyke and Ch'en (1936) found 40-50% more glycogen in the myometrium than in the endometrium, and that the tunica muscularis of the uterine body contains significantly more glycogen during the corpus luteum stage than during the preceding stage of follicular growth; a sharp decrease in amount occurs during menstruation. Although Brody (1958) did not determine the amounts of endometrial glycogen in the human during the menstrual cycle, he did find a considerable reduction in the myometrial content during the period of bleeding. He observed no difference in glycogen levels between the proliferative and luteal phases of the cycle. Walaas (1952) reported that myometrial glycogen content in the rat always exceeds that of endometrial glycogen during the estrus cycle, and Bo and Atkinson (1952) described for this same animal a progressive diminution of myometrial glycogen from proestrus to diestrus. Also, Bo and Atkinson (1952) noted consistently larger deposits of glycogen in the outer longitudinal myometrium than in the inner circular layer during all stages of the cycling rat. In the two sample non-pregnant hamster uteri (estrus stage undetermined) included by York and Hillemann (1968), one contained more glycogen in the myometrium than in all of the other regions of the uterus combined, while the other showed no myometrial glycogen at all. The present study on the hamster demonstrates that the greatest amounts

of uterine glycogen are located in the myometrium throughout the entire estrus cycle, and that the amount of this material is minimal during metestrus, that it increases during both diestrus and proestrus, and that it reaches a peak at estrus. Glycogen is consistently more plentiful in the outer than in the inner myometrium.

These cyclic variations of glycogen in the uterine myometrium appear, as previously suggested by others, to be directly related to the working capacity of the uterine muscle during uterine muscle contraction. For example, in an in vitro study on the rat uterus, West and Cervoni (1955) demonstrated a concentration threshold for glycogen below which the maintenance of contractility becomes critical. Bo and Atkinson (1952) reported that estrogenic stimulation increases both motility and glycogen content of the myometrium, whereas ovariectomy or treatment with progesterone decreases both. They concluded that glycogen functions as a source of readily useable energy for motor activity. In contrast, Reynolds (1949) stated that the period of greatest muscular activity in the monkey uterus (follicular maturation phase) coincides with low amounts of glycogen, and conversely, when the activity is least, the glycogen content is highest (luteal phase). The relationship between the ovarian hormones, muscular contraction, and glycogen deposition in the hamster uterus has not been studied. The fact that the longitudinal and circular layers of uterine muscle in this animal differ in their content of glycogen, leads

one to suspect a difference in metabolic activity between these two layers.

Reports on the storage of glycogen in the stroma of various animals present interesting differences. The absence of glycogen in the connective tissue of the endometrium has been reported by Corner (1921) in the sow, by Morris (1957) in the hedgehog, and by Hall (1965) in the mouse. In a series of 35 dogs, Fitch (1963) found the staining reaction for glycogen to be moderately strong during proestrus, and weaker at both estrus and metestrus. During anestrus, glycogen is deposited in moderate amounts. Burgos and Wislocki (1956) reported only traces of glycogen in the periglandular stromal cells of the guinea pig uterus during both estrus and metestrus, while van Dyke and Ch'en (1936) working on the monkey, and Zondek and Stein (1940) investigating man, concluded that very little glycogen is associated with the endometrial stroma. Rosenbaum and Goolsby (1957) found glycogen-bearing cells in the subepithelial and periglandular stroma of the cycling rat and none in the stratum spongiosum. The staining reaction of these cells is moderate in intensity during proestrus, estrus, and diestrus, with only a faint staining reaction occurring during metestrus. Walaas (1952) observed low but constant levels of glycogen in the endometrial stroma of the rat throughout its cycle. York and Hillemann (1968) observed in the endometrial stroma of the uterus in one of two non-pregnant hamsters some dense, deeply staining glycogen granules,

with none in that of the other. In the present study, glycogen if not entirely absent, appears only in diffuse form throughout the estrus cycle. Except for a weak PAS-positive reaction during estrus, the stratum spongiosum is consistently void of all glycogen, while the subluminal stroma stains lightly for this substance in all stages, except estrus when the reaction is intense.

Glycogen in the superficial portions of the glands lying in the body of the uterus of the monkey has been reported by van Dyke and Ch'en (1936) and in that of man by Zondek and Stein (1940). In both animals, only traces of glycogen exist during both the menstrual and estrogenic phases, but during the luteal phase of the cycle, glycogen is present in large amounts. During the luteal phase (in man) the glands become dilated and coiled, and in them two different products are elaborated, both mucus and glycogen (Zondek and Stein, 1940). The absence of glycogen in the endometrial glands was reported by Dawson and Kosters (1944) for the cat, by Corner (1921) for the sow, and by Morris (1957) for the hedgehog. Burgos and Wislocki (1956) found the endometrial glands of the guinea pig to be devoid of glycogen throughout the cycle, and that they do contain mucus on the free border of their component epithelial cells, as well as in their lumina. Fitch (1963) found glycogen in moderate amounts in the glandular epithelium of the proestrus dog. During estrus, glycogen increases in abundance in the secretory parts of the glands, but is only faintly

evident or absent in the gland necks. At metestrus, glycogen is markedly represented in the deeper portions of the glands and reappears in moderate amounts in the gland necks. At anestrus all parts of the uterine glands stain deeply for glycogen. No glycogen was demonstrated in the lumina of the glands at any stage of the cycle, although diastase-resistant mucus plugs were seen in the ducts throughout the estrus cycle. Buchanan (1966) found moderate amounts of glycogen in the cells of the gland necks in the ferret uterus at estrus, but none in the cells of the gland fundi. This author identified the major secretory product of the uterine glands as a glycoprotein and/or mucoprotein, both in the lumina of the glands, and adherent to the apical end of the glandular epithelium. Rosenbaum and Goolsby (1957) reported the occasional appearance of glycogen in the glandular epithelium in the proestrus rat, while at estrus the material may be found in both the luminal and epithelial areas of the glands. At metestrus, glycogen is either absent, or present in small amounts in these glandular areas, but at diestrus it is absent. Throughout the cycle, mucin was found in association with the free surface of the glandular epithelium, and in the gland lumen. York and Hillemann (1968) reported heavy deposits of apically located glycogen in the glands of one of two sample nonpregnant hamster uteri, and only small amounts of dispersed glycogen granules in the other. In the present study, glycogen in the endometrial glands of the hamster uterus is either absent or present in

insignificant amounts except at estrus, when a marked increase occurs in the apical portion of the glandular epithelium. The gland lumina, as well as the free border of the glandular epithelium, have a diastase-resistent material (PAS-positive) throughout the cycle, with especially high concentrations of the luminal material appearing at both estrus and metestrus. No glycogen was noted in the gland lumina.

The function of glycogen, and of mucus (if this is the PAS-positive-diastase-resistent material), remains unknown. In primates, the glandular secretions are presumed to be adaptations essential to both the nourishment and development of the fertilized ovum, since its secretion is most active when the endometrium is in preparation for egg reception (Zondek and Stein, 1940). However, it is certainly not true that all mammal species provide these substances for the development of the early egg cylinder, since glycogen production to any considerable degree in the luteal phase is encountered solely in primates (Zondek and Stein, 1940).

Glycogen in the superficial epithelium of the uterine lumen of the monkey has been reported by van Dyke and Ch'en (1936), and of man by Zondek and Stein (1940). In both animals a relative glycopenia exists during both the menstrual and proliferative phases, and a marked glycocopia during the secretory phase. According to Burgos and Wislocki (1956), only traces of glycogen in the cycling guinea pig uterus are seen in the surface epithelial cells during both estrus and

metestrus. During diestrus, glycogen-bearing leukocytes accumulate amongst the epithelial cells. Throughout the estrus cycle, mucus occurs on the free border of the surface epithelial cells. Hall (1965) has similarly reported glycogen-bearing leukocytes in the mouse uterus (leukocytes were mobilized by estrogen injection). Fuxe and Nilsson (1963) have observed an intensely staining "gluey secretion" on the apical surface of the uterine luminal epithelium in mice. Isolated clusters of glycogen granules in the luminal epithelium (with an apical mucus layer) were reported by Rosenbaum and Goolsby (1957) at all stages of the cycling rat. Buchanan (1966) demonstrated glycogen in the uterine epithelium of the ferret uterus at estrus, in addition to a thin diastase resistant apical cap on the epithelial cell. Glycogen was reported absent from the above tissue in the cat (Dawson and Kosters, 1944), in the dog (Fitch, 1963), in the hedgehog (Morris, 1957), and in the sow (Corner, 1921). York and Hillemann (1968) found heavy deposits of glycogen in the luminal epithelium in one of two sample non-pregnant hamster uteri (cycle stage not determined) but only small amounts in the other. The uterine epithelium of the hamsters used in the present study demonstrates little glycogen during the estrus cycle, except during estrus when small granular deposits of the substance appear. Throughout the cycle, the luminal border of the epithelium is covered by a PAS-positive diastase-resistant material. Glycogen-bearing leukocytes are also present

during both estrus and metestrus.

Fuxe and Nilsson (1963) reported the presence of a PAS-positive substance in the lumen of the mouse uterus at estrus, a substance they assumed to be a watery fluid. No diastase controls were used. In the hamster, an irregularly distributed, granular PAS-positive, diastase-labile material is present in the lumen throughout the estrus cycle, in great amounts at estrus and in smaller amounts during the other three stages. This same material also adheres to the free border of the uterine epithelium. Since the substance is either completely, or partially digested by diastase, as the case may be, it is accordingly assumed to be all or partly glycogen.

According to Fuxe and Nilsson (1963), the basement membrane of the uterine epithelium in mice reacts positively to PAS but they used no diastase controls to identify specifically the material. York and Hillemann (1968) found no glycogen in this membrane in either of the two sample non-pregnant hamster uteri included in their study. But in the present study, glycogen was either absent or present in varying intensities and in the diffuse form only.

In the two sample non-pregnant hamster uteri (York and Hillemann, 1968), there were heavy deposits of glycogen in the walls of the blood vessels. In the present study on the hamster, moderate amounts of glycogen occur during both estrus and proestrus, while small amounts are detectable at both metestrus and diestrus. As seen

in section, consistently heavier deposits of glycogen appear in veins than in arteries, the significance of which is not known.

Glycogen in the Post Partum (Non-lactation) Hamster Uterus

Studies concerning the pattern of glycogen distribution in animals at or after parturition are few. Godlewski (1962), using histochemical methods, found no glycogen in the tunica muscularis of the rabbit uterus on day 20 of gestation. However, this polysaccharide is present in great quantities in both layers of the myometrium on the first day after parturition, and on the third day it is absent. In the mouse myometrium Hall (1966) noticed heavy deposits of glycogen (especially in the circular layer), shortly before parturition. During parturition, the circular muscle fibers reacted intensely for glycogen, while glycogen in the longitudinal muscle layer was either absent, or present in trace amounts. On day one post partum (lactating), moderate amounts of this substance were still found in the circular fibers, but 24 hours later no glycogen could be detected in either layer of muscle. At term in man, Brody (1958) reported the average glycogen content (per tissue unit of the myometrium) to be approximately 10-15 times that found in the myometrium of non-pregnant (cycling) women. At parturition there is a sharp fall in glycogen content, but by four days post partum, its concentration is still considerably above that of the non-pregnant uterus. According to York and Hillemann (1968), a

four-hour post partum lactating hamster possessed less myometrial and epithelial glycogen than did a two-hour post partum lactating animal; but both uteri contained scattered, dense glycogen granules in the cells of the uterine epithelium and endometrial glands, and in the tunica media of the uterine blood vessels. Glycogen was absent from both the perimetrium and endometrial stroma. In a series of post partum lactating hamsters (representing days 1-10, 12, 14), Wicklund (1968) found glycogen to be either absent or present in diffuse form in the perimetrium, in the endometrial stroma, in the uterine epithelium proper and its associated basement membrane, and in the vascular tunic throughout this period. According to Wicklund, diffuse glycogen occurs randomly throughout this period in the glandular epithelium, except on day nine on which glycogen in granular form appears in moderate amounts. He states that, except for days one, four, and seven, the uterine lumen contains no glycogen. According to him, glycogen in the myometrium is more abundant on days one, two, three, and nine, than on any other day; he noted no difference in glycogen deposition between the inner and outer layers of the myometrium.

In the present study, consistently smaller amounts of glycogen are seen in the myometrium, the endometrial epithelium, the endometrial glands, and the vascular tunic, than in the corresponding tissues of the two- and four-hour post partum (lactating) hamsters of

York and Hillemann (1968).

In comparing present observations made on non-lactating hamsters with those made on the lactating series (Wicklund, 1968), myometrial glycogen deposits are always slightly greater in the former than in the latter, except on day two on which equal amounts are detected in both, and except on day three when significantly more myometrial glycogen occurs in the lactating animal. During the first four days post partum (non-lactating) heavier deposits of glycogen occur in the inner myometrium than in the outer, a pattern which is the reverse of that seen during the estrus cycle. Both patterns suggest that the individual muscle layers differ in both their capacity to store glycogen, and in their metabolic activity. There also appears to be slightly more of both glandular and uterine epithelium glycogen in the non-lactating series, than in the lactating group, throughout the period studied. An irregular pattern of glycogen distribution occurs in the uterine lumen of both lactating (Wicklund, 1968) and non-lactating post partum animals, although glycogen deposition appears more frequently in the latter. There is consistently more glycogen in the vascular tunic in non-lactating than in lactating (Wicklund, 1968) animals, the difference being especially noticeable from day one through day six post partum. No differences in glycogen content of endometrial stroma and basement membrane were noted between the two groups of animals.

In comparing the slides made of the post partum non-lactating hamster uterus used in the present study, with those made by York and Hillemann (1968) for the term uterus, the glycogen levels in the former are relatively low. The present study also shows that although there is a fall in uterine glycogen content after parturition, the amounts for the first seven days after parturition are still above those found in the uteri of the individual cycling hamsters, except for those in estrus. Glycogen levels on days 8, 9, 10, 12, and 14 post partum approach those for animals in the estrus cycle. No vaginal smears were made on the post partum animals.

The decrease in glycogen following parturition may be due to conversion to lactic acid in consequence of providing energy for muscular work.

In comparison with lactating post partum hamsters (Wicklund, 1968), uterine glycogen content is generally higher in the non-lactating animals for all of the days studied. This reduction of the cellular glycogen level in the lactating non-cycling hamsters may be in consequence of a lack of hormonal stimulation.

SUMMARY

1. The periodic acid-Schiff technique was used for the histochemical localization of glycogen in the uterus of the immature, cycling and non-lactating post partum (days 1 through 10, 12, and 14) hamster. Diastase controls were employed to determine the specific sites of glycogen throughout this organ.

2. During the sexual cycle, the total uterine glycogen content is low in both metestrus and diestrus uteri, slightly higher in the proestrus uterus, and maximal in the estrus uterus. The glycogen content in every phase of the cycle exceeds that found in the immature hamster uterus.

3. In proestrus, glycogen is present in the outer myometrium and blood vessel tunic in moderate amounts, and in minimal amounts in the inner myometrium, endometrial glands, and uterine lumen. The uterine epithelium stains lightly for diffuse glycogen at this time.

4. During estrus, glycogen increases to large amounts in both the endometrial glands and uterine lumen, but to small amounts at most in both the inner myometrium and uterine epithelium. Glycogen remains in moderate amounts in the outer myometrium and blood vessel tunic.

5. Decreases in glycogen content are seen in all areas of the uterus during metestrus: the outer myometrium contains small amounts of glycogen; the endometrial glands, vascular tunic and uterine lumen

all demonstrate minimum amounts of glycogen; and both the inner myometrium and uterine epithelium stain lightly for diffuse glycogen.

6. During diestrus, glycogen content increases slightly in both the inner myometrium and uterine lumen, but remains unchanged in the outer myometrium, blood vessel tunic, and uterine epithelium. The endometrial glands contain no glycogen at this time.

7. Throughout the estrus cycle, glycogen is either absent or present in varying amounts of the diffuse form in both the endometrial stroma and basement membrane of the uterine epithelium. No glycogen is present in the perimetrium.

8. During the first six days after parturition, there is a rise in the amounts of myometrial glycogen above those found in the myometrium of the uteri of both cycling and immature hamsters. Throughout the rest of the post partum period however, myometrial glycogen deposits return to estrus cycle values. The vascular tunic contains markedly more glycogen during the first seven days post partum than in the last half of the period studied, while glycogen content in the uterine lumen fluctuates greatly during the post partum period studied. Relatively insignificant amounts of glycogen occur in the endometrial stroma, endometrial glands, and the uterine epithelium and associated basement membrane, throughout the post partum period studied. Glycogen is absent from the perimetrium during this post partum period.

9. In comparison with the amount of uterine glycogen at term as

given by York and Hillemann (1968), the postparturient content is relatively low; however, during the first seven days after parturition, the glycogen levels are still above those found in the uteri of all cycling hamsters except those in estrus. Throughout the post partum period studied, total uterine glycogen is slightly higher in amount in non-lactating (post partum) animals, than in the lactating post partum animals as studied by Wicklund (1968).

10. The suggested relationship of glycogen and uterine energy requirements is discussed.

11. The distribution of glycogen in the uterus of the immature virgin, cycling virgin, and post partum (non-lactating) hamster is given in detail in Table 1.

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