HISTOCHEMICAL STUDIES OF UTERUS OF THE INTACT CYCLIC RATS AND THE HORMONE-TREATED CASTRATES

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By

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Introduction

Histochemical studies of glycogen in the uterus have been made by many investigators: Müller (19), Bo and Atkinson (8), and Rosenbaum and Goolsby (23) using rats; Aschheim (2), Zondeck and Stein (31), Spyker and Filder (26), Arzac and Blanchet (1), and Senarcleus (24) with humans; Hisaw and Greys (15) with monkeys; and Moss *et al.* (18) with cows. From their reports, it is evident that the endometrial glycogen has been found primarily in the epithelium and in the superficial portions of glands, and the amount present varies considerably according to the stages of sexual cycles.

By means of chemical methods, Boettiger (9) and Kostyo (16) showed that the total glycogen content of the rat uterus varies according to the stages of the estrous cycle, reaching the maximum during proestrus and the minimum during diestrus. This result clearly endorses the histochemical observations mentioned above.

A quantitative fluctuation of alkaline phosphatase in the uterus has been demonstrated histochemically by several workers: Atkinson and Elftman (3) using mice; Leathem *et al.* (17) with cats and rabbits; Atkinson and Engle (4) using humans and monkeys; Atkinson and Gusbery (6) using humans. Moreover, the quantitative fluctuation of alkaline phosphatase has been studied in relation with that of glycogen by some investigators as follows; Arzac and Blanchet (1) studied the fluctuations of alkaline phosphatase and glycogen in human endometrium, and stated that the amount of enzyme increases during the estrogenic phase and declines during the progestational phase, whereas the opposite happens for glycogen. Moss *et al.* (18), using the bovine uterus, reported that the highest activity of alkaline phosphatase was seen during the mid-cycle, and that, in contrast with phosphatase distribution, the

largest amount of glycogen was found at the begining and at the end of the cycle, but no glycogen during the mid-cycle.

Concerning the fat in the uterus, histochemical studies of fat in the rabbit uteri have been made by Reese (22) and Gilbert (13). Also, Okey *et al.* (20), who used chemical methods, reported that the total fat in the endometrium of pigs and monkeys fluctuates during their menstrual cycles.

As for RNA, histochemical studies of it in the human endometrium were done by Atkinson *et al.* (5), and using quantitative methods, by Herrmann *et al.* (14), showing no changes in RNA content during the menstrual cycle.

In spite of the vast literatures mentioned above, morphological alterations of mitochondria and Golgi apparatus seem to have received little attention in relation with glycogen, fat, RNA and alkaline phosphatase during the sexual cycle.

This investigation deals with the fluctuations of glycogen, RNA, fat and alkaline phosphatase in the rat uterus during the estrous cycle in relation with the morphological alterations of the mitochondria and Golgi apparatus, and further, with all of these substances in the uterus of the castrates and the hormone-treated castrates, to examine the influence of ovarian hormone on the histochemical substances.

Materials and Methods

Adult female rats of 10 months of age, obtained from our laboratory colony which had been maintained as a closely inbred strain for many years, were daily given vaginal lavages, and their smears were made for several days to determine their stages in the estrous cycle.

Four rats in diestrus, four in proestrus, three in estrus and three in metestrus were killed by a blow on the head. Their uteri were removed, and each of them was immediately fixed in Champy's fluid, Zenker-formalin, Regaud's fluid, 10 per cent neutral formalin, cadmium-formalin and 95 per cent alcohol. After embedded in paraffin, the sections were cut at 7μ.

For the histological observations of the uterine tissues, Zenker-formalin fixed sections were stained with hematoxylin-eosin, and with Mallory-Azan, and sometimes by the Van Gieson's method ; for the detection of mitochondria, Champy fixed and Regaud fixed sections were stained by the Heidenhain's iron hematoxylin method, and sometimes by the Altman-Kull's method; for Golgi apparatus, cadmium-formalin fixed sections by Aoyama's method; for glycogen, 95 per cent alcohol fixed ones by the periodic acid-Schiff method, later identification of glycogen in them was made by means of the salivary test at 37°C in an incubator ; for the demonstration of DNA, 95 per cent alcohol fixed sections by the Feulgen's method ; for RNA, 95 per cent alcohol fixed ones with thionin, following by confirmation with RNA by ribonuclease treat-

ment; for fat, 10 per cent neutral formalin fixed ones with Sudan black B; for alkaline phosphatase, 95 per cent alcohol fixed ones by the Gomori's revised method by using sodium glycerophosphate as a substrate.

For endocrinological data, seven female rats were castrated at 10 months of age. On the 10th day following the operation, four of them were injected subcutaneously with 0.2 mg estradiol benzoate (Teikoku Hormone MFG. CO. LTD.) daily for three days (25). Three uninjected castrate rats were used as controls. Approximately 24 hours after the last injection, the animals were killed by a blow on the head, and their uterine tissues were treated in the same ways as mentioned above.

Results

A. Observations on the intact cyclic animals

1. General histology

In the uterus during diestrus, three types of epithelial cells are found; normal columnar cells, thinner ones and hypertrophied ones (Figs. 1 and 4). The epithelial cells which occupy a greater part of epithelium are columnar in shape, measuring 16–23μ×5–8μ, and their oval nucleus contains a spherical nucleolus. A large number of mitochondria, whose shapes are small granules, are scattered evenly throughout the cytoplasm (Fig. 2). The Golgi apparatus which takes the form of a network of black, irregular, wavy threads is definitely located above the nucleus (Fig. 3). Among the columnar cells just stated, there are a considerable number of epithelial cells which are much thinner than the others in the width, measuring 16–26μ×3–5μ. They are usually gathered together. Their cytoplasm is stained stronger than the others with Heidenhain's iron hematoxylin, safranin or eosin, and the elongated nucleus, stained also very dense with hematoxylin, contains a spheroidal nucleolus. In those cells are found a number of mitochondria and supranuclear Golgi apparatus in almost the same form as found in the normal columnar cells. The hypertrophied cells, measuring 18–25μ×11–13μ, usually appear alone or a few together among the columnar cells (Fig. 4). Their nucleus takes a position near the basement membrane. In the cytoplasm of these cells, various sizes of vacuoles are found, small ones appearing frequently in the perinuclear region and large ones anywhere and often containing degenerated lymphoid cells (Fig. 4). The mitochondria scatter among these vacuoles. The Golgi apparatus which is broken up into small particles or granules is distributed among the vacuoles at the supranuclear region.

In the uterus during proestrus, estrus and metestrus, the normal columnar cells, measuring 13–23μ×6–10μ, mainly compose the epithelium, with a few thinner cells and hypertrophied ones as seen during diestrus (Fig. 5). Some of the mitochondria in the columnar cells take the form of short rods, while

others remain as small granules, but the Golgi apparatus does not show a marked variation. Mitotic nuclei are frequently found among the epithelial cells (Fig. 6), whereas mitosis is rare during diestrus.

In the uterus during diestrus, the invasion of the lymphoid cells, measuring about 6.5–8.0μ, is seen in the epithelium adjacent to the basement membrane (Fig. 8). The invasion of neutrophils is rarely found, whereas never that of eosinophils. The invading lymphoid cells are spherical in shape and their nuclei are densely stained with hematoxylin. A small amount of mitochondria are distributed evenly throughout the cytoplasm. The Golgi apparatus, the shape of which is a collection of small spheres, occupies a portion directly near the nucleus. No glycogen or RNA are contained in these cells. Degenerating lymphoid cells are seen among and in the epithelial cells, especially they are abundant in the hypertrophied cells. The degenerating features of the lymphoid cells are as follows. The cytoplasm hypertrophies and reaches about 13μ in diameter. The nucleus shows piknosis and then disperses to form various sizes of globules, which are positive to Feulgen's stain (Fig. 9). PAS positive, eosinophilic small granules, considered as a product from the cytoplasm during the degenerating process, appear around these Feulgen positive globules (Figs. 4 and 7). Since the PAS reactive substance is not digested by saliva, it is not glycogen. During the degeneration the mitochondria and Golgi apparatus completely disappear. Though rare, masses of the Feulgen-reactive material are seen in the uterine lumen.

To investigate whether the lymphoid cells have a phagocytic faculty, vital staining of the endometrium was carried out on two intact cyclic rats by the method of intraperitoneal injections with 0.5 *ml* of 0.5 per cent trypan blue every day for a week. The results were as follows. Neither the lymphoid cells which invaded in the epithelium nor those present in the stroma store any trypan blue, though the macrophages and monocytes in the stroma store this dye abundantly in their cytoplasm. From the fact stated above, it is shown that the lymphoid cells in the endometrium have no phagocytic faculty.

Those invading lymphoid cells begin to appear, directly near the basement membrane, during metestrus, and increase in number during diestrus. They are found rarely during proestrus and estrus.

Uterine gland cells are columnar in shape, measuring 10–13μ. The spherical large nucleus possesses one nucleolus. A large number of mitochondria, which are larger than those in the epithelial cells, are distributed evenly throughout the cytoplasm. The supranuclear Golgi apparatus is found as a network of black, irregular threads resembling that in the epithelial cells (Fig. 3). No marked variations are found in the morphological features of gland cells through the estrous cycle. During diestrus and metestrus, no mitotic nuclei are found in the gland cells, whereas such nuclei are frequently

found during proestrus and estrus. A similar relation is also observed in the epithelial cells, as has been mentioned already.

The uterine stroma contains a large amount of wandering lymphoid cells, a small to a large amount of eosinophils, and a moderate amount of pigment cells and of neutrophils, but no mast cells.

The myometrium contains a large amount of mast cells, and a small to a large amount of pigment cells.

No fluctuations in number of these cells are found through the estrous cycle.

2. Glycogen

Fine granules of glycogen are found in the epithelial cells and gland cells, especially abundantly in the superficial portion of these cells (Figs. 10 and 11), and the coarse granules are found in the muscularis through all the stages of the estrous cycle (Figs. 12 and 13). The amount of the glycogen during the estrous cycle is given in Table 1.

Table 1. The amount of glycogen during the estrous cycle.

Tissues	Stages of the estrous cycle			
	Diestrus	Proestrus	Estrus	Metestrus
Epithelium	+	++	+ to ++	+
Gland	+	++	+	+
Stroma	−	−	−	−
Circular muscle	+ to ++	++	+ to ++	+ to ++
Longitudinal muscle	++	+++	++	++

As shown in Table 1, glycogen appears abundantly during proestrus, and in a moderate amount during estrus, and it reaches the minimal at metestrus and diestrus. Much less glycogen is consistently observed in the epithelia and glands than in the muscularis. In most cases, glycogen appears uniformly distributed in the epithelia and glands, whereas its distribution shows a marked difference in the muscularis; it is more concentrated in the longitudinal muscle than in the circular muscle. Frequently, glycogen is contained also in the endothelium of the blood capirallies at any stage of the estrous cycle.

3. RNA

The amount of RNA in the uterus through the stages of the estrous cycle is given in Table 2.

As shown in Table 2, a large amount of RNA is evenly spread throughout the cytoplasm of the epithelial cells and gland cells and a small amount of it is found in the cells of stroma and muscularis (Fig. 14). No fluctuation in the amount of RNA is found throughout the estrous cycle.

Table 2. The amount of RNA during the estrous cycle.

Tissues	Stage of the estrous cycle			
	Diestrus	Proestrus	Estrus	Metestrus
Epithelium	++ to +++	+++	+++	+++
Gland	++ to +++	+++	+++	++ to +++
Stroma	+	+	+	+
Circular muscle	+	+	+	+
Longitudinal muscle	+	+	+	+

4. Fat

The amount of fat in the uterus is given in Table 3.

Table 3. The amount of fat during the estrous cycle.

Tissues	Stages of the estrous cycle			
	Diestrus	Proestrus	Estrus	Metestrus
Epithelium	+++ to +++	+++ to +++	+++	+++
Gland	+ to ++	+ to ++	+ to ++	+ to ++
Stroma	−	−	−	−
Circular muscle	+	+	+	+
Longitudinal muscle	+ to ++	+	+	+

As shown in Table 3, a large amount of fat, the shape of which is coarse globules, appears in the supra- and infra-nuclear portions of the epithelial cells, a moderate amount of it, the shape of which is fine globules, in the supra-nuclear portion of the gland cells, and a small amount of it in coarse globules in the cells of muscularis (Fig. 15). No fluctuation in the amount of fat in these tissues is found throughout the estrous cycle.

5. Alkaline phosphatase

An intense alkaline phosphatase reaction is found in the free surface of

Table 4. The intensity of alkaline phosphatase reaction during the estrous cycle.

Tissues	Stages of the estrous cycle			
	Diestrus	Proestrus	Estrus	Metestrus
Epithelium	+	++	+	+
Gland	+	++	+	+
Stroma	−	−	−	−
Circular muscle	−	−	−	−
Longitudinal muscle	−	−	−	−
Endothelium	+++	+++	+++	+++

the epithelial cells and gland cells and also in the endothelial cells of blood capirallies of both the endometrium and myometrium (Figs. 16 and 17). The intensity of a lkaline phosphatase reaction in various cells is given in Table 4.

As shown in Table 4, in the epithelium and glands the alkaline phosphatase reaction appears more intensely during proestrus than during the other stages of the estrous cycle. In the endothelium, however, the alkaline phosphatase reaction is constant throughout the stages of the estrous cycle.

6. Acid polysaccharides

Acid polysaccharides, which should be demonstrated by metachromasia of thionin, have been proved to be absent either in the endometrium or myometrium throughout the estrous cycle.

B. Observation on the castrates and the hormone-treated ones

1. General histology

The height of uterine epithelial cells of castrates is lower than that of the intact cyclic animals, measuring 8–16μ, but the width of the cells is almost the same, measuring 5–8μ (Fig. 18). The thinner cells and the hypertrophied cells as seen in the epithelium during diestrus of intact rats are not found in the castrates. A large number of mitochondria in the form of small granules scatter evenly throughout the cytoplasm, resembling that of the intact animals during diestrus. The Golgi apparatus above the nucleus has transformed from the network of wavy threads which has been already described in the non-castrate uteri into a collection of irregular small rodlets (Fig. 20). No mitotic nuclei are found, which feature is similar to that of a uterus during diestrus. A small number of invading lymphoid cells are found adjacent to the basement membrane. In the uterus of a certain castrate, a large number of lymphoid cells and of glycogen-bearing neutrophils are found among the epithelial cells. In the epithelium of another castrate, vacuolated cells which contain a yellow pigment are frequently found among the normal epithelial cells.

In the hormone-treated castrates, the height of the uterine epithelial cells is extremely larger than that of the castrates, measuring 26–40μ (Fig. 19). Further, it exceeds that of the intact cyclic animals. However, the width of epithelial cells does not differ between the castrates and hormone-treated castrates or intact cyclic animals, measuring 5–8μ. The nuclei of epithelial cells of hormone-treated castrates, containing a prominent spherical nucleolus, occupy a basal portion of the cytoplasm. Mitotic nucleus, which is abundantly found in the estrus, is not found in these cells. There are a large number of mitochondria in the form of rods and granules. The Golgi apparatus appears above the nucleus taking the form of a network of long and fine, irregular wavy threads, resembling that during estrus. No invasion of

lymphoid cells is seen in the epithelium. Secreting droplets that are not stained with eosin, thionin nor by the PAS method appear abundantly in the uterine lumen adjacent to the free surface of the epithelial cells.

Uterine gland cells of the castrated rats are almost the same in size and in shape as those of the intact cyclic ones (Fig. 18). As seen in the gland cells of intact animals during diestrus, no mitotic nucleus is found. A large number of mitochondria whose shape is small granules scatter evenly throughout the cytoplasm, resembling that of intact cyclic animals during diestrus. The Golgi apparatus takes the form of a collection of irregular rodlets just as seen in the epithelial cells (Fig. 20). The rat which had a yellow pigment in its epithelium also had it in its gland cells. And the rats which had invading lymphoid cells and glycogen-bearing neutrophils also had both of those in the gland cells.

In the hormone-treated castrates, the height of gland cells is slightly higher than that of the castrates, measuring 13–15μ, but the width of them does not differ between the castrates and hormone-treated castrates or intact cyclic animals, measuring 6–8μ (Fig. 19). Different from the feature of gland cells in estrus, no mitotic nucleus is found. A large number of mitochondria in the form of rods or granules appear in the gland cells, too. The Golgi apparatus takes the form of a network of irregular, wavy threads (Fig. 21) similar to that during estrus. Also similar to the feature during estrus, no invasion of lymyhoid cells is seen in the gland cells.

No differences in the number of wandering cells appearing in the stroma and in the muscularis are found among the intact cyclic animals, castrates and hormone-treated castrates.

2. Glycogen

In the uterus of castrates, coarse granules of glycogen are found in the epithelial cells and gland cells, mainly located in the supranuclear portion (Fig. 22), while in the uterus of hormone-treated castrates, fine granules of glycogen are found throughout the cytoplasm of the epithelial cells and gland

Table 5. The amount of glycogen in the uteri of castrates and hormone-treated castrates.

Tissues	Animals	
	Castrates	Hormone-treated castrates
Epithelium	+	++ to +++
Gland	+	++
Stroma	−	−
Circular muscle	+ to ++	+ to ++
Longitudinal muscle	+ to ++	+++

cells (Fig. 23). Coarse granules appear in the muscularis of both the castrates and hormone-treated castrates. The amount of glycogen in the uteri of castrates and of hormone-treated castrates is given in Table 5.

As shown in Table 5, the amount of glycogen in the epithelial cells and gland cells is more abundant in the hormone-treated castrates than in the non-treated castrates. The amount in the longitudinal muscle cells is also more in the hormone-treated castrates than in the non-treated ones, while the amount in the circular muscle cells does not vary between the former and the latter.

3. RNA

The granules of RNA are found abundantly in the cells of epithelium and glands spread throughout the cytoplasm, but scarcely found in the cells of stroma and of muscularis (Figs. 24 and 25). The amount of RNA in the uteri of the castrates and hormone-treated castrates is given in Table 6.

Table 6. The amount of RNA in the uteri of castrates and hormone-treated castrates.

Tissues	Animals	
	Castrates	Hormone-treated castrates
Epithelium	⧺	⧺
Gland	⧺	⧺ to ⧺
Stroma	+	+
Circular muscle	+	+
Longitudinal muscle	+	+

As shown in Table 6, the amount of RNA in the epithelial cells and gland cells is more abundant in the hormone-treated castrates than in the non-treated castrates. No difference in the amount is found in the cells of stroma and of muscularis.

4. Fat

A large amount of fat, the shape of which is coarse globules, appear in

Table 7. The amount of fat in the uteri of castrates and hormone-treated castrates.

Tissues	Animals	
	Castrates	Hormone-treated castrates
Epithelium	⧺	⧺ to ⧺
Gland	+	+ to ⧺
Stroma	−	−
Circular muscle	+	+
Longitudinal muscle	+ to ⧺	+ to ⧺

the epithelial cells, while a small to a moderate amount of it in the cells of gland and of muscularis (Fig. 26). The amount of fat in the uteri of castrates and hormone-treated castrates is given in Table 7.

As shown in Table 7, no marked difference is found in the amount of fat in these tissues between the castrates and the hormore-treated castrates.

5. Alkaline phosphatase

The alkaline phosphatase reaction appears in the free surface of the epithelial cells and of gland cells, and also in the endothelial cells of blood capirallies (Figs. 27 and 28). The amount of alkaline phosphatase in the uteri of the castrates and hormone-treated castrates is given in Table 8.

Table 8. The amount of alkaline phosphatase in the uteri of castrates and hormone-treated castrates.

Tissues	Animals	
	Castrates	Hormone-treated castrates
Epithelium	+	++
Gland	+	++
Stroma	−	−
Circular muscle	−	−
Longitudinal muscle	−	−
Endothelium	+++	+++

As shown in Table 8, the alkaline phosphatase reaction in the epithelial cells and gland cells is more intense in the hormone-treated castrates than in the non-treated castrates. No difference in the intensity is found in the endothelial cells of blood capirallies between the castrates and the hormone-treated castrates.

6. Acid polysaccharides

No acid polysaccharides are found in the uterine tissues of either the castrates or the hormone-treated castrates.

Discussion

1. The fluctuations of glycogen, alkaline phosphatase, RNA and fat in the uterus of the intact cyclic animals.

The fluctuation in the amount of endometrial glycogen of humans has been described by Arzac and Blanchet (1) and Senarcleus (24) using the histochemical method, by Zondeck and Stein (31) using chemical analysis. In these investigations, it was ascertained that the amount of glycogen in the epithelium and the superficial portion of glands increases during the progestational phase and declines during the estrogenic phase. Moss *et al.* (18) who used

bovine uteri reported that their glycogen distribution shows a prominent cyclic variation in the surface epithelium ; the largest amount of glycogen was seen for several days at the begining and at the end of the cycle, but no glycogen during the mid-cycle. Takamine *et al.* (27) also reported on the appearance of glycogen in the epithelium of bovine uterus during the estrus. Breipohl and Schmitz (11) studied the appearance of glycogen in the uterus of pigs, cows and horses, and recorded only incomplete and casual description of the fluctuation in its amount according to the stages of the sexual cycle. Bo and Atkinson (8) stated that, contrary to the condition prevalent in the hunam uterus, no glycogen is deposited in the endometrium of the rat, and its deposition is limited to the muscle cells in the myometrium. Using rats, Rosenbaum and Goolsby (23) stated that glycogen is found in the smooth muscle of the myometrium through all the stages of the estrous cycle, and the amount is abundant at proestrus, moderate at metestrus and diestrus, and small at estrus.

In the present investigation, fine granules of glycogen are found in the epithelium and glands, and coarse granules in the muscularis throughout the estrous cycle. This disagrees with the report of Bo and Atkinson and of Rosenbaum and Goolsby in rats. The result on the amount of glycogen in the rat uterine epithelium and glands coincides rather well with the report of Moss *et al.* in cows, and of Takamine *et al.* in cows, in the point of view that glycogen appears abundantly during estrus. On the contrary, the result of this investigation disagrees with that of several workers in human uteri; they stated that the glycogen in the epithelium and glands increases during the progestational phase and declines during the estrogenic phase. These histochemical results on the amount of glycogen are roughly endorsed by the results obtained through chemical analyses of Boettiger (9), Kostyo (16), Van Dyke and Chen (28), and Walaas (29).

Arzac and Blanchet (1) studied the alkaline phosphatase in human endometrium, and reported that the amount of enzyme increases during the estrogenic phase and declines during the progestational phase. Moss *et al.* (18), studying the alkaline phosphatase in bovine uteri during estrous cycle, reported that a distinct cyclic variation of alkaline phosphatase activity is present, though only at the surface epithelium ; the highest activity is seen during the mid-cycle, while little or no activity for several days at the begining and at the end of the cycle, whereas the opposite happens for glycogen.

In the present investigation, the alkaline phosphatase reaction appeared more intensely in the free surface of epithelial cells and gland cells during proestrus than other stages of the estrous cycle, agreeing with the fluctuation of glycogen.

Atkinson *et al.* (5) histochemically demonstrated a large amount of RNA

in the epithelium and glands of human endometrium. Herrmann *et al.* (14) chemically carried out the determination of the amount of RNA per cell, showing no significant fluctuation throughout the menstrual cycle.

In the present investigation, also, a large amount of RNA was found spreading evenly throughout the cytoplasm of epithelial cells and gland cells without fluctuation, nearly coinciding with that of Herrmann.

Takamine *et al.* (27) studied the distribution of acid polysaccharides in the female genital tract of cows, and stated that, in spite of the presence of acid polysaccharides in the uterine lumen, no acid polysaccharides were found in the epithelial cells. Zachariae (30) reported that acid polysaccharides appear in the glandular cells and lumina of human uterus for three days after ovulation.

In the present investigation, no acid polysaccharides were found in rats' uterine tissues at any stage of the estrous cycle.

Reese (22), using rats, stated that when the uterine epithelium was full of fat globules, the amount of fat in the gland cells was very little, and also that there was no evidence of cyclic change in fat distribution in the glands. On the contrary, Bourg (10) noted an almost complete disappearance of fat granules from the cells of the uterine epithelium of the mouse during estrus, and its reappearance in metestrus in the form of fine granules, principally in the base of the cells. Van Dyke and Chen (28) also reported that in normal female monkeys the total amount of fat in the uterine mucosa is the highest during the secretory stage.

In the present investigation was found a large amount of fat in the epithelium, a moderate amount in the glands, and a small amount in the muscularis. No marked fluctuation was found according to the stages of estrous cycle, agreeing with that of Reese, but disagreeing with that of Bourg.

2. The effect of estrogen on the appearance of glycogen, alkaline phosphatase, RNA and fat in the uteri of castrates.

The histochemical changes of uterus caused by the experimental control of the ovarian hormones have been reported by the following investigators. Ortiz (21) studied the effect of castration on the reproductive system of the golden hamster, and reported that abnormal glands showing very limited growth in the length and an extremely wide lumen are found in the uterus of all the castrates. Bell *et al.* (7), studying the effect of estrogen and progesterone on the genital tract of the ewes, reported that estradiol caused an increase in the height of the epithelium of the uterine glands and of uterine surface of spayed ewes.

In the present investigation, the height of uterine epithelium became smaller by castration, but abnormal glands as described by Ortiz were not

found, while the height of uterine epithelum became heigher by estrogen injection, agreeing with the report of Bell *et al.*

Hisaw and Grey (15) found that in the castrated monkeys suficiently large amounts of estrogen may bring about the deposition of glycogen in the uterine glands. Rosenbaum and Goolsby (23) stated that the estrogen is able to cause glycogen deposition in the myometrium of the castrated rat uterus, but the effect of estrogen on glycogen deposition in the endometrium is not so marked as in the muscularis ; in the estrogen-treated animals uterine growth increases, while glycogen deposition does not occur there. Müller (19) also reported that castrated rats which had been treated with folliculin alone never showed glycogen. From these observations, it may be considered that glycogen deposition, in the case of rats, is not under physiological control of the ovarian hormones.

In the present investigation, however, the injection of estrogen was responsible for a heavy accumulation of glycogen in the uterine epithelium and glands as well as the longitudinal muscles of castrated rats, disagreeing with the report of Rosenbaum and Goolsby and of Müller in rats, but agreeing with the report of Hisaw and Greys in monkeys. This result clearly shows the fact that the glycogen deposition in the rat uterus is under the control of the ovarian hormones.

More recently, an increase of uterine alkaline phosphatase by estrogen treatment has been proved by Atkinson and Engle (4) in mice, Leathem *et al.* (17) in cats and rabbits, Atkinson and Elftman (3) in mice and Fuenzalida (12) in guinea pigs.

In this investigation, the injection of estrogen resulted the increase of alkaline phosphatase activity in the uterine epithelium and glands, agreeing with the reports described above.

Bourg (10) in rats and Gilbert (13) in rabbits experimentally carried out the distribution of fat by the estrogen injection, and stated that only the estrogen in large quantity causes the fat to disappear from the surface epithelium and from the stroma cells.

In the present investigation, no marked difference in the amount of fat in the uterus was found between the castrates and hormone-treated castrates, disagreeing with the reports of Bourg and of Gilbert.

From the results obtained in this investigation, it is clearly confirmed that the amount of glycogen and the alkaline phosphatase activity in the epithelium and glands fluctuates according to the stages of the estrous cycle. By the estrogen injection the similar relation is also observed. This shows that glycogen and alkaline phosphatase are under the physiological control of ovarian hormone. The amount of RNA and of fat showed no marked fluctuations.

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Summary

The results obtained in this investigation are summarized as follows.

1. In the intact rats during diestrus, the uterine epithelium is composed of columnar cells, thinner ones and hypertrophied ones, but in other stages of the estrous cycle it is composed of columnar cells alone. No marked variation is found in the size of epithelial cells, measuring 16–23$\mu \times$5–8μ during diestrus, and 13–23$\mu \times$6–10μ in other stages. In the hormone-treated castrates, the uterine epithelial cells are extremely higher than those of the castrates, measuring 26–40μ; they further exceed those of intact cyclic animals. However, the width of epithelial cells does not differ among the intact cyclic animals, castrates and hormone-treated castrates, measuring 5–8μ. The gland cells show features similar to those of epithelial cells. Invading lymphoid cells are seen in the epithelium during diestrus.

2. Glycogen appears in the uterine epithelium and glands, abundantly during proestrus, moderately during estrus and scantly during metestrus and diestrus. Only a little glycogen is found in the epithelium and glands of castrates, but it extremely increases in those of hormone-treated castrates. Glycogen appears abundantly in the muscularis. Fluctuation in its amount in the longitudinal muscle nearly coincides with that in the epithelium and glands, while no difference is found in the circular muscle among the intact cyclic animals, castrates and hormone-treated castrates.

3. A large amount of RNA appears in the epithelium and glands, and a small amount in the stroma and muscularis, showing no fluctuation during the estrous cycle. The amount of RNA in the epithelium and glands is more abundant in the hormone-treated castrates than in the non-treated ones. No difference in the amount is found in the stroma and muscularis.

4. A large amount of fat appears in the epithelium, a moderate amount in glands, and a small amount in muscularis, showing no fluctuation among the intact cyclic animals, castrates and hormone-treated castrates.

5. Alkaline phosphatase reaction is found in the free surface of the epithelium and glands, and in the blood capirallies. The fluctuation of its amount in the epithelium and glands of the intact cyclic animals, castrates and hormone-treated castrates nearly coincides with that of glycogen, but the intensity of the alkaline phosphatase reaction in the blood capirallies is always constant.

6. No acid polysaccharides are found in the uterine tissues of the intact cyclic animals, nor castrates nor hormone-treated castrates.

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Plate 1

Explanation of Figures

Fig. 1. Endometrium of diestrus. Hematoxylin-eosin stain. ×400.
The epithelium is composed of normal columnar cells and thinner cells.

Fig. 2. Uterine epithelium of diestrus. Heidenhain's iron-hematoxylin stain for mitochondria. ×600.
A large number of mitochondria in the form of small granules are scattered evenly throughout the cytoplasm.

Fig. 3. Endometrium of diestrus. Aoyama's method for Golgi apparatus. ×400.
The Golgi apparatus in the form of a network of black, irregular, wavy threads is definitely located above the nucleus in the cells of epithelium and glands.

Fig. 4. Endometrium of diestrus. PAS stain after Champy fixation. ×400.
Various sizes of vacuoles are found in the cytoplasm of epithelial cells, and some of them contain piknotic nuclear globules.

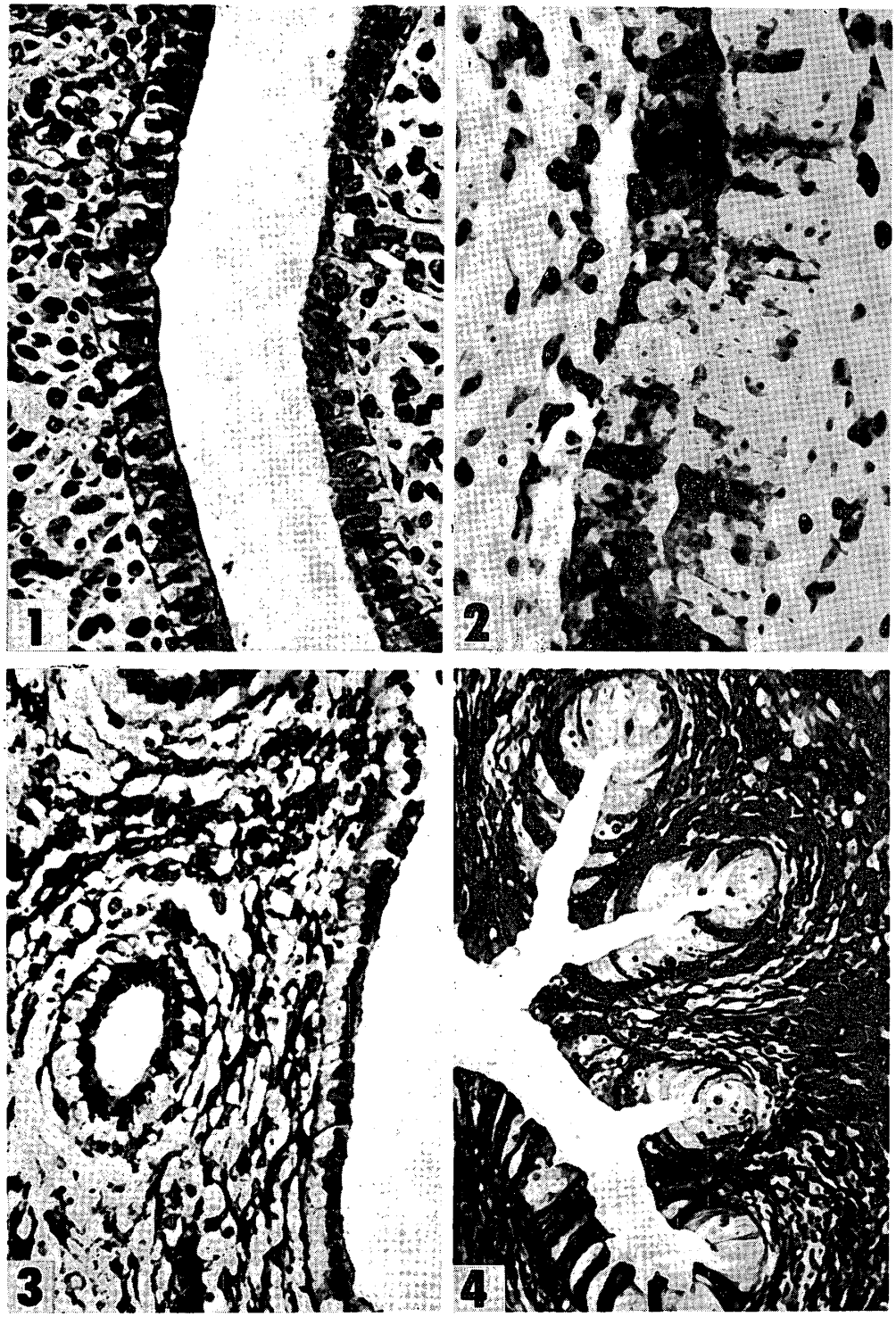
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Plate 2

Explanation of Figures

Fig. 5. Endometrium of Proestrus. Hematoxylin-eosin stain. ×400.
The epithelium is mainly composed of normal columnar cells.

Fig. 6. Uterine epithelium of proestrus. Hematoxylin-eosin stain. ×600.
A mitotic nucleus is seen in the epithelium.

Fig. 7. Uterine epithelium of diestrus. Hematoxylin-eosin stain. ×400.
A degenerating lymphoid cell contains a piknotic nucleus and eosinophilic granules.

Fig. 8. Uterine epithelium of diestrus. Hematoxylin-eosin stain. ×400.
An invading lymphoid cell is seen in the epithelium adjacent to the basement membrane.

Fig. 9. Uterine epithelium of diestrus. Feulgen's stain for DNA. ×600.
A piknotic nuclear globule resulted from the degenerating lymphoid cells is seen in the epithelium.


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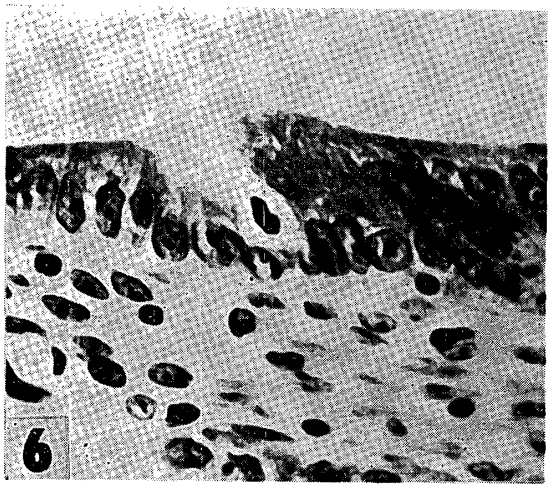
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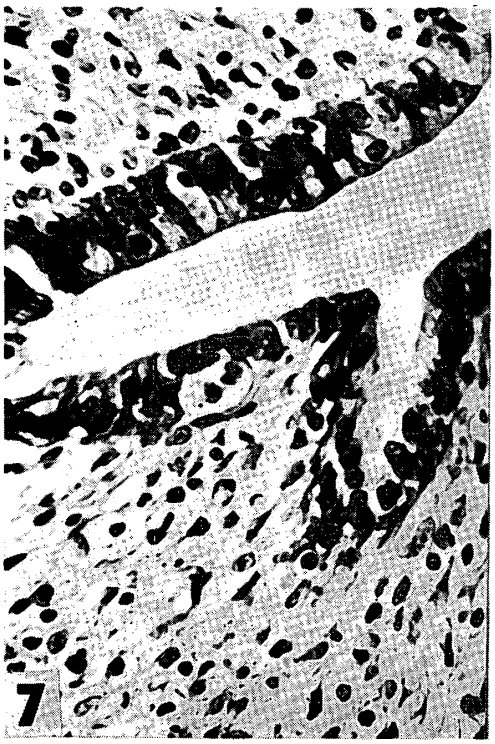
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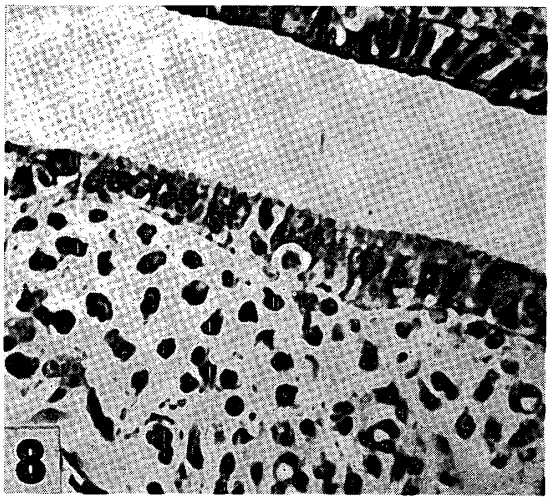
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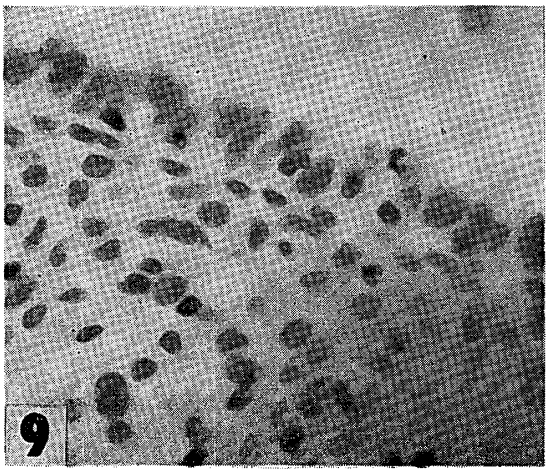
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Plate 3

Explanation of Figures

Fig. 10. Endometrium of diestrus. PAS stain. ×200.
A small amount of glycogen is seen in the epithelium and gland

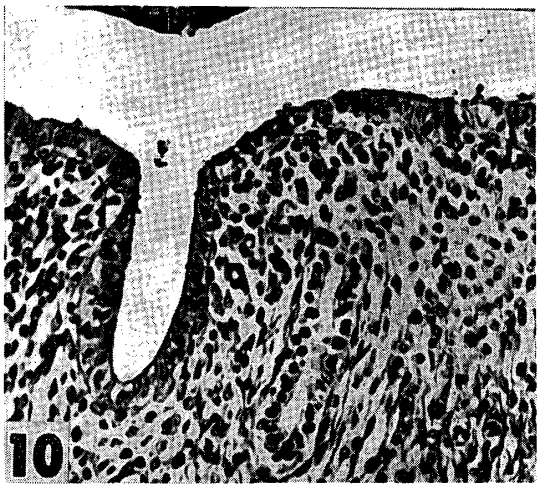
Fig. 11. Endometrium of proestrus. PAS stain. ×200.
A large amount of glycogen is seen in the epithelium and glands.

Fig. 12. Myometrium of diestrus. PAS stain. ×100.
A moderate amount of glycogen appears in the circular and longitudinal muscles.

Fig. 13. Myometrium of proestrus. PAS stain. ×100.
A large amount of glycogen appears in the longitudinal muscle, and a moderate amount in the circular muscle.

Fig. 14. Endometrium of proestrus. Thionin stain. ×200.
A large amount of RNA appears in the epithelium and glands.

Fig. 15. Endometrium of proestrus. Sudan black B stain. ×200.
A large amount of fat appears in the epithelium, and a moderate amount in the gland.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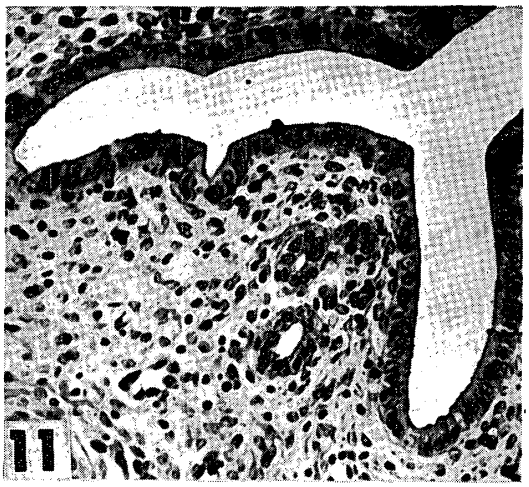
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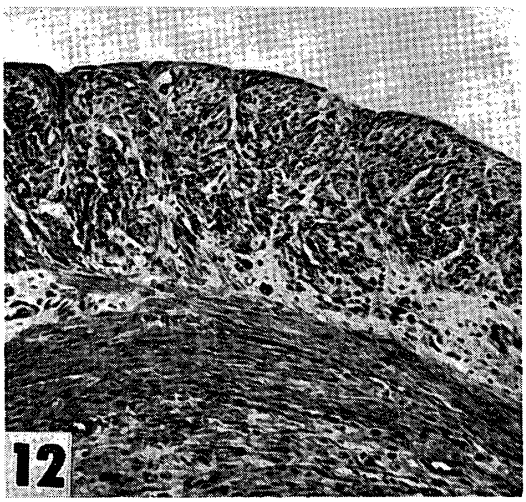
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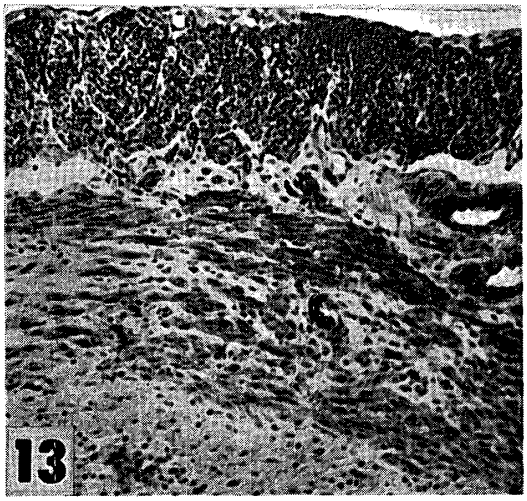
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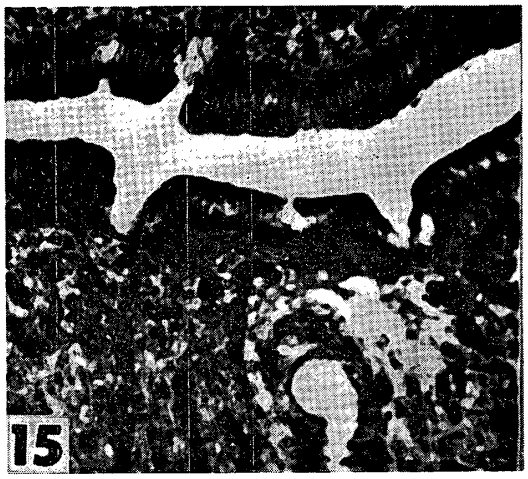
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Plate 4

Explanation of Figures

Fig. 16. Endometrium of diestrus. Gomori's revised method. ×200.
A weak alkaline phosphatase reaction is found in the free surface of epithelial cells, and an intense reaction in the endothelial cells of blood capirallies.

Fig. 17. Endometrium of proestrus. Gomori's revised method. ×200.
An intense alkaline phosphatase reaction is found in the free surface of epithelial cells and in the endothelial cells of blood capirallies.

Fig. 18. Endometrium of a castrate. Hematoxylin-eosin stain. ×400.
Epithelial cells which are small in the height are seen.

Fig. 19. Endometrium of a hormone-treated castrate. Hematoxylin-eosin stain. ×400.
Epithelial cells which are extremely large in the height are seen.

Fig. 20. Endometrium of a castrate. Aoyama's method for Golgi apparatus. ×400.
The Golgi apparatus which takes the form of a collection of irregular small rodlets is seen in the cells of epithelium and gland.

Fig. 21. Endometrium of a hormone-treated castrate. Aoyama's method for Golgi apparatus. ×400.
The Golgi apparatus which takes the form of a network of irregular threads appears above the nucleus of gland cells.


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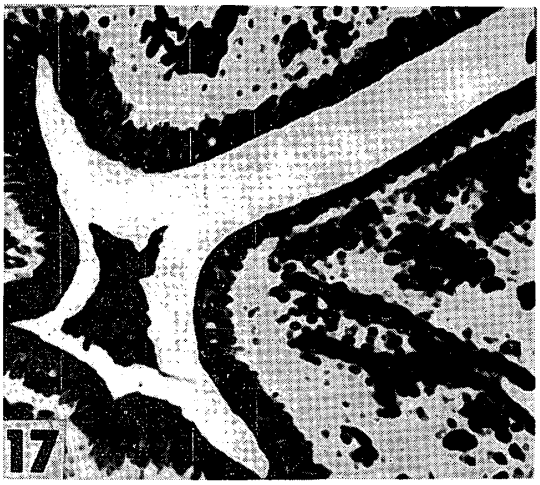
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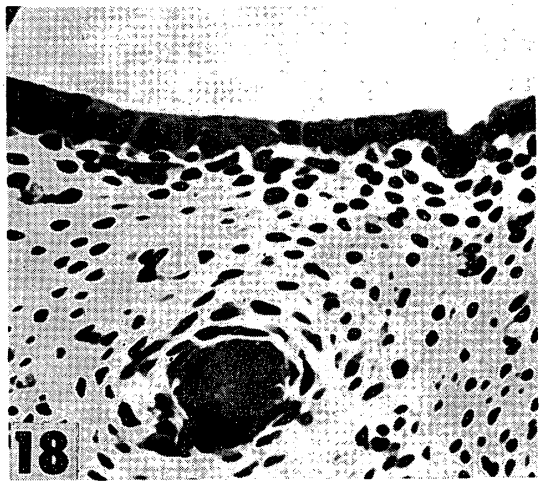
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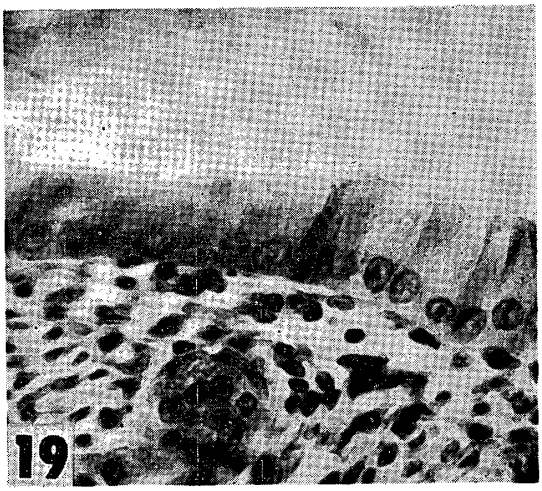
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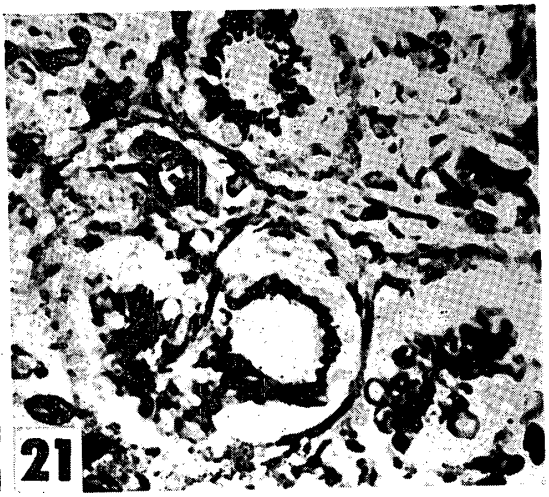
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Plate 5

Explanation of Figures

Fig. 22. Endometrium of a castrate. PAS stain. ×200.
A small amount of glycogen is seen in the epithelium and glands.

Fig. 23. Endometrium of a hormone-treated castrate. PAS stain. ×200.
A large amount of glycogen is seen in the epithelium and glands.

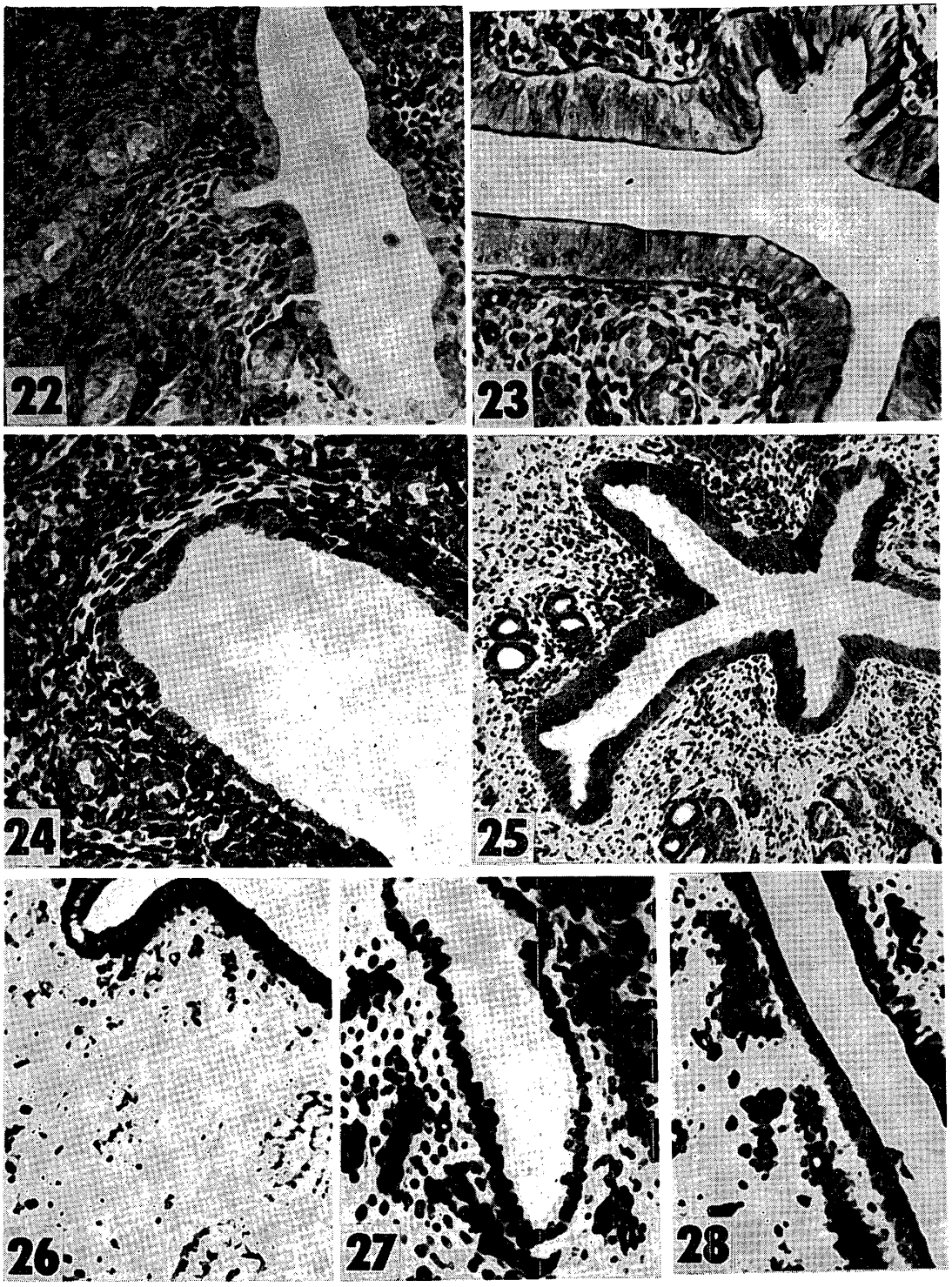
Fig. 24. Endometrium of a castrate. Thionin stain. ×200.
A moderate amount of RNA is found in the epithelium and glands.

Fig. 25. Endometrium of a hormone-treated castrate. Thionin stain. ×200.
A large amount of RNA appears in the epithelium and glands.

Fig. 26. Endometrium of a castrate. Sudan black B. ×200.
A large amount of fat appears in the epithelium, while a small amount of it in the glands.

Fig. 27. Endometrium of a castrate. Gomori's revised method. ×200.
A weak reaction of alkaline phosphatase is found in the free surface of the epithelium, and an intense reaction in the blood capirallies.

Fig. 28. Endometrium of a hormone-treated castrate. Gomori's revised method. ×200.
An intense reaction of alkaline phosphatase is found in the free surface of the epithelium and in the blood capirallies.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