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A Histological and Histochemical Study Of the Bovine Oviducts, Uterus And Placenta

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ABSTRACT

An attempt has been made to provide a more complete basis from which to interpret reproductive phenomena in the cow. Oviducts, uteri, and placentas removed at slaughter from reproductively normal cows in known stages of the estrous cycle and gestation were studied histologically. In addition, the tissues were examined histochemically for type of connective tissue, alkaline phosphatase, lipid, and glycogen.

The oviducts are active during the follicular phase of the cycle, as evidenced by the high level of alkaline phosphatase activity and the concentrated cytoplasmic basophilia. Indications of regression are seen during the luteal phase. The mucosal epithelium shows increased pseudostratification, goblet-like cells, and extruding nuclei during early diestrus. Glycogen and small amounts of lipid accumulate during the luteal phase. The oviducts seen during gestation resemble the oviducts of diestrus.

Changes which occur in the uterus during the estrous cycle are not marked. Progestational proliferation and glandular activity continue throughout diestrus as shown by intense alkaline phosphatase activity in the epithelium at this time. Very little epithelial lipid or glycogen is seen during the luteal phase. The uterine surface shows localized desquamation during estrus or metestrus, and blood becomes trapped in the stratum compactum during this regressive stage. Should conception occur, the entire uterus continues to hypertrophy. The uterine glands continue secretion, with some decrease in activity in late gestation.

The uterine part of the placentome is an outgrowth from the stratum compactum of the caruncle. It forms crypts which surround the villi of the fetal cotyledon. A low syncytium of undetermined origin covers the septa of the caruncular crypts everywhere except on their distal ends. This syncytium contains a lipid, and it forms a complete alkaline phosphatase barrier between maternal and fetal bloods.

The trophoblast of the villi is an irregular syncytium in which are characteristic giant cells. The giant cells react heavily for alkaline phosphatase and glycoprotein. The trophoblast between the bases of the villi is predominantly irregular, tall columnar cells. These cells absorb histotroph from the

space which separates them from the distal ends of the caruncular septa. Red blood corpuscles absorbed by the columnar trophoblast give rise to yellow-brown, lipoprotein pigment deposits.

The columnar trophoblast of the intercotyledonary chorion resembles that seen in the placentome; however, acetone-soluble lipid granules were definitely identified here.

Two postpartum uteri were observed. At eight days postpartum, the uterus contained an amorphous mass of degenerating placentomes. Regression was not completed in the 25-day postpartum uterus.

INTRODUCTION

Certainly one of the most perfectly adapted environments of any organism is that of the developing mammal *in utero*. Even before the fragile, single-cell, fertilized egg has begun to cleave, preparations have been made for its reception in the uterus. Escape from the environment has been prevented by a tight sealing of the exit (*os uteri*). The environmental temperature is closely regulated. Since the eggs of placental mammals contain little stored energy, the uterine glands have begun to secrete their nourishing fluid. The endometrium has been conditioned to permit an intimate attachment of the extra-embryonic membranes; following which, it and its profuse vascular system will serve as a provider of nutrients and a remover of wastes. The quiescence of the myometrium aids in protecting the delicate blastocyst. All uterine tissues are prepared to undergo a phenomenal growth and hypertrophy to provide adequate space for the rapidly growing conceptus and its fluids. Such a complexity of biological adaptations require acute coordination, the function of the reproductive endocrines, the pituitary, ovary, and later the placenta.

It is fitting that so vital a link in the preservation of placental mammals as the uterus and its functioning should receive thorough investigation. It is also imperative for a more complete knowledge of the biological mechanisms involved in an adequate intrauterine life that all resources and techniques of the various researches be utilized. Primary among these investigations is a detailed histological study. Morphogenesis of the reproductively normal tract should be related accurately to the various physiological stages of the estrous cycle. The early microscopic reactions of the gravid uterus to the blastocyst, the beginning of placenta formation and the rapid expansion of the placenta to meet the growing needs of the embryo and fetus, the slow aging of the placenta with advancing gestation, preparturient changes in the placenta—all need to be described. To gain further morphological evidence into the functioning of the uterus and placenta, the techniques of histochemistry are of value. Localization of the metabolities, hormones, and enzymes of reproduction, estimates of their quantitative variations, and interpretation of their interrelationships in maintaining the environment of the conceptus will materially aid in the understanding of the reproductive process. Logically such investigations need to be applied first to females which are normal by known criteria,

such as history of normal estrous cycles and reproduction, freedom from genital diseases, and macroscopically normal genitalia. The animal should be of normal physical appearance and have been fed adequately on a complete diet. Furthermore, the stage of the estrous cycle or of gestation needs to be accurately known so that microscopic observations may aid in the interpretation of other concurrent biological changes. Certainly, without this accurate depiction none but the more exaggerated morphological changes, be they experimentally or pathologically induced, can be recognized.

Comparative morphogenesis of placentas has long been of interest to the zoologist and important evolutionary relationships among the orders of *Placentalia* have been observed. Gross species differences in the structure of the uterus and in the nature of the reproductive cycle are well recognized. Even among ruminants important differences in reproductive processes necessitate the investigation of each species.

Because of the importance of the cow to our nutritional welfare, it appears unusual that cyclic and gestational microscopic changes in its reproductive tract are not thoroughly described in the literature. However, the high economic value of the cow which meets the above listed criteria of normality and the necessity of killing the animal for tissue sections may have limited studies. Artificial insemination of dairy cows has focused considerable attention upon the study of reproductive physiology of cattle, but most investigations have been made upon the male, especially upon semen, its dilution, storage, and insemination. Meanwhile, the cow, probably the main offender in lowered reproductive efficiency, has been relatively neglected. For example, during the years of 1947 through 1951 the Journal of Dairy Science published 61 original papers treating with reproduction in the bull and only 20 concerned with the cow. In the more recent years the trend has been changing. There apparently is an increasing awareness of the dual nature of reproductive problems. Nevertheless, it will take a continued, concerted effort in a variety of endeavors before there is real understanding. Without pretense of finality, this histological and histochemical study has been undertaken with the intent of making a contribution to this cause.

REVIEW OF LITERATURE

Some appreciation of mammalian female reproductive physiology undoubtedly antedates any organized investigation, for Assheton (1906) reports the practice of infant ovariectomy in certain semi-barbaric tribes in Asia. However, Knauer (1900), who began his ovarian extirpation and replacement experiments with rabbits in 1895, was the first to conclusively demonstrate an ovarian-uterine relationship. He observed castrate atrophy of the rabbit uterus and prevented it by successful ovarian transplants. Heape's definitions (1901) of the phases of the sexual season in mammals and his relating these to various periods in uterine morphogenesis stimulated investigation of many species.

Marshall and Jolly (1906) in a study of the estrous cycle of the dog concluded that the ovary was an organ of internal secretion. He injected ovarian extracts and blood serum from bitches in estrus into anestrous bitches and produced estrus in these animals. In a later study of the dog, Marshall and Halnan (1917) observed that numerous, developing ovarian follicles were associated with proestrous changes in the uterine glands. It was not until the work of Allen and Doisy, in 1923, however, that the secretory nature of the ovarian follicle was irrefutably established. Unlike previous investigators, they used ovariectomized test animals, injecting *liquoris folliculi* removed from sows' ovaries into ovariectomized mice and rats to produce full estrus and typical estrous conditions in the uterus and vagina. The active extract was a lipid material free from protein. Murphey, *et al.* (1925b) claimed the first successful production of the uterine congestion, edema, and vaginal secretions in a spayed cow by the injection of *liquoris folliculi* from oxen. They termed the substance an "oestrual" hormone.

Probably its extended persistence during the estrous cycle and during pregnancy led early to the establishment of the secretory nature of the corpus luteum. Fraenkel, in 1903, investigating the hypothesis of this teacher, Gustav Born, concerning the secretory nature of the corpus luteum concluded the following:

> "Das Corpus luteum bezitzt die Function, die befruchteten in der Tube bezw. im Uterus befindlichen Eier zur Insertion gelangen zu lassen. Der Wegfall der Corpora lutea verhindert das Zustandekommen der Graviditat."

If corpora lutea of rabbits were removed from the ovary or destroyed before implantation, pregnancy was terminated. It was also observed by Marshall and Jolly (1906) that following ovulation the corpus luteum is formed as an organ of internal secretion, and that this secretion provided a stimulus necessary for the nourishment of the embryo during early gestation. Loeb, in 1908, stated that the production of deciduomatas in the rabbit uterus required a "preparing substance" which was believed to be a secretion of the corpus luteum. Hammond (1917) observed that these deciduomatas were practically identical in structure to the maternal placenta, thus confirming Fraenkel's experiments. The corpora lutea were also found to be necessary for uterine gland hypertrophy and endometrial hyperemia. Cyclic changes in the uterus were related to the growth and regression of the corpus luteum (Hammond, 1927) and these changes were likened to pregnancy on a small scale. The more apparent pregnancy changes are due to the continued action of the corpus luteum. It was intimated by Beaver (1922) that the active secretion of the corpus luteum inhibits the ovarian function controlling estrus and ovulation, for removal of the pathologically retained corpus luteum in cows caused estrus to appear. It was not until 1929 that the secretory nature of the corpus luteum was definitely established, by Corner and Allen. Lipid extracts of corpora lutea from pregnant sows caused progestational proliferation in the rabbit endometrium.

The injection of an extract of follicular fluid caused no decidual reaction. Therefore, it was demonstrated that the corpus luteum secretes a progestational hormone and this hormone differs from estrogen. Following the establishment of the follicular and luteal secretory functions of the ovary, extirpation and replacement techniques were used to extend interpretations of uterine physiology.

The fimbriated, anterior end of the oviduct is active during proestrus and estrus (Reynolds, 1949). The smooth muscle found in the suspensory ligament of the ovary is so attached to the peritoneal wall, ovary, upper uterus, and ampulla of the tube that its action is capable of approximating the fimbria and surface of the ovary. During the period of greatest tubal activity the ovum travels rapidly through the ampulla, but it traverses the isthmus of the tube when activity is least. The alteration in tubal activity which comes about at this time coincides with the formation of the corpus luteum. Injection of cows in the luteal phase with pregnant mare's serum, which has both follicle stimulating and luteotrophic activity (Cole, 1946), followed by expression of the corpus luteum causes not only multiple ovulations but also accelerated passage of the ova down the oviduct (Dowling, 1949). These ova are of low viability. If the oviduct is over-stimulated by estrogenic substances while the ova are traversing it, the ova may become tube-locked (Whitney and Burdick, 1936).

One of the first uterine indications of follicular activity is an increase in uterine weight due to water edema (Astwood, 1939; Boettiger, 1946; Carroll, 1945). Treatment of castrate rats with estradiol dipropionate causes a rapid influx of water specifically in the uterus, with no qualitative difference in the edema of the myometrium or endometrium (Carroll, 1945). This edema, which is both intra- and extra-cellular, precedes mitotic activity by several hours and it appears to be favorable for growth. Estrogenic stimulation of ovariectomized animals also causes a sudden increase in uterine vascularity (Allen, 1937). This is followed by growth of the glandular epithelium. Epithelial mitosis progresses toward the uterine surface. Finally, glandular mitosis stops and secretion begins.

Bell, *et al.* (1941) observed the histological changes induced in the uterus of spayed yearling ewes by estrogen, progesterone, and estrogen plus progesterone. While estrogen and progesterone alone caused some increase in uterine gland and surface epithelium height, maximal epithelium height was produced by use of the combined steroids. In ewes treated with estradiol plus high levels of progesterone an increased coiling of the uterine glands was seen, and nuclei appeared to be more basally located than in the spayed or estradiol treated ewes. Uterine glands were rather straight in spayed ewes killed during induced heat. Asdell, *et al.* (1949) have similar studies with ovariectomized heifers. They observed stromal edema with both estrogen and progesterone injection. Progesterone injection produced the tallest epithelial

cells, with large, ovoid, coarsely granular nuclei. Estrogen alone or estrogen plus progesterone caused slight tissue destruction and epithelial fragility, especially at the margins of the caruncles. Some epithelial denudation was seen. Estrogen produced large, clear stromal nuclei. It required both estrogen plus progesterone injected in a balanced amount to produce the typical progestational endometrium in the ovariectomized heifers.

The myometrium is very sensitive to changes in the hormonal activity of the ovary (Reynolds, 1931). Maximal muscular activity is seen during proestrus, estrus, and metestrus; then it subsides in diestrus (Cupps and Asdell, 1944). Asdell, *et al.* (1945) have shown with ovariectomized heifers that this is a follicular influence followed by luteal stimulation. It has also been shown that the progestational response of the myometrium is one not only of hypertrophy, but also, hyperplasia of the smooth muscle cells (Allen, *et al.* 1937).

In addition to the effect of estrogen upon the stromal connective tissue cells (Muller, 1951), it also alters the type of connective tissue (Burack, *et al.* 1942). Estrogen induces a hastening of the transformation of endometrial reticular fibers in immature rats into collagenous fibers. The dense, subepithelial reticular zone or stratum compactum is transformed into a collagenous zone. Also, reticular fibers in the myometrium tend to be changed to collagenous fibers. With prolonged injection the stroma becomes dense and hyaline-like, and the ordinarily rounded nuclei of the stratum compactum become shrunken like fibroblasts.

The vascular system of the uterus is differentially effected by the ovarian steriods. In the rat uterus, treatment with estrogen causes vasodilatation in all parts (Williams, 1948), but if it is supplemented with progesterone the vasodilatation is confined to the endometrium; the myometrial capillaries will be constricted. For the maximal dilatation of the subepithelial endometrial capillaries the two must be used synergistically. In the cow (Hansel and Asdell, 1951) the endometrial arterioles largely disappear following ovariectomy, and estrogenic hormones appear to be more effective than progesterone in maintaining these arterioles.

Estrogen injections increase the rate of uptake of phosphorus by the uterus (Grauer, *et al.* 1950), and the rate of turnover of energy rich phosphates, such as adenosinedi- and adenosinetri-phosphate and hexose diphosphate, by the myometrium (Borell, 1951). Progesterone, on the contrary, appears to increase the amount of slowly hydrolysable phosphate esters, and this may be correlated with the increase in myometrial glycogen and hyperplasia.

Because of the apparent importance of phosphatase in protein synthesis, carbohydrates and lipid metabolism, and solute transfer (Atkinson and Elftman, 1947; Bradfield, 1950; Dempsey and Wislocki, 1946; Moog, 1946) the effects of the ovarian hormones upon its distribution and relative concentration have been studied histochemically. Alkaline phosphatase activity is low in the castrate uterus (Atkinson and Elftman, 1946, 1947). Activity in the myo-

metrium is usually low (Atkinson and Engle, 1947; Pritchard, 1949), and appears to be altered very little or not at all by the ovarian steroids (Atkinson and Elftman, 1946, 1947). Pritchard's (1947, 1949) observations on alkaline phosphatase distribution in the rat uterus are difficult to interpret. He states that cytoplasmic alkaline phosphatase activity appeared to be high during specific functional activity of the cell (1947), yet he observed a disappearance in histochemically demonstrable alkaline phosphatase in the epithelium when these cells were under progesterone stimulation. It has been well established that the uterine glands are secretorially active during the luteal phase of the estrous cycle (Asdell, 1946; Reynolds, 1949).

The influence of the ovarian steriods on the distribution of lipids in the rat uterus has been investigated by Alden (1946, 1947). Using osmicated and sharlach R stained uteri, he found fat in the surface epithelium, but only rarely in the glands. In the normal rat no lipid was detected in proestrus, but then it began to increase up to a maximum during diestrus. Lipid accumulated at the implantation site; this may be due to degeneration of the surface epithelium in this area (Cain, 1950). Alden (1947) observed that estradiol completely and rapidly eliminated the surface epithelium fat, but progesterone brought about a distribution in the castrate uterus which was similar to that seen in early pregnancy.

Prolonged injection of high levels of estrin in the monkey causes the uterine epithelial nuclei to migrate distally in the columnar cells (Overholser and Nelson, 1936). Lendrum and Hisaw (1936) have produced the same phenomenon with progesterone. The infranuclear space was found to be filled with glycogen. With continued treatment the glycogen appears to move around the nucleus and escape into the lumen. Similar fluctuations in epithelial glycogen have been observed in the human during the menstrual cycle (Hughes, 1945; Spyker and Fidler, 1942).

It appears that the cyclic changes, and the gestation changes, which occur in the uterus cannot be ascribed as being purely estrogenic or progesteronic, but rather, a balanced synergism is required. Corner and Allen (1929) in their original demonstration of the luteal hormone noted that both stimuli were necessary for progestational proliferation. The quantitative nature of the balance is indicated by Leonard, *et al.* (1932) who were able to produce the typical progestational endometrium in castrate rabbits with theelin (estrogen) and corporin (progesterone), but only if an excess of theelin (10 r. u.) to corporin (1. r.u.) was avoided. Murphey, *et al.* (1925a) were unable to duplicate the estrous condition in spayed cows by use of ovarian follicular fluids.

The changes occurring in the cow's uterus between estruses do not appear to fit Heape's (1901) classical description of the diestrous, or, as it is commonly called, estrous cycle. McNutt (1924) has observed the corpora lutea at known stages of the cycle, and he detected only slight morphological evidence of regression at 20 days postestrus in this animal which has a normal

estrous cycle of 21 days (Roark and Herman, 1950). Elder (1925) and Hammond (1927) likewise found that regression did not begin until 2 or 3 days before the next estrus. Spontaneous uterine motility, which is a characteristic of estrus, begins to increase at about 16 days post- or 5 days pro-estrus (Evans and Miller, 1936). Further evidence of this telescoping of cycles is the fact that the vaginal bleeding which is occasionally seen in the cow will occur after that stage of the cycle at which the greatest hypertrophy of the uterus occurs; this happens about the 20th day after ovulation and about the time of the next estrus (Hammond, 1927). During proestrus the myometrium shows a diaphasic response to epinephrin (Asdell, 1946a). The same response can be induced in the ovariectomized cow by injection of 250 r. u. of estrogen and 18 Rab. u. of progesterone daily. This clearly indicates the overlapping of cycles; however, quantitative estimates of normal cyclic variations in ovarian secretory activity are needed for a more precise interpretation.

The uterus of the cow is bicornuate, the cornua being about 35 cm. long (Beaver, 1922). They join posteriorly into a short corpus uteri of 3 to 4 cm. length. The horn or cornu curves forward, upward, and outward, and then turns backward and downward forming a spiral coil. In the non-gravid uterus the mucosal walls touch. On the mucosal surface small round elevations are seen, the caruncles. They number at least 80 and are arranged in rows parallel to the long axis of the cornu (Hammond, 1927). The caruncles disappear from the surface on the anterior 3 to 4 cm. of the cornu (Andersen, 1928). There is a slight constriction at the tubo-uterine junction; however, it apparently does not prevent flow of fluid from the uterus into the oviduct.

The oviduct is long, 21 to 28 cm., thin, and tortuous; it opens into a fimbria which lies close to the ovary (Beaver, 1922). It rests in a mesenterial fold, the mesosalpinx, which is intimately associated with the broad ligament of the uterus (Arey, 1946). The tunica muscularis of the oviduct consists of a rather strong, inner, circular layer of smooth muscle fibers with a few external longitudinal fibers (Beaver, 1922). The mucosa itself consists of thin longitudinal folds which decrease posteriorly in height and intensity (Roark and Herman, 1950). The mucosa shows marked congestion and edema from the 16th day on in the cycle (Murphey, 1924). The ciliated, tall columnar epithelium of the folds shows morphological changes associated with the estrous cycle. Cell and cilia height are maximum at estrus and minimum about mid-cycle (Asdell, 1946b; Murphey, 1924; Roark and Herman, 1950).

These cells appear to have a characteristic secretory process which has been described variously as cellular desquamation, protein secretion, and nuclear extrusion. More epithelial cells are involved in this phenomenon at mid-cycle than at other times (Roark and Herman, 1950.)

The serous coat or perimetrium of the bovine uterus is a semi-dense, collagenous tissue containing very few reticular or argyrophilic fibers (Weber, *et al.* 1948). Just internal to this lies a longitudinal muscle layer, the *stratum*

subserosum of the myometrium (Maximow and Bloom, 1949). Next, inward, is the vascular bed of the myometrium, the *stratum vasculare*. The irregular arrangement of the smooth muscle bundles and the numerous large blood vessels give this layer a spongy appearance. The innermost layer of the myometrium is the circular *stratum submucosum* (Kingman, 1944). Hammond (1927) and Cupps and Asdell (1944) present data showing cyclic hypertrophy in the myometrial muscle cells. The latter found that although there were large standard deviations in cell lengths, there was a tendency for cells to be longer at the time of estrus, smaller about the end of diestrus, and renewed growth began about 2 days proestrus.

Kingman (1944) has conveniently distinguished 4 zones or areas of the bovine endometrium. These are, proceeding from the myometrium to the mucosal surface: (1) *stratum basalis*, in which the uterine glands terminate; (2) *stratum spongiosum*, a loose connective tissue area in which the glands ramify; (3) *stratum compactum* which is a very dense connective tissue area; and (4) the *lamina propria* of the surface epithelium. The s. compactum of the caruncle is much wider than that of the intercaruncular area, as is well illustrated by Cole's (1930) Figure 20, and no glands penetrate the s. compactum of the caruncle (Beaver, 1922). The endometrial stroma becomes very edematous and congested around the time of estrus (Asdell, *et al.* 1949; Cole, 1930; Roark and Herman, 1950; Weber, *et al.* 1948), but it increases rapidly in density in early postestrus (Asdell, *et al.* 1949; Cole, 1930). The edema has subsided by 8 days postestrus (Hammond, 1927). Kolster (1903) observed extravasation of red blood corpuscles, especially near the surface epithelium, following estrus in a heifer. Similar extravasation of blood from subepithelial vessels has been noted 2 to 3 days postestrus by Hammond (1927). Murphey (1924) noted on the second day postestrus that the caruncles in a heifer were red and covered with blood. Weber, *et al.* (1948) noted massive accumulations of blood in the lamina propria of the heifer at 2 days postestrus. Asdell, *et al.* (1949) observed vesicle formation in the lamina propria around the time of estrus. The blood which is extravasated at this time may give rise to a yellow-brown, amorphous pigment (Hammond, 1927; Kolster, 1903). Murphey (1924) believed this hematogenous pigment was formed intracellularly in large phagocytes, and Kolster (1903) detected iron in the pigment, indicating its red blood corpuscle origin.

Hammond (1927) observed that glandular hypertrophy was associated with the development of the corpus luteum, and although there are slight time differences, the works of Cole (1930), Asdell, *et al.* (1949), Roark and Herman (1950), and Weber, *et al.* (1948) confirm this. Furthermore, atrophy of the glands (Cole, 1930; Hammond, 1927; Roark and Herman, 1950) appears to be associated with the morphological regression of the corpus luteum as shown by Asdell, *et al.* (1949). The latter investigators have noted that the ratio of epithelial cell height to nuclear length follows a cylic pattern with a

ratio of 3 or 4 to 1 indicating quiescence and a ratio of 2 or 1½ to 1 indicating a state of high secretory activity. Weber, *et al.* (1948) noted in heifers that many ciliated cells were present in the necks of the uterine glands.

The uterine surface epithelium is tall columnar and generally shows considerable pseudostratification in late diestrus (Hammond, 1927; Murphy, 1924) or proestrus (Cole, 1930; Roark and Herman, 1950). In addition, at this time (Roark and Herman, 1950) or a little later (Asdell, *et al.* 1949; Murphey, 1924) the nuclei become elongated and there may be evidence of pyknosis and cytoplasmic vacuolation. Asdell, *et al.* (1949) and Weber, *et al.* (1948) have noted some destruction of surface epithelium around or shortly after estrus, and this is indicated in Figure 21 of Roark and Herman (1950). Asdell, *et al.* (1949) observed minimal height of surface epithelium at 2 days postestrus. The distinction between surface epithelium of the caruncular and intercaruncular areas has not been too clearly noted, however differences in time (Asdell, *et al.* 1949) and magnitude (Roark and Herman, 1950) of changes in the two have been reported.

Studies of normal cyclic changes in the morphology of the non-gravid uterus are of importance only insofar as they give some insight into the biological mechanisms whereby the uterus provides a favorable environment for the nutrition of the free-living blastocyst and for its eventual attachment to the uterus. That these functions are delicate ones is indicated by the apparently high ovum or embryonic mortality rate. In cows with macroscopically normal reproductive tracts 30 to 40 per cent of the breedings prove infertile (Laing, 1945, 1949), and this appears to be due to physiological aberrations rather than any pathological changes (Hammond, 1950). In "repeat-breeding" cows (Christian, *et al.* 1951; Tanabe and Casada, 1949) and sows (Warnick, *et al.* 1949) the embryonic death rate appears to be about 65 per cent. Kidder, *et al.* (1951) observed that the incidence of multiple ovulations in cows in a large dairy herd was 13.1 per cent, but the incidence of twinning was only 1.9 per cent. The possible causes of this "featureless" infertility (Laing, 1949) appear to be diverse; a small excess of estrogen in early gestation terminates pregnancy (Allen, 1932; Ingelman-Sundberg, 1950; Parkes and Bellerby, 1926), ova may descend the oviduct too rapidly (Dowling, 1949) or become tube-locked, (Whitney and Burdick, 1936), the follicle or ovum may have been abnormally formed (Laing, 1949), or some genetic lethal may be involved (Gregory, *et al.* 1951).

The bovine blastocyst appears to be free-living and dependent upon glandular secretions as a source of energy for some time. The fertilized ovum reaches the cornu 3 to 4 days postestrus at which time it has 8 to 16 blastomeres (Laing, 1945; Winters, *et al.* 1942). Once within the uterus, the chorion elongates rapidly so that by the 17th day it extends throughout the pregnant horn. There appears to be no unanimity of opinion about just when and how the conceptus becomes attached to the uterine wall. Mossman (1937)

describes the implantation as being caruncular and only superficial, that is, the trophoblast does not invade the uterine stroma. However, the surface epithelium may be eroded away by its contact with the trophoblast (Beaver, 1922; Hallman, 1925; Melton, *et al.* 1951), as is illustrated in Hammond's Plate XXII (1927). Hamilton and Laing (1946) found no specialized cells in the covering of a 190-hour blastocyst, and the 12-day, 15-hour specimen figured by Winters, *et al.* (1942) appears to have no special attachments or processes. In their 13-day, 14-hour specimen the trophectoderm appears to be tall columnar with some evidence of local accumulations of trophotoblastic elements. This specimen could not be flushed from the uterus, but was recovered by dissection. However, Hammond (1927) found the fetal membranes had not become attached to the caruncles by fetal projections at the end of the first month of gestation. Kingman (1948) designated the 50th to the 90th days as the period of implantation. Melton, *et al.* (1951) observed the first definite evidence of attachment at 31 days following ovulation. They state that the caruncular surface becomes undulated at this time in response to cone-like formations of trophoblastic cells. Villi penetrate the uterine submucosa by 33 days to establish a fragile union, and a mesodermal core appears in the villi by 35 days. By 38 days the villi have lengthened and formed a network of lateral branches. Hallman (1925) stated that the villus stimulates the uterine mucosa to grow up around the villus, thus forming the uterine crypt. The early placentome is sessile, but as it continues to grow it becomes pedunculated (Beaver, 1922).

The classification of the bovine placenta according to the scheme of Grosser (see Amoroso, 1952), that is, by the types of tissue separating the maternal and fetal blood vascular systems, has been difficult. That this is an important consideration is indicated by the study of Flexner and Gellhorn (1942) who found a correlation between the rate of transfer of radioactive sodium per unit weight of the placenta and the number and kinds of tissue layers between maternal and fetal circulations. They found that the rate of transfer in the syndesmochorial placenta of the goat was only one-fifteenth as fast as that in a hemochorial placenta. It appears that the connective tissue of the uterine septa and the uterine endothelium offer considerable resistance to the transfer of sodium. Ratner, *et al.* (1927) in a review stated that the bovine placenta appears to be impermeable to antibodies. This may be related to the finer morphology of the placenta which Mossman (1937) has described as both syndesmochorial and epitheliochorial. The uterine epithelium undergoes syncytial degeneration near the mouths of the crypts and appears to be lost in later gestation, but whether it actually does or not is, according to Mossman, open to question. Assheton (1906) and recently Wimsatt (1950) have shown in the sheep that the placental-vascular relationship is syndesmochorial with the crypt lining being formed by characteristic, often binucleated, giant cells. These large trophoblastic cells may phagocytose the uterine epithelium follow-

ing which they develop a syncytium on the connective tissue septa of the caruncle. In the cow, however, both authors observed a simple, uniform cuboidal lining in the crypts which was assumed to be of maternal origin. Kolster (1903), Jenkinson (1906), Hallman (1925), Kingman (1948), and Drieux and Thiery (1951) have all described a similar crypt lining of maternal origin, or an epitheliochorial relationship. Hallman (1925) stated, however, that the uterine epithelium is usually eroded and then a new epithelium grows over the septa, and Melton, *et al.* (1951) also noted that the trophoblast removed the uterine surface epithelium. Jenkinson (1906) observed giant cells in the space between the villus and crypt wall. Kolster (1903), Drieux and Thiery (1951), and Amoroso (1952) describe and figure giant cells in the crypt lining. Yet, no phagocytic function such as that postulated for the sheep has been ascribed to these giant cells in the bovine placenta. Fraenkel (1898) in an early study stated that the uterine epithelium in the placentome is degenerate or entirely missing. Beaver (1922) described the destruction of the uterine epithelium where it contacted the trophoblast. In the intercaruncular area it regenerated by the third month of gestation, but there was no regeneration in the placentome according to this investigator. Hammond (1927) likewise describes a syndesmochorial placentome relationship, but he considers the cells seen lining the uterine crypts to be tissue plasma or lamellar cells whose function is nutritional.

The stroma of the distal ends of the maternal septa may be directly exposed to a cavity bounded by the arcade or basal trophoblast of the chorion (Drieux and Thiery, 1951). Some maternal blood may be liberated into this resorption cavity where it is absorbed by tall columnar, vacuolated cells of the basal trophoblast. Red blood corpuscles so absorbed may give rise to a hematogenous pigment, or pigments (Jenkinson, 1906).

Kolster (1903) has figured the general trophoblast of the villus as a syncytium and in this lie the giant cells. Hammond (1927) described the formation of giant cells from normal epiblastic cells of the trophoblast. The giant cells appear to undergo mitosis without cytokinesis, therefore they are believed to be unable to reproduce (Wimsatt, 1951). The giant cells give reactions for glycoprotein, cytochrome oxidase, alkaline phosphatase, and acid phosphatase. They are not believed to have any significant role in lipid metabolism and their function in the cow is unknown. The mesenchymal core of the villus has been described as collagenous and containing stellate-shaped cells (Drieux and Thiery, 1951). Intratrophoblastic capillaries have been noted. On its internal or fetal side the chorion is covered either by the endoderm of the allantoic membrane or by the amnion whose cuboidal epithelium has localized thickenings which are very rich in glycogen.

With advancing gestation there is continued hypertrophy of the myometrium, especially in the vascular zone (Hammond, 1927). The uterine glands continue to hypertrophy and appear to remain active. The uterine wall be-

comes thin and the enlarged glands are stretched out parallel to the uterine surface. Drieux and Thiery (1951) describe typical villi in the lumina of the dilated uterine glands. Small accessory or adventitious placentomes have also been described (Hammond, 1927; Jenkinson, 1906; Kolster, 1903).

Lipids have been detected histochemically in the placentome of the cow (Drieux and Thiery, 1951; Kolster, 1903; Wimsatt, 1951) and in the interplacentome trophoblast (Jenkinson, 1906). Both the fetal and the maternal parts of the placentome have rather high estrogenic potencies (Allen, 1927; Parkes and Bellerby, 1927); and the urinary excretion of an estrogenic hormone is high during gestation, but drops rapidly following parturition (Turner, *et al.* 1930).

The mechanism allowing separation of the fetal membranes following birth is unknown. Hammond (1927) has suggested that muscular contraction is important since pressure at the base of the caruncle is effective in forcing out the fetal parts. Furthermore, the collapse of the two blood systems aids separation. Necrobiosis in the caruncles begins rapidly following parturition (Beaver, 1922) and appears to be completed by 30 to 40 days postpartum (Casida and Wisnicky, 1950; Roark and Herman, 1950).

MATERIALS AND TECHNIQUES

The genitalia studied were obtained from apparently reproductively normal animals upon slaughter. The animals were culled for a variety of reasons, such as low production, mastitis, poor udders, and old age. Heifers were not excluded, however, those used were sexually mature and exhibiting normal estrous cycles. Three dairy breeds were represented, Guernsey, Holstein, and Jersey; and ages at time of slaughter ranged from 1½ to 12 years. The reproductive status of the 10 non-pregnant and 8 pregnant animals involved in the study is given in Table 1. Following removal of the genital tract, small sections were taken from the mid-region of the oviduct, the upper uterine horn, and the caruncular and intercaruncular regions of the mid and lower horn. In the non-gravid uteri these sections were cut from the side of the currently active ovary, that is, the ovary containing the developing Graafian follicle or corpus luteum. The sections were taken from the antimesometrial aspect of the uterus. The intercotyledonary fetal membranes were not sectioned routinely. The tissues were transferred to appropriate fixatives as rapidly as possible, there being an elapse of 30 to 45 minutes from exsanguination to placing of tissues in fixatives. Following this, a brief macroscopic examination of the tract was made.

Tissues to be used for general histological observations and for connective tissue differentiation were fixed in Zenker's fluid. For glycogen and mucin staining the tissue blocks were fixed in an alcoholic-picro-formalin fixative (Bensley, 1939). For the detection of alkaline phosphatase cold acetone was used as a fixative. Paraffin sections of 8 micra thickness were prepared in the usual manner. A small dissecting needle bent at an angle of

70° two centimeters from its pointed end was found very useful for cutting the paraffin ribbons which were floated in a water bath. The bent end of the needle is dipped in xylene, the excess is shaken off and then it is touched to the ribbon. This cuts the paraffin ribbon cleanly without disturbing the embedded tissue. For the study of lipids, formalin fixed, frozen sections were cut at a thickness of 15 to 25 micra.

For routine histological observations, Mallory's (1938) phloxine-methylene blue staining technique was used. This technique stains basophilia a deep blue and the plasmal ground substance a bright red. The color contrast is reputed to be sharper than that of the commonly used hematoxylin-eosin stain. For phloxine, a 1 per cent solution of phloxine in 0.05 per cent aqueous oxalic acid as suggested by Delez and Davis (1950) was used. This phloxine solution is stated to improve the quality and uniformity of the acidophilic staining over that of Mallory's 2.5 per cent aqueous phloxine solution. It does save stain and reduces staining time. All microscopic measurements were made on phloxine-methylene blue stained tissues.

Connective tissue was localized and differentiated satisfactorily by Wilder's (1935) silver impregnation technique for reticular fibers, followed by counter-staining deeply with alum hematoxylin (1 hour) and then for 5 minutes with Van Gieson's picro-acid fuchsin solution. The counter-staining with Van Gieson's differentiates the pink to red staining collagenous fibers from the black, silver impregnated reticular fibers (Foot, 1928).

The Bauer-Feulgen technique as given by Lillie (1948) was used to demonstrate glycogen and mucin. The Schiff's reagent was prepared fresh before using by the method of Lillie and Greco (1947). Chromic acid was used to free the aldehyde groups of the polysaccharide to provide the chromophore for the leuco base of the fuchsin. The technique does not distinguish between glycogen and certain glycoproteins, such as mucin (Gomori, 1950); however, the differentiation was made on selected sections by digestion in saliva for one hour at 37° C. If the tissue gave a delicate pink or red staining reaction following the salivary digestion, the reaction was assumed to be due to glycoprotein since the glycogen is readily hydrolyzed by salivary amylase (Hawk, *et al.* 1949).

Alkaline phosphatase was identified histochemically by the method of Gomori (1941) as modified by Kabat and Furth (1941) and by Wilmer (1944). The pH of the sodium-beta-glycerophosphate substrate ranged from 9.0 to 9.3. Calcium nitrate (0.1 per cent) was used as a source of calcium ions in the incubation medium of the control sections which were otherwise treated as the experimental sections. The extended incubation period, eight to 14 hours, suggested by Wachstein (1944) was used to develop maximal enzymatic reactivity. Some of the sections were counterstained in 0.1 per cent safranine Y for one minute. This light counterstaining did not interfere with the detection of the dark cobalt sulfide precipitate.

In the early part of this study lipids were stained with sudan IV using equal parts of alcohol (70 per cent) and acetone as a vehicle for the dye (Mallory, 1938). Control sections were routinely immersed in acetone for five minutes. During the course of this work Chiffelle and Putt (1951) published their technique for sudanophilia which uses ethylene or propylene glycol as the dye solvent. This technique appeared to localize more lipid, therefore, it was adopted. The frozen-cut sections were affixed to slides before staining. Frozen-cut sections mounted in glycerine were also observed between crossed Nicols for birefringent crystals. Lipid birefringence was determined by treating control sections in acetone. Occasionally frozen-cut sections were stained in alum hematoxylin to aid in localization of the birefringent material.

OBSERVATIONS

To facilitate description of cyclic changes in the non-gravid uterus and oviduct the nomenclature of Heape (1901) will frequently be used. The first three days postestrus are considered as metestrus, the next 14 days as diestrus, and from the 18th day postestrus to estrus as proestrus. The word *lipid* is used to designate a broad group of naturally occurring substances characterized by their insolubility in water and their solubility in fat solvents (Hawk, *et al.* 1949). They are usually both sudanophilic and birefringent (Cain, 1950). All photomicrographs reproduced herein have been reduced 40 per cent from the original print. The times-magnification given with each figure represents magnification on the original print.

No detailed macroscopic study was made, however, some salient observations are noted in Tables 1 and 2. The right ovary and oviduct of Cow 12 were nonfunctional, but this aged cow had been reproducing normally. The chorions of the 58-day twin fetuses of Cow 9 had completely fused. The kidney-shaped amnions, which could be held in the palm of the hand, were separated from one another and from the chorio-allantois. By the 98th day of gestation the amnion had made a loose, gelatinous contact with the chorio-allantois. Small patches of aberrant fetal epidermis were noted on the inner, or fetal, aspect of the amnion. These patches of amniotic epidermis were most numerous near the umbilical cord. Cow 6, pregnant 112 days, had two large, apparently functional corpora lutea on the same ovary; however, she had only one fetus and it appeared normal. The placentomes, or cotyledons, remain smaller in the uterine horn opposite the side of original attachment of the trophectoderm to the endometrium. In the 25-day post-partum uterus, regression was more advanced in one horn than in the other.

Mid-Region of the Oviduct.

The longitudinal folds of mucosa are covered with a columnar epithelium which showed maximal height, about 34 micra, at estrus and minimal height, 18 micra, at 15 days postestrus. Considerable pseudo-stratification was seen during early diestrus, but this decreased by late diestrus and very little was

TABLE 1 -- REPRODUCTIVE STATUS AND GROSS APPEARANCE OF GENITALIA OF COWS AND HEIFERS AT TIME OF SLAUGHTER.

Cow No.	Days Post-Estrus or Pregnant	Macroscopic Observations		
		Ovaries	Uterus	Remarks
16[a]	Estrus	Mature follicle, both ovaries.	Mucosa slightly red. Vascular system and caruncles small.	Mucus in vigina. Cervix relaxed.
2	1	Ovulated.	Mucosa hyperemic.	Oviducts Hypertrophied.
18	4	Corpus luteum about 1.3 cm. maximum diameter each ovary.	Thin walled. Atrophic. Evidence of intercaruncular extravasation.	Bleeding at 2 days postestrus.
4	8	Corpus luteum about 1.3 cm. diameter.	Vascular system and caruncles prominent.	Left cornu slightly enlarged.
13	11	Corpus luteum 2.5 cm. maximum diameter.	Hypertrophied and vascular.	Entire tract rather turgid.
12	14	Corpus luteum 2.5 cm., left ovary.	Moderate edema.	Fibrous adhesions, right ovary and fimbria.
7	15	Active corpus luteum 2.0 cm. Regressing corpus luteum 1.5 cm.	Moderate stromal edema. Mucosa dry.	One large ovarian follicle 1 cm.
20	18	Corpus luteum 2.6 cm. Large follicle 1.5 cm.	Caruncles small. Mucosa avascular with thick mucus on surface.	Cervix constricted. Some mucus, anterior vagina.
9[b]	58	Large corpus luteum 1.9 cm. each ovary. Many small follicles.	Cotyledons loosely attached. Fetal fluids thin, colorless.	Twin fetuses, male and female. Chorions completely fused.
14	79	Corpus luteum 2.5 cm.	Cotyledons loosely attached; largest 3.2 cm. diameter. S. spongiosum very loose.	Amnion loosely attached to chorion in places.
15	98	Corpus luteum 2.9 cm., recessed into ovary.	Cotyledons up to 5.4 cm., firmly attached. Many adventitious cotyledons.	Loose, gelatinous chorio-amniotic fusion has been made.
17	102	Corpus luteum 2.5 cm. Many follicles, both ovaries.	Uterus thin-walled. Cotyledons large, firmly attached. Many adventitious cotyledons.	Amnion and chorion loosely fused. Allantoic fluid murky, urine odor.
6	112	Two corpora lutea, 2.5 cm. same ovary, both functional.	Most placental attachments appear to have been made.	Cotyledons smaller in cornu opposite side of original attachment.
3	142	Corpus luteum 2.5 cm.,	Cotyledons firm. Adventitious cotyledons seen.	Amnion and chorion fused.
10	178	Corpus luteum 2.4 cm., recessed into ovary. Many small follicles.	Cotyledons firmly attached. Larger ones about 7.0 cm. in diameter.	Cotyledons in upper cornu opposite corpus luteum are small.
8	241	Corpus luteum 2.3 cm., appears active.	Cotyledons very strongly attached.	Small cotyledons upper cornu opposite side of original attachment.
19[c]	8	Corpus luteum 1.5 cm. Also follicle 1.5 cm., in opposite ovary.	Uterus thin-walled, flaccid. Mucosa red-brown color, appears edematous.	Uterus contains mass of degenerating placentomes and about 1 liter of red-brown fluid.
5	25	Very recent ovulation site, left ovary.	Regression incomplete. Intercaruncular mucosa has red-brown color.	Abundant, thick mucus in vagina and cervix. Cervix relaxed.

[a] Cows 16 through 20 as listed in table were non-pregnant.
[b] Cows 9 through 8 as listed in table were pregnant.
[c] Cows 19 and 5 were postpartum cows.

TABLE 2 -- CROWN-RUMP MEASUREMENTS ON BOVINE FETUSES OF KNOWN AGES.

Cow No.	Breed	Age of Fetus Days	Sex	Crown-Rump Length, cm.	Remarks
9	Holstein	58	Male Female	9.2 8.5	Twin fetuses. Chorions fused. Amnions separated.
14	Guernsey	79	Female	12.1	Eyes closed. No hair.
15	Guernsey	98	Female	17.2	Eyes closed. Muzzle and lower jaw hairs.
17	Holstein	102	Female	19.4	Eyes closed. Muzzle hairs.
6	Holstein	112	Male	25.0	Fetus appeared normal.
3	Holstein	142	Female	36.8	Fetus appeared normal.
10	Holstein	178	Male	52.1	Eyes opened. Muzzle, eyebrow, and switch hairs. No body hairs.
8	Holstein	241	Female	78.0	Fetus appeared normal. Body covered with hair.

seen during estrus. The epithelial cells with distally located nuclei were goblet-shaped. Their nuclei tended to be round and vesicular, and their cytoplasm vacuolated (Fig. 3). A few nuclei in the process of being extruded were seen at 2 and 4 days postestrus (Fig. 2). Cilia were never well developed or evenly distributed on the plicae in the mid-region, but rather, they were often bunched into brush-like tufts. Such tufts gave a strong alkaline phosphatase reaction, were acidophilic, and became silver impregnated with Wilder's technique. Cilia were very sparse in late diestrus (Fig. 1). A moderate amount of fine granular cytoplasmic basophilia was seen at estrus and this increased up to four days postestrus. Only a few granules were seen in late diestrus and proestrus. The surface epithelium showed a very faint lipid reaction from two to 11 days postestrus, but was otherwise negative except for a diffuse birefringence at 18 days postestrus. No epithelial glycogen was seen at estrus, but by eight days postestrus the cells gave a strong reaction (Fig. 4). By late diestrus only a very faint glycogen reaction was seen. Moderate to heavy alkaline phosphatase activity, as shown by the black cobalt sulfide deposits, was seen during estrus. This decreased during early diestrus, but actively increased again with approaching proestrus.

The connective tissue of the plicae and that surrounding the tube was predominantly reticular fibers of medium thickness. Some collagenous fibers were seen in the wider folds. Reticular fibers were most prevalent at estrus, a time when only a few collagenous fibers were observed. The stroma of the mucosa gave a slight to moderate, diffuse alkaline phosphatase reaction.

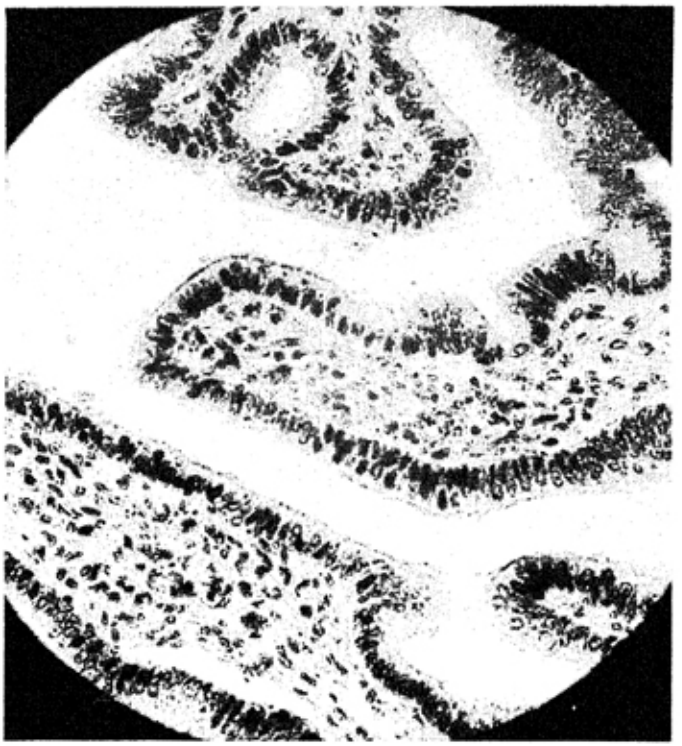


Fig. 1.—Oviduct at 15 days postestrus. Epithelium shows minimal height. Cilia are sparse. Note small epithelial-lined pocket. Mallory's phloxine-methylene blue. X 228.

Fig. 2.—Oviduct near fimbria at four days postestrus. Several nuclei in process of extrusion are shown. Cilia are poorly developed. Mallory's phloxine-methylene blue. X 592.

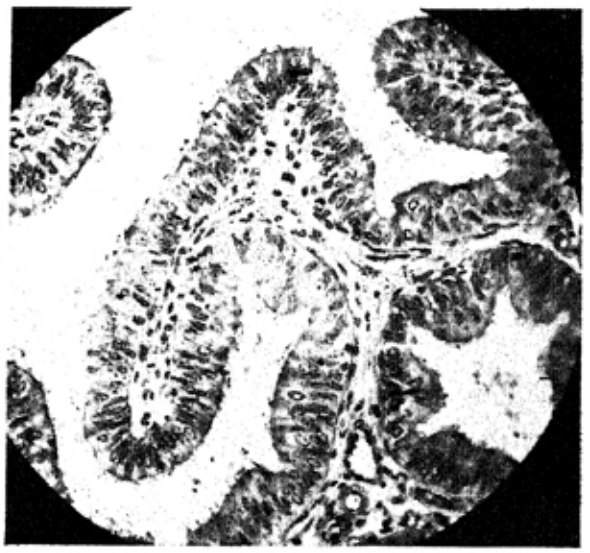


Fig. 3.—Oviduct at four days postestrus. Darkened epithelial cytoplasm indicates glycogen. Note also pseudostratification and goblet-like cells. Schiff's leucofuchsin and Lillie's acid hemalum. X 207.

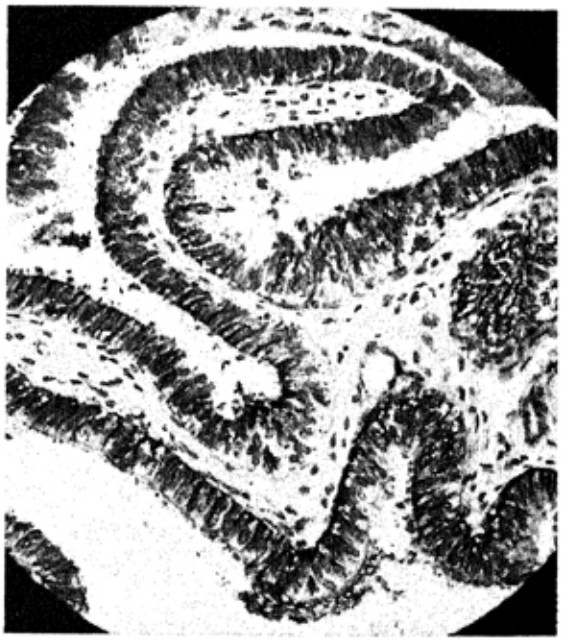


Fig. 4.—Oviduct at eight days postestrus. Maximal epithelial glycogen, shown by darkened areas of the cytoplasm. Schiff's leucofuchsin and Lillie's acid hemalum. X 207.

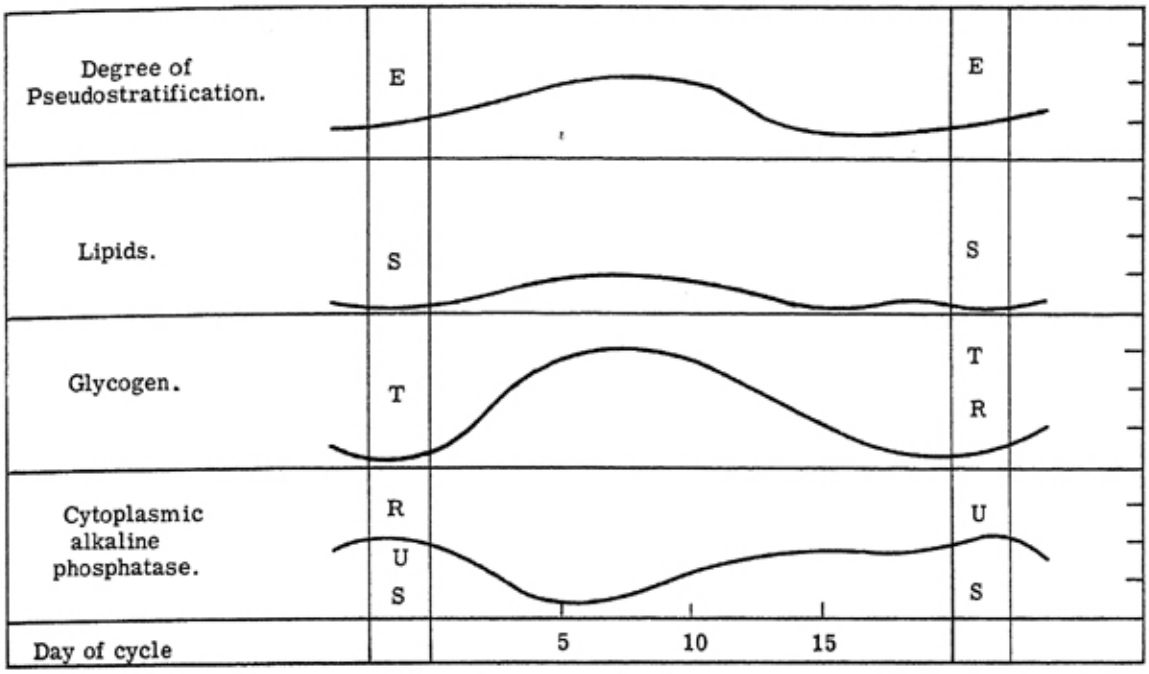


Fig. 5.—Graphic representation of microscopic changes occurring in the bovine oviduct epithelium during the estrous cycle.

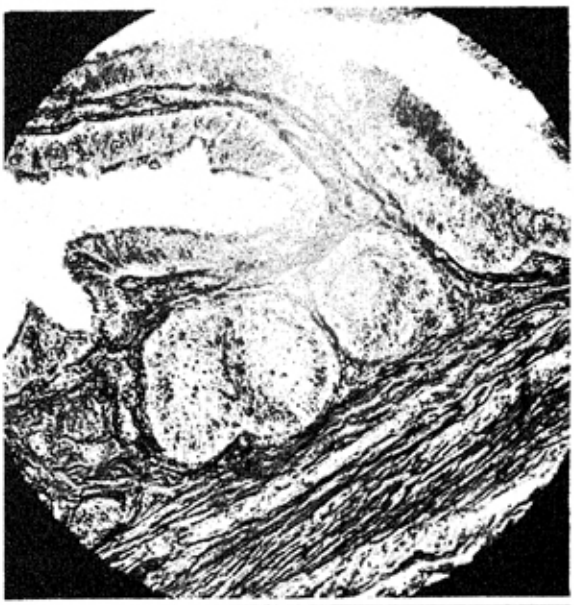
No pronounced gestational trends were seen in the mid-region of the oviduct. The surface epithelium was consistently about 20 micra high and had only a slight pseudostratified appearance. Nuclear extrusions and goblet-like cells were seen in the epithelium and the two appeared to be related. Cilia were sparse, poorly distributed and often non-existent. Acidophilic cytoplasmic projections were more numerous during pregnancy than in the non-gravid oviduct. A light sudanophilia and small birefringent crystals were seen in the epithelium during the latter half of gestation. Small amounts of epithelial glycogen were detected throughout gestation. This glycogen was located predominantly at the bases of the plicae. Alkaline phosphatase activity resembled that seen during diestrus with most of the color (cobalt sulfide) localized in a distal band of the cytoplasm.

The stroma of the mucosal folds appeared avascular during gestation, especially after the 100th day. It was of moderate density and, except in early pregnancy, collagenous fibers were more prominent than in the non-gravid (cyclic) oviduct.

Small, epithelial-lined pockets were seen in the folds toward the periphery of the tube (Figs. 6, 7, 8). These pockets appear first as solid nests of epithelial cells. The pocket may have a depth of up to 140 micra, and it opens into the central lumen. Some pockets open anteriorly—others posteriorly. Throughout most of their length they are completely surrounded by the connective tissue stroma of the mucosa.

The Perimetrium.

The perimetrium or serosa is composed of a simple cuboidal, epithelial covering and a narrow connective tissue area. In this connective tissue, next



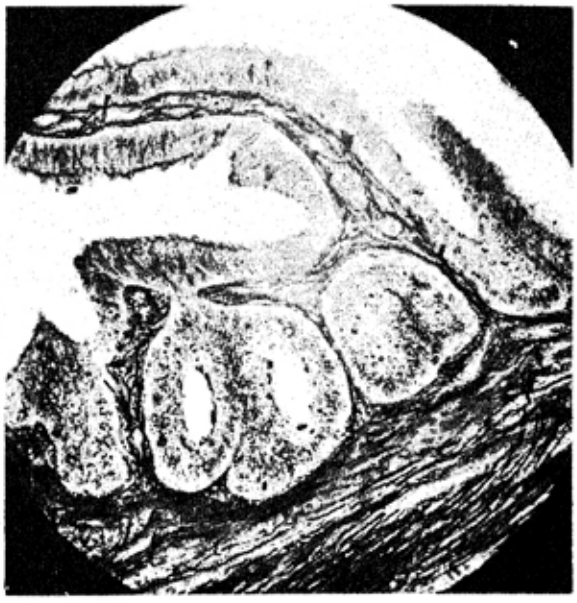


Fig. 6.—Oviduct at eight days postpartum. Serial sections (Figs. 6, 7, 8) showing development of three epithelial-lined pockets. Wilder's silver impregnation and Van Gieson's. X 220. (Upper left)

Fig. 7.—Two epithelial-lined pockets have developed lumina, and connective tissue of the mucosa no longer surrounds these two. Wilder's silver impregnation and Van Gieson's. X 220. (Upper right)

Fig. 8.—The third epithelial-lined pocket has developed a lumen. Wilder's silver impregnation and Van Gieson's. X 220 (Lower left)

to the myometrium, is a dense bed of small blood vessels. The connective tissue is acellular, especially in latter gestation, and hyaline-like in appearance. During estrus and for 8 days postestrus, the connective tissue contained both argyrophilic and collagenous fibers. By 11 days postestrus, argyrophilic reticular fibers were decreased and there was an increase in collagenous fibers. Throughout pregnancy the connective tissue stained heavily with Van Gieson's and only a few argyrophilic fibers were detected (Fig. 9). A diffuse Schiff's positive reaction was seen in all perimetria. In the non-gravid uterus it appeared abundant during mid-diestrus. In the gravid uterus it varied inconsistently from slight to moderate. The reaction was altered very little by salivary digestion.

The Myometrium.

The uniformly longitudinal muscle fibers of the s. (stratum) sub-serosum are organized into large fasciculi by inroads of connective tissue from the perimetrium (Fig. 9). This stratum was atrophic at one day postestrus, and then it increased in size to 18 days postestrus when a slight edema was apparent. There was decreased edema at estrus. The thickness of the connective tissue of the area showed a similar trend. A slight to moderate Schiff's reaction was seen during diestrus, but at other stages the reaction was negative. No lipid or alkaline phosphatase was detected in the smooth muscle cells (Fig. 10).

The muscle fibers of the appropriately named s. vasculare are irregularly arranged, and the interfascicular connective tissue is prominent. Cyclic variations are similar to those of the s. subserosum. The approximate center of the stratum is largely a mass of blood vessels surrounded by collagenous tissue. The larger, thinner walled vessels have strongly Schiff's positive tunics.

The inner fibers of the s. vasculare blend inconspicuously with the uniformly circular muscle fibers which comprise the thin s. sub-mucosum. This internal stratum of the myometrium shows little or no cyclic changes in morphology. In the non-gravidae it gave negative reactions for glycogen, alkaline phosphatase, and lipids. No collagenous fibers were detected in this stratum.

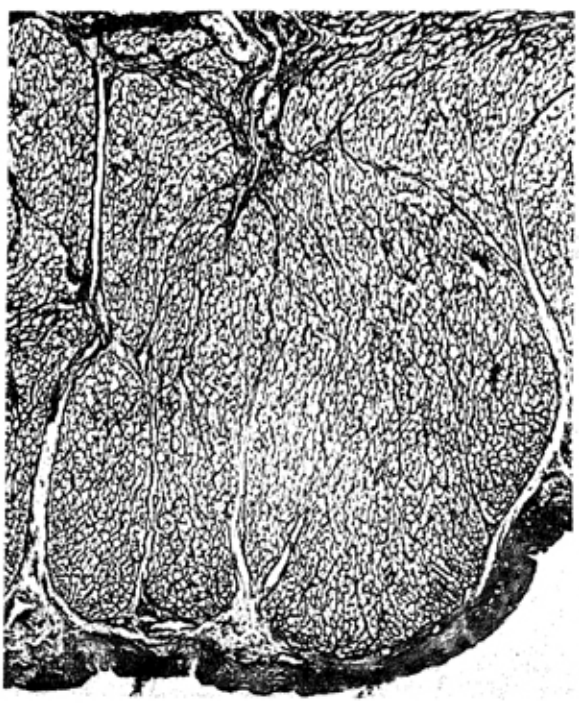


Fig. 9.—Cross section of stratum suberosum at 241 days gestation. Note the extreme hypertrophy of muscle cells and collagenous fibers. Wilder's silver impregnation and Van Gieson's. X 61.

Fig. 10.—Cross section of stratum suberosum at eight days postestrus. Blackened lines indicate alkaline phosphatase activity of vascular endothelium. Smooth muscle is negative. Gomori's alkaline phosphatase. X 33.

The myometrial hypertrophy and edema seen during the late luteal phase is minor when compared to that seen in the gravid uterus. In the pregnant animal, the muscle cells in all strata become very hypertrophied and the tissue becomes extremely loose (Fig. 9), especially in the s. vasculare. Due to the great edema, the tissue appears increasingly hypoplastic. There was no accumulation of histochemically demonstrable glycogen. Slight alkaline phosphatase activity was apparent in both the muscle nuclei and sarcoplasm in the s. vasculare and s. submucosum. The activity was heavier in early gestation. The muscle bundles became widely separated by loose connective tissue. This tissue was predominantly short, reticular fibers in early pregnancy; but during the latter half, wide collagenous fibers became very apparent.

The Endometrium.

Stromal edema in the s. functionalis, which includes both the s. basalis and s. spongiosum, was not a marked characteristic of the non-gravid uterus (Fig. 13). However, moderate looseness of the stroma was seen during proestrus and estrus. The connective tissue reached maximum density by four to eight days postestrus. Consistent with these observations was the hypoplastic appearance of the stroma during proestrus and estrus. Cellularity then increased during diestrus. Irregular reticular fibers were rather dense during metestrus. These then decreased during diestrus and delicate, wavy collagenous fibers became predominant.

Only a small cyclic change was noted in the normally profuse vascular system of the s. functionalis. There was a constant increase in vascularity up to 18 days postestrus. The s. functionalis in cows often contains very heavily walled vessels (Fig. 12). In many of these, the diameter of the vessels' tunics is four times that of the lumen. A zone of particularly heavy vascularization is found just below the thick, conical-shaped s. compactum of the caruncle (Fig. 11).

The periglandular stroma gave a slight Schiff's reaction at all times. The general stroma was negative, except at one, four, and eight days postestrus scattered, discrete deposits of about 12 micra in diameter were seen. Salivary digestion did not remove these deposits. Siimlar deposits of an acetone-insoluble, birefringent, yellow-brown pigment were seen in the same area during diestrus (Fig. 15). The thickened connective tissue in the s. basalis and in the periglandular and perivascular regions gave a moderate to heavy alkaline phosphatase reaction, but the general stroma appeared negative.

During early pregnancy, the s. functionalis becomes extremely loose. Continued distention of the uterus and glandular hypertrophy tend to condense the fibers slightly in latter gestation. Also, with advancing gestation, reticular fibers appear to be replaced by collagenous fibers. A moderate number of large, acetone-insoluble pigment deposits was seen in early pregnancy. By 112 days these were gone and none was observed thereafter.

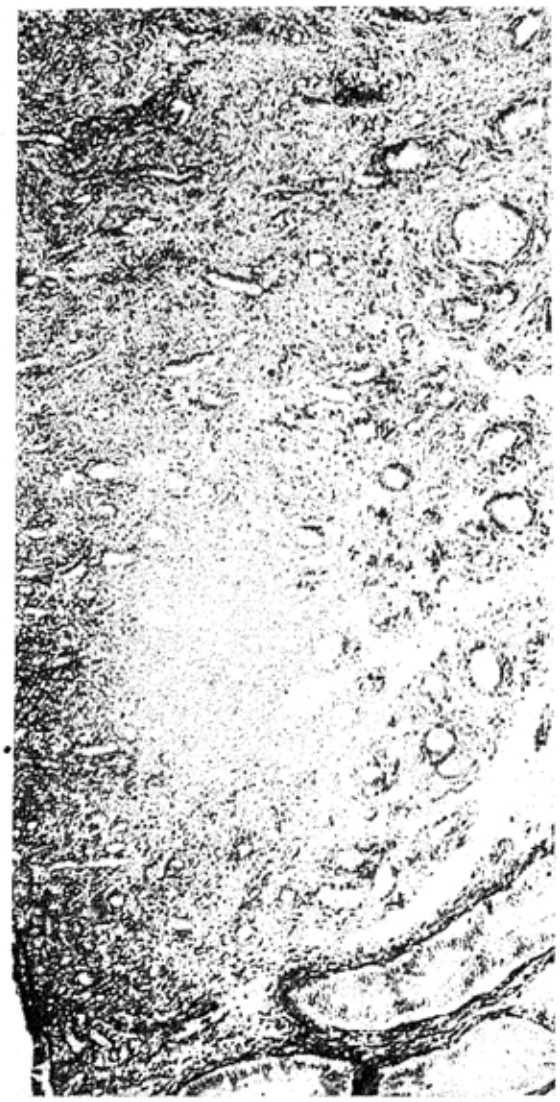


Fig. 11.—Periphery of a caruncle at four days postestrus. Stratum compactum shows dense reticular fibers. Surface epithelium appears degenerate. Wilder's silver impregnation and Van Gieson's. X 340.

Fig. 12.—Small caruncle at 11 days postestrus. Note dense stratum compactum and thick-walled blood vessels in underlying stratum spongiosium. Mallory's phloxine-methylene blue. X 49.

The s. compactum is a normally dense, connective tissue zone which lies on the luminal side of the s. spongiosum, separated from the surface epithelium by the narrow lamina propria. The s. compactum of the caruncle is wider and its tissue is denser than that of the intercaruncular area (Figs. 11, 13, 16). The intercaruncular s. compactum shows very little cyclic variation in tissue density or cellularity; but in the caruncle it shows mild hypertrophy and hypoplasia at estrus; then becomes very dense and hyperplastic

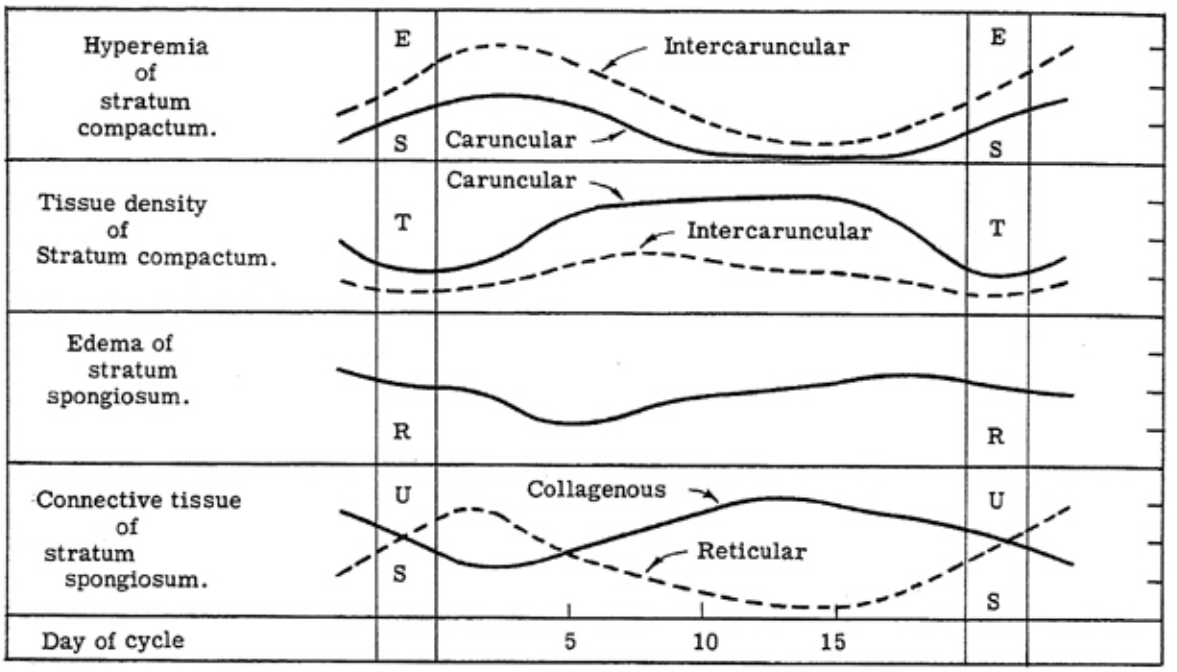


Fig. 13.—Graphic representation of microscopic changes occurring in the bovine endometrial stroma during the estrous cycle.

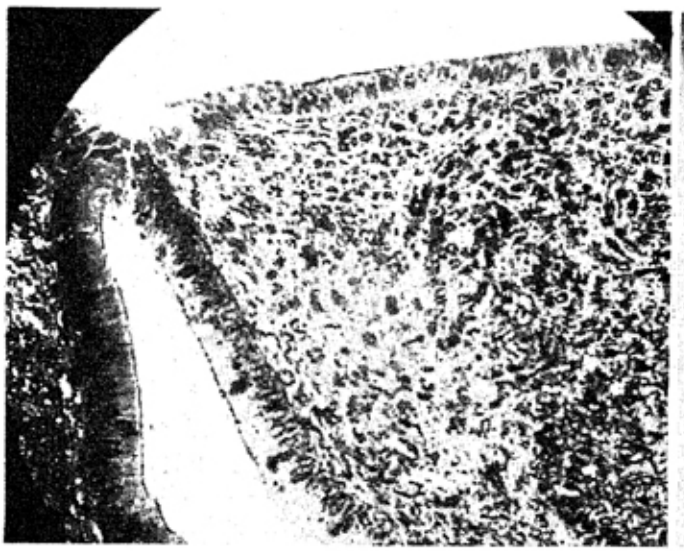


Fig. 14.—Intercaruncular stratum compactum at four days postestrus. A gland neck, surface epithelium, and a very dense stroma are shown. Note discrete stromal cells. Mallory's phloxine-methylene blue. X 243.

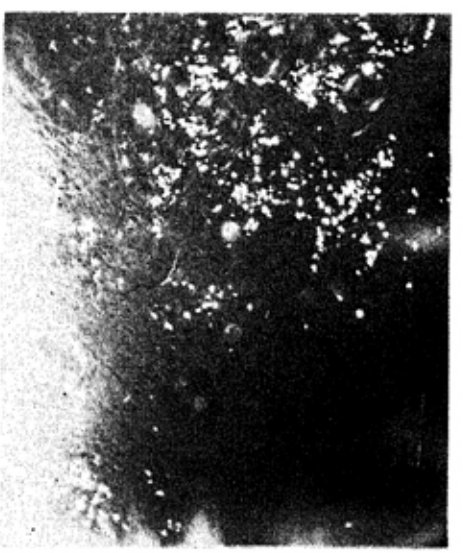


Fig. 15.—Stratum spongiosum at 15 days postestrus. Phomicrographed through crossed Nicols. Birefringent spheres are acetone-insoluble, stromal pigment. Unstained. X 268.

in late diestrus. The stromal cells of the s. compactum differ from the characteristic fibroblast in that their nuclei are large, ovoid, and rather vesicular (Fig. 14). Furthermore, when treated by Wilder's technique the cells tend to be discretely surrounded by reticular fibers.

The s. compactum of the intercaruncular area shows more tendency toward hyperemia and extravasation of red blood corpuscles than does that of the caruncle. Considerable hyperemia and extravasation were noted around estrus, and this was most apparent in the lamina propria which encircled the caruncle. Pigment deposits were concentrated in the same area, although these became maximum during diestrus. The pigment varied in color from light yellow to dark brown. A few deposits appeared to be removed by acetone or xylene. They were birefringent, only rarely sudanophilic, and in some uteri, they appeared to give a Schiff's positive reaction.

Alkaline phosphatase activity in the stroma of the s. compactum varied during the estrous cycle from very heavy at estrus to only a moderate activity during diestrus. No activity was detected in the lamina propria.

The s. compactum of the intercaruncular area remains dense during gestation. It becomes ischemic in latter pregnancy and pigment has disappeared by the 112th day. By late gestation the reticular fibers have become

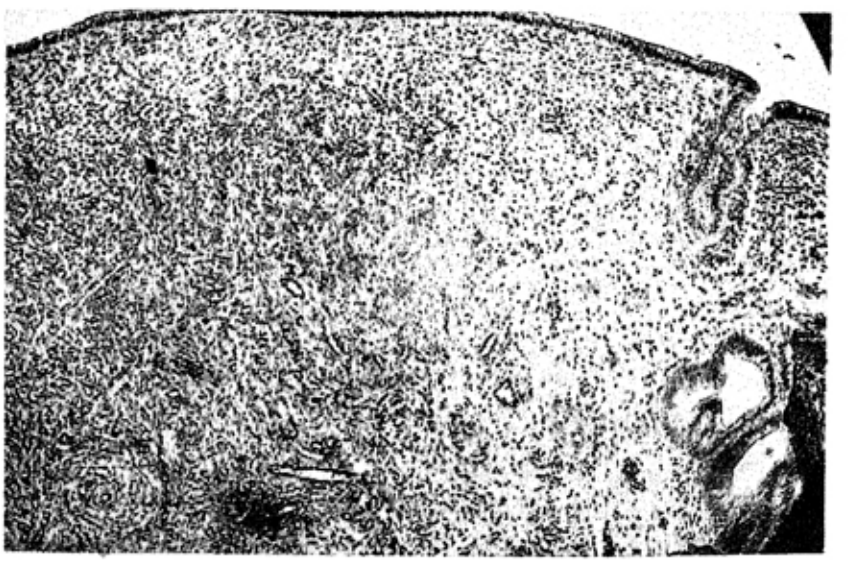


Fig. 16—Junction between caruncular and intercaruncular stratum compactum at 18 days postestrus. Note difference in cellularity in the areas. Mallory's phloxine-methylene blue. X 92.

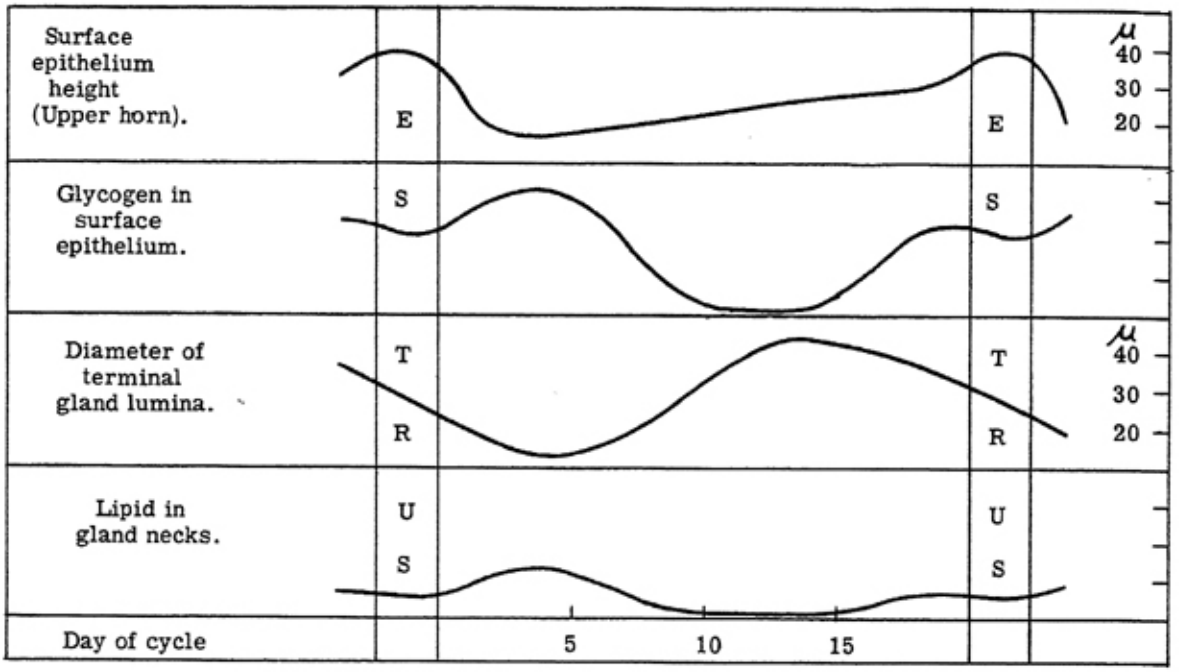


Fig. 17.—Graphic representation of microscopic changes occurring in the bovine uterine epithelium during the estrous cycle.

less prominent, and at 241 days a moderate collagenous reaction was detected (Fig. 21).

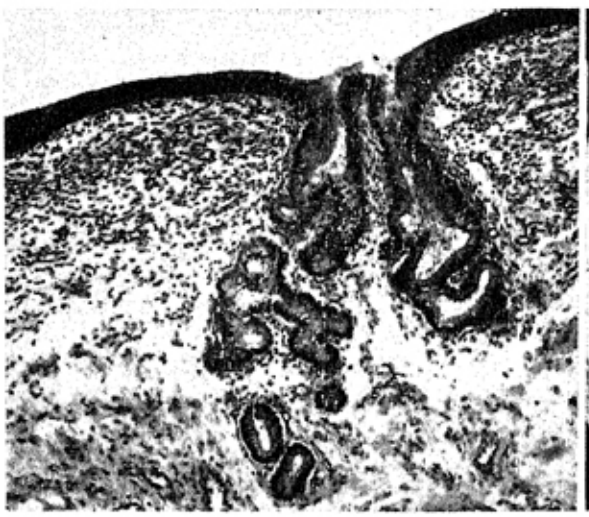
Unlike the s. compactum in the intercaruncular area of the non-gravid uterus, this stratum exhibited little or no alkaline phosphatase activity during gestation. The lamina propria, which had a hyaline, acellular appearance, was consistently negative for alkaline phosphatase activity.

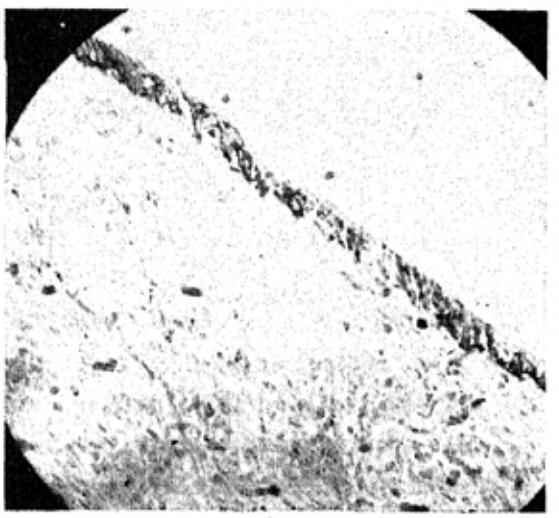
The uterine glands are coiled, compound, tubular glands. They terminate in coils in the deep s. spongiosum or in the s. basalis. As the glands pass superficially they become straighter and their lumina become larger. The gland necks do not penetrate the thickened s. compactum of the caruncle, and they can be seen taking diagonal courses around this area (Fig. 11). Many, but not all, gland terminals hypertrophy under luteal stimulation. During late diestrus some measure up to 45 micra in luminal diameter. Around metestrus the lumina are atrophic (Fig. 17). The lumina continue to enlarge during gestation (Fig. 20), and by 142 days, lumina of 90 micra diameter were seen in the mid and lower cornua. There appeared to be very little further increase in luminal size of the endometrial glands during the latter half of gestation. The walls of the hypertrophied glands may collapse, and when these are examined microscopically, islands of glandular epithelium are often seen in the lumina (Fig. 23). The glandular epithelium is simple columnar, with

consistently ovoid, basal, rather vesicular nuclei. The acidophilic ground substance of the cytoplasm contained slight amounts of basophilic granules in all uteri, with no cyclic or gestational trends being observed. Maximal height of the epithelium in the terminals, 20 micra, was noted during diestrus. During pregnancy the cells were low columnar and varied little from 15 micra in height. The epithelial height in the gland necks was about 5 micra taller than in the terminals.

Lipids were detected in the glandular epithelium, however, the reaction was slight and confined to the most superficial gland necks. In the cyclic glands, epithelial lipid was maximal at 4 days postestrus and none was detected at 11, 14, or 15 days postestrus. In the gravid state, lipid was first seen at 102 days. The relative amount appeared to increase slightly with advancing gestation, but the epithelial cells in the gland terminals remained negative.

As with lipids, glandular glycogen was largely limited to the gland necks. Very little or no glycogen was detected from 8 to 15 days postestrus. There-





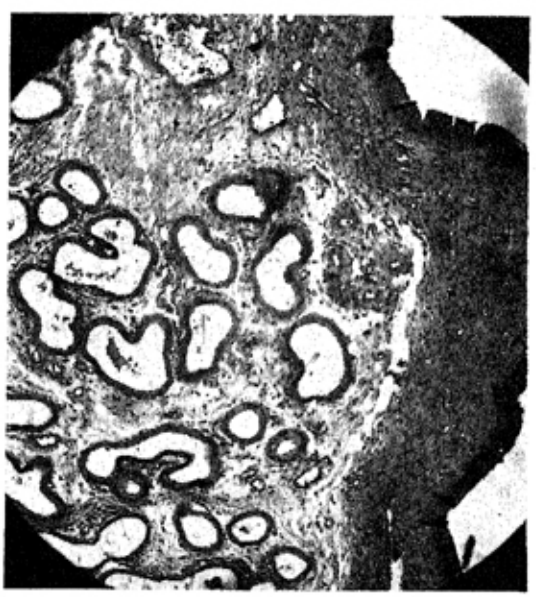


Fig. 18.—Endometrium at 18 days postestrus. Darkened epithelial cytoplasm indicates the high glycogen content at this time. Schiff's leucofuchsin and Lillie's acid hemalum. X 72. (Upper left)

Fig. 19.—Superficial endometrium of caruncle at one day postestrus.. The low columnar surface epithelium has a high glycogen content (darkened cytoplasm) and shows degeneration. Schiff's leucofuchsin. X 200. (Upper right)

Fig. 20.—Endometrium at 142 days gestation. Hypertrophied uterine glands are shown in a loose hypoplastic stroma. Mallory's phloxine-methylene blue. X 50. (Lower left)

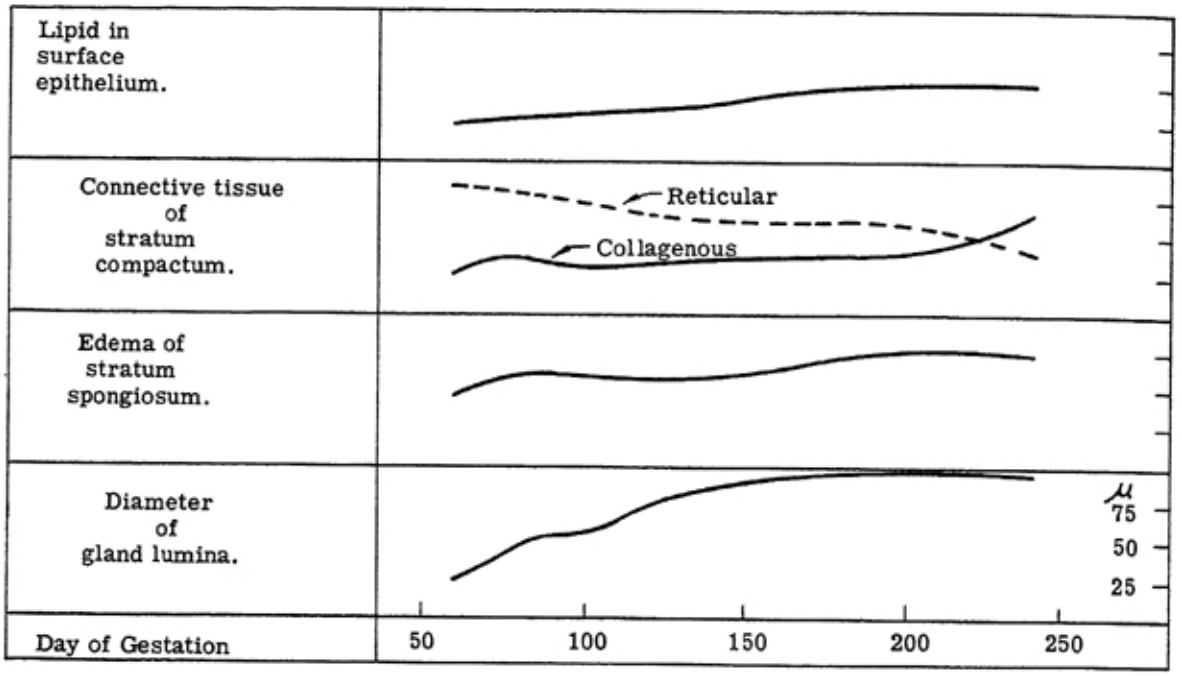


Fig. 21.—Graphic representation of microscopic changes occurring in the bovine intercaruncular endometrium during gestation.

after, small, red, granular fuchsinophilic deposits were seen, becoming of moderate intensity around estrus. No glycogen was detected in the glandular epithelium of the mid and lower uterine horns during gestation. A moderate, granular reaction was observed in the upper horn during early pregnancy, but by 178 days the reaction had disappeared.

Alkaline phosphatase activity was noted in the uterine gland epithelium. Nuclear activity was seen as coarse deposits of cobalt sulfide, the general cytoplasmic reaction was rather light or negative, and a distal band concentration was characteristic. The overall glandular activity was slightly increased at mid-diestrus and reduced at estrus. Activity appeared to be heavier in the superficial glands than in the terminal glands. In the gravidae, the activity varied between slight and moderate with evidence of decreased phosphatase activity at 241 days.

Cyclic changes in height of the columnar surface epithelium are shown graphically in Figure 17. The cell height was more variable on the caruncle than in the intercaruncular areas. The surface epithelium on the caruncle often appeared degenerate and the nuclei here tended to exhibit karyorrhexis with coincident cytoplasmic basophilia. This was most pronounced during estrus and in the early cycle (Figs. 11, 19). In the intercaruncular areas, pseudostratification was apparent when the surface epithelium was tall, that is, during

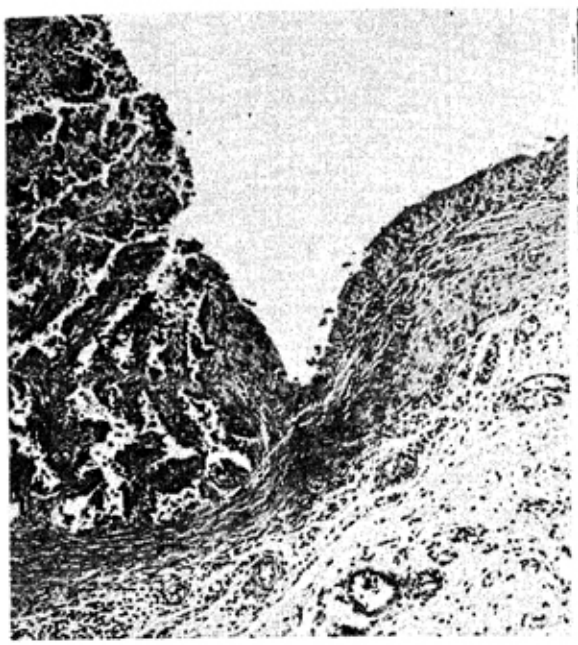


Fig. 22.—Junction of small placentome with intercaruncular endometrium. Gestation, 79 days. Dense subplacentome tissue continuous with stratum compactum of intercaruncular area. Mallory's phloxine-methylene blue. X 59.

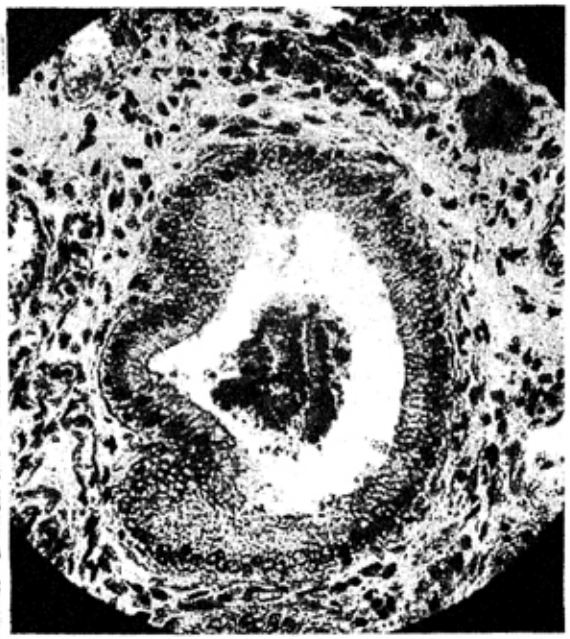


Fig. 23.—Hypertrophied uterine gland at 112 days gestation. Wall is folded in and a portion appears as an island in the lumen. Mallory's phloxine-methylene blue. X 211.

proestrus and estrus. During metestrus, when cell height had decreased, most nuclei had migrated to the mid or distal cytoplasm, giving a vacuolated appearance to the proximal cytoplasm. During the first half of gestation the surface epithelium varied markedly in height, from completely absent to 50 micra. It also showed karyorrhexis and pseudostratification at this time. Later in gestation it became more uniform in height, with nuclei being predominantly basal and ovoid.

Small amounts of lipid were seen in the surface epithelium at 1, 4, and 18 days postestrus, and throughout pregnancy. There was some increase with advancing gestation (Fig. 21). More lipid was demonstrated in the interplacentome areas than in the upper uterine horn where placental attachments had not been made. Heavy reactions for surface epithelial glycogen were elicited at and around estrus, that is, pro-, met-, and early di-estrus (Figs. 18, 19). However, little or no glycogen staining was seen from eight to 14 days postestrus. A moderate glycogen reaction was seen in the surface epithelium throughout pregnancy. The surface epithelium gave a moderate to heavy alkaline phosphatase reaction. The enzymatic activity appeared predominantly in a distal band of the columnar epithelium. Cyclic and gestational trends were not too apparent. High activity was the rule during diestrus. In the gravid endometrium, the surface epithelium showed less activity, and some decrease in alkaline phosphatase activity was noted in late pregnancy.

In a few gravid uteri, the surface epithelium which approaches the placentome was seen. It appeared extremely pseudostratified and variable in height. In this region, it showed considerable degeneration and was often completely missing (Figs. 22, 24).

The Placenta.

Under the caruncular or maternal part of the placentome, the s. functionalis remains uninvaded by any fetal elements (Fig. 26). Superficially, there is a cellular zone which is continuous with the s. compactum of the intercaruncular area (Figs. 22, 24). This zone morphologically and histochemically resembles the adjacent s. compactum. Connective tissue septa extend radially inward from this subplacentome s. compactum to form the stroma of the uterine crypt walls. The small placentome lies flat on the uterine surface. With continued growth of the fetal cotyledon at the periphery, and with coincidently stimulated growth of the caruncle, the placentome assumes a pedunculated, mushroom shape. Separation of the fetal

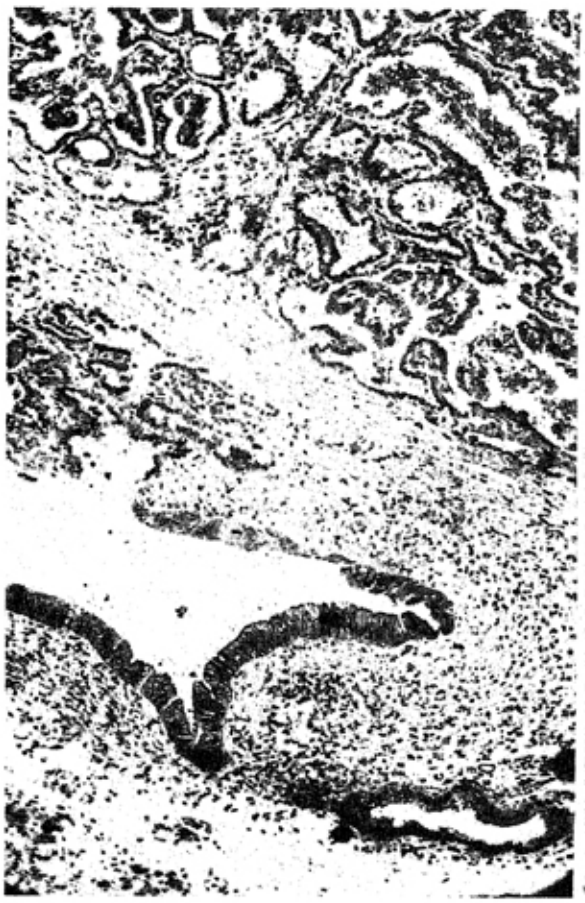


Fig. 24.—Endometrium at peduncle of placentome, 98 days gestation. Note degenerate surface epithelium which appears missing just next to the caruncular septa. Mallory's phloxine-methylene blue. X 66.

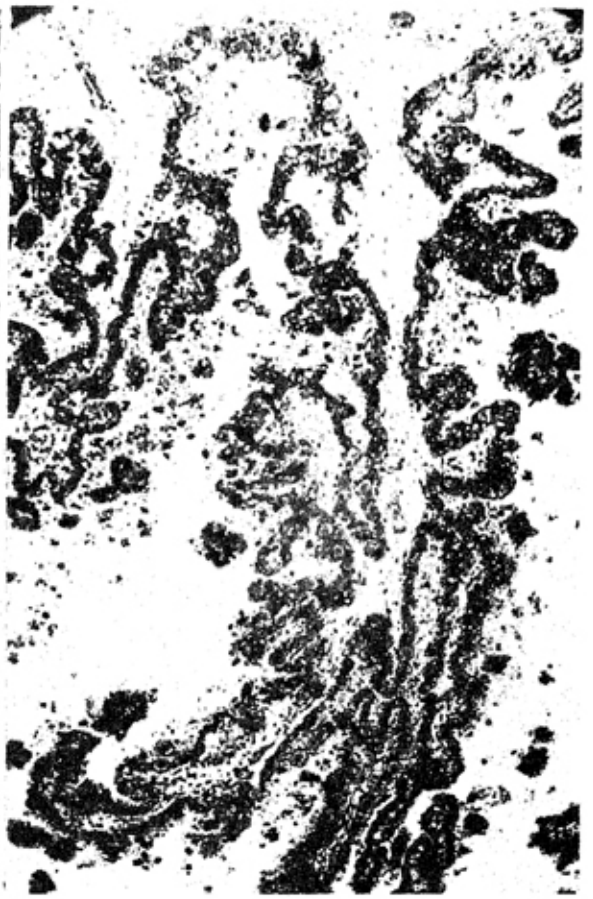


Fig. 25.—Full length of average villus at 58 days gestation. Note loose mesenchymal core and basophilic trophoblastic covering. Columnar arcade trophoblast shown. Mallory's phloxine-methylene blue. X 66.

and maternal tissues reveals a honeycombed caruncular surface, the mouths of the crypts which surround the fetal villi. The connective tissue of the septa is hypoplastic throughout gestation. On their distal ends, the septa are often enlarged and appear anastomosed. Here, the connective tissue is more cellular and closely resembles that of the s. compactum. The septal stroma is composed predominantly of argyrophilic fibers of medium thickness and density. No collagenous fibers were observed in early pregnancy, but in the average sized placentome examined at 102 days a few, delicate collagenous fibers were stained. Collagen appeared to increase slightly with aging of the placentome (Fig. 28). The septa are not particularly vascular; however, the blood vessels of the septa are very thin-walled, and lacunar blood spaces were often seen (Fig. 30).

A diffuse, apparently saliva resistant, Schiff's positive material was seen in the septal stroma of the uterine caruncle. The estimated amount increased from slight to moderate during gestation. Within a uterus, amounts were heavier in the larger caruncles. The septal stroma was generally negative for alkaline phosphatase activity, however, in the narrower septa and on the distal ends a slight activity was detectable (Fig. 27).

Fig. 26.—Placentome formation superficial to the stratum compactum, 53 days gestation. Note stroma of radial septa formed from proliferation of stratum compactum. Mallory's phloxine-methylene blue. X 66.

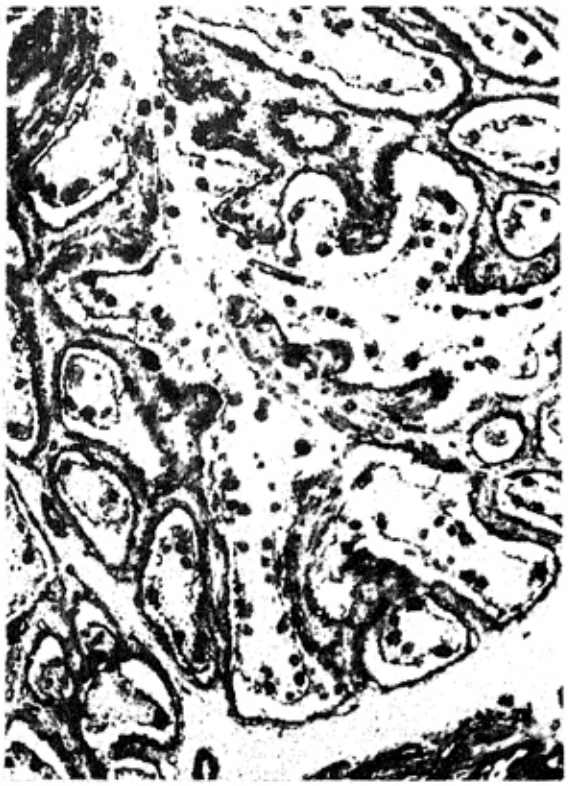
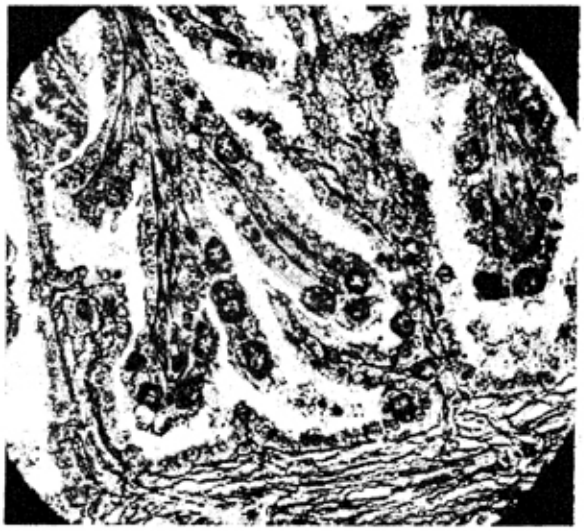
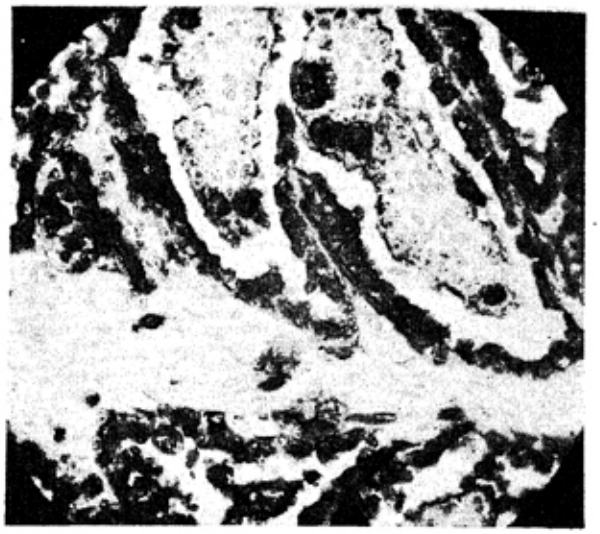


Fig. 27.—From large placentome, 241 days gestation. Dark color indicates alkaline phosphatase activity. Note reactivity of giant cells and crypt lining syncytium. Gomori's alkaline phosphatase. X 66.





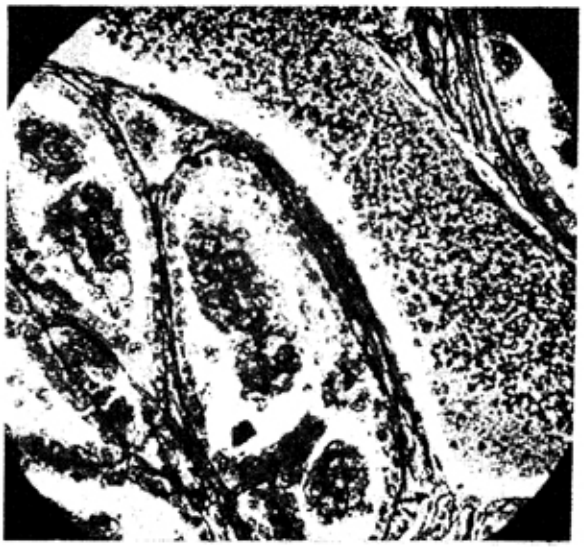


Fig. 28.—Branching fetal villus in uterine crypt, 112 days gestation. Note synctyium of trophoblast and crypt lining. Two large nuclei are in crypt lining syncytium. Wilder's silver impregnation and Van Gieson's. X 200. (Upper left)

Fig. 29.—Section of placentome, 112 days gestation. Darkened areas indicate alkaline phosphatase activity of giant cells and crypt lining syncytium. Note evidence of shrinkage. Gomori's alkaline phosphatase. X 200. (Upper right)

Fig. 30.—Section of average size placentome at 112 days gestation showing large, lacunar maternal blood vessel and its relationship to the villi. Wilder's silver impregnation and Van Gieson's. X 200. (Lower left)

A low, epithelial-like syncytium covers the connective tissue stroma of the uterine septa or crypts. In the crypts, it measures about 10 micra in height. On the distal septa, it may be attenuated or completely missing. The syncytial nuclei measure from 5.4 to 7.8 micra in diameter, and in the crypts, most of the syncytial cytoplasm lies between them and the septal stroma. The short reticular fibers of the septa are slightly thickened below the syncytium, (Fig. 28); however, in places these fibers appear discontinuous and here no "basement membrane" was noted. The syncytium gives a uniformly moderate acidophilic reaction. In this ground mass is a relatively heavy concentration of fine granular basophilia. No gestational trends in acido- or baso-philia were observed. The syncytium gave a heavy alkaline phosphatase reaction in all placentomes (Fig. 29). In latter pregnancy, some decrease in activity was evidenced. Histochemically demonstrable, acetone-soluble lipid was more abundant in this syncytium than in any other uterine or placental tissue (Figs. 31, 32). These sudanophilic and birefringent deposits ranged in size from

Fig. 31.—Frozen-cut section of large placentome at 241 days gestation, showing some fetal villi in uterine crypts. Unstained. X 352.

Fig. 32.—Sale area as Figure 31, but showing birefringent, acetone-soluble lipid crystals in the crypts' walls of the caruncle. Unstained. Photomicrographed through crossed Nicols. X 352.

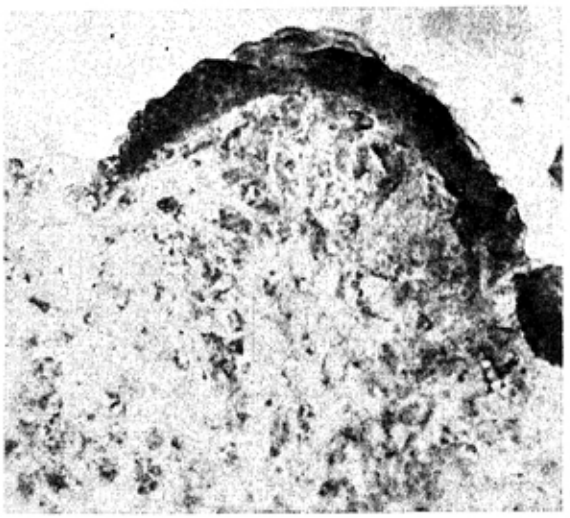


Fig. 33.—Distal, enlarged tip of uterine caruncular septum, 112 days gestation. Frozen-cut section. Syncytial covering is strongly basophilic. Methylene blue. X 352.

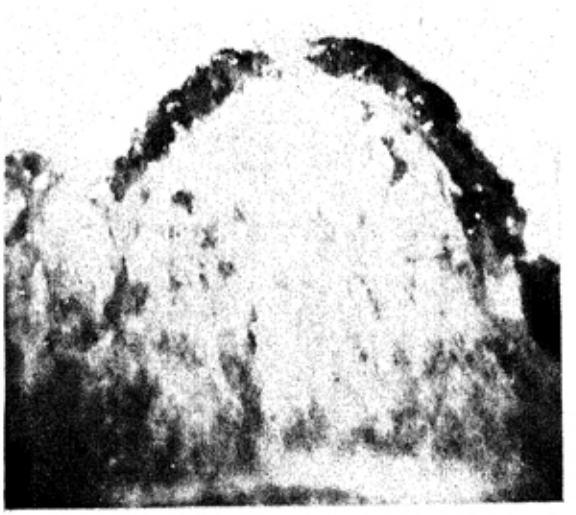


Fig. 34.—Shows tissue seen in Figure 33, but photomicrographed through crossed Nicols. Birefringent lipid crystals are shown in the syncytium covering the septum. Methylene blue. X 352.

barely microscopic up to about 10 micra. Their color with sudan IV varied from orange for the smaller droplets to a bright red for the larger. The reactivity was especially heavy on the distal ends of the septa (Figs. 33, 34). There was a general increase in syncytial lipids with advancing gestation (Fig. 35). No glycogen or glycoprotein was detected in the syncytium covering the uterine septa of the caruncle.

The villi of the cotyledon consist of an outer trophoblastic layer and a mesenchymal core (Fig. 25). Primary villi, that is, villi without a mesenchymal stroma were not seen. With advancing gestation, the villi lengthen and branch profusely, so that the originally loose fetal-maternal attachments become very firm. The mesenchyme of the chorio-allantois or membranous chorion, which fuses with the amnion, is essentially the same as that of the villi. The tissue changes from extremely loose in early gestation to moderately dense at 241 days. The stellate-shaped mesenchymal cells also increase in numbers, although the tissue remains hypoplastic. The irregular, short mesenchymal fibers are silver impregnated with Wilder's technique. As the placentome ages, wavy collagenous fibers appear, especially toward the

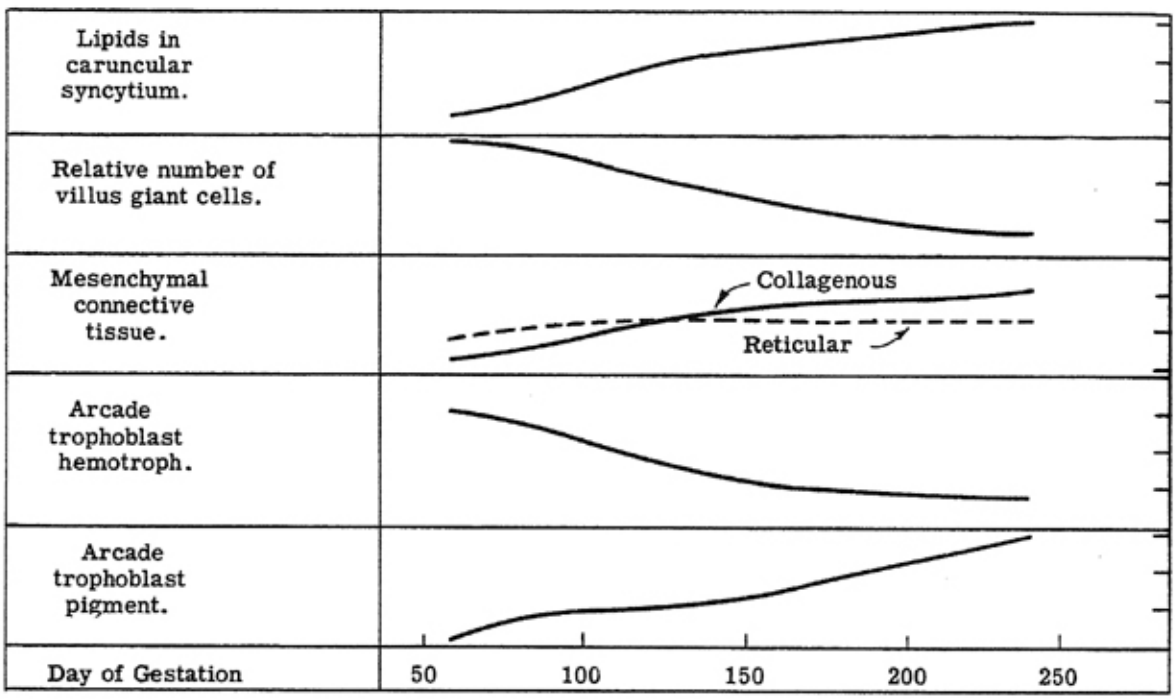


Fig. 35.—Graphic representation of microscopic changes occurring in the bovine placentome during gestation.

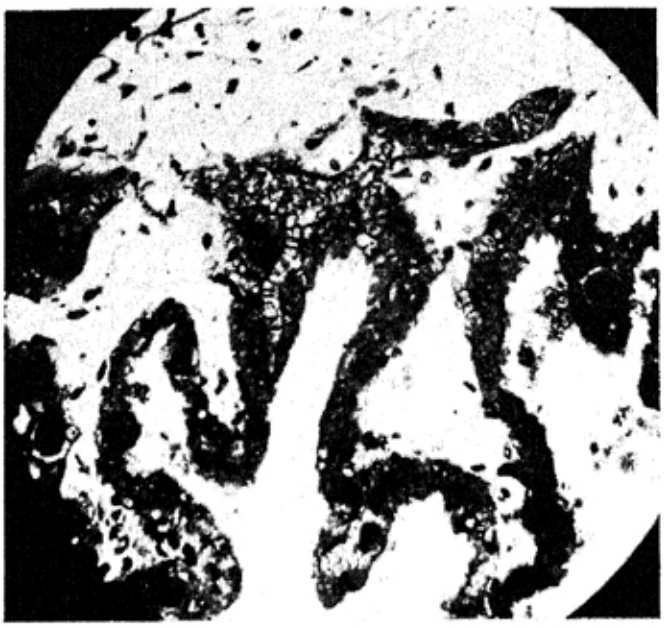


Fig. 36.—Section of basal chorion at 112 days gestation, showing short villi. In the arcade trophoblast, near the center, is an intratrophoblastic capillary. Mallory's phyloxine-methylene blue. X 226.

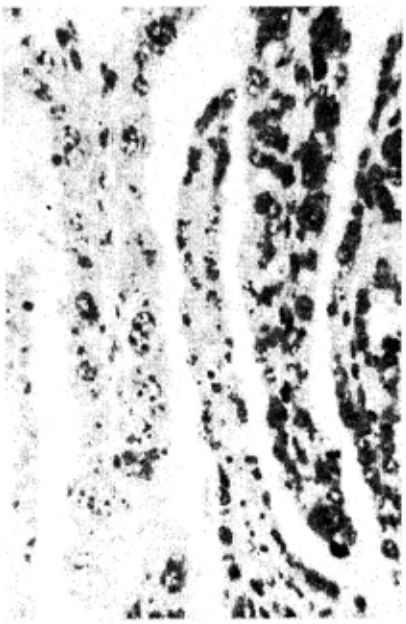


Fig. 37.—From placentome at 112 days gestation showing villi, with giant cells projecting into uterine crypts. Note syncytial appearance of trophoblast and crypt lining tissue. Mallory's phloxine methylene blue. X 262.

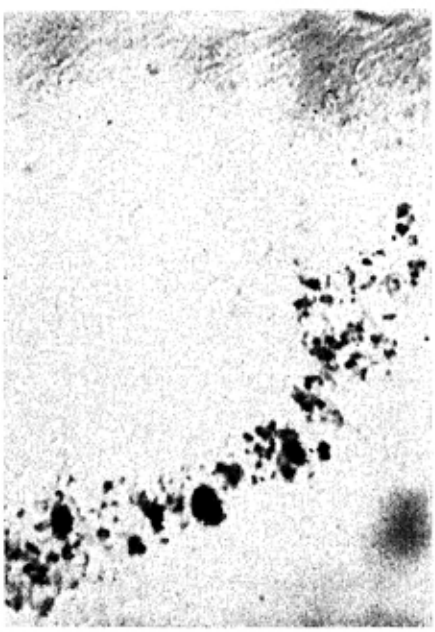
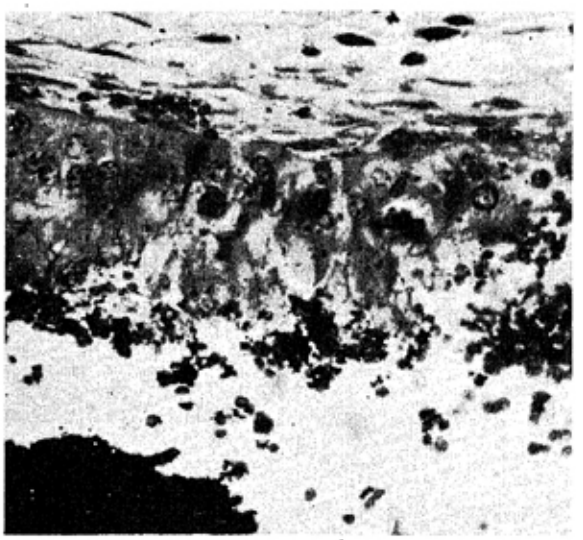
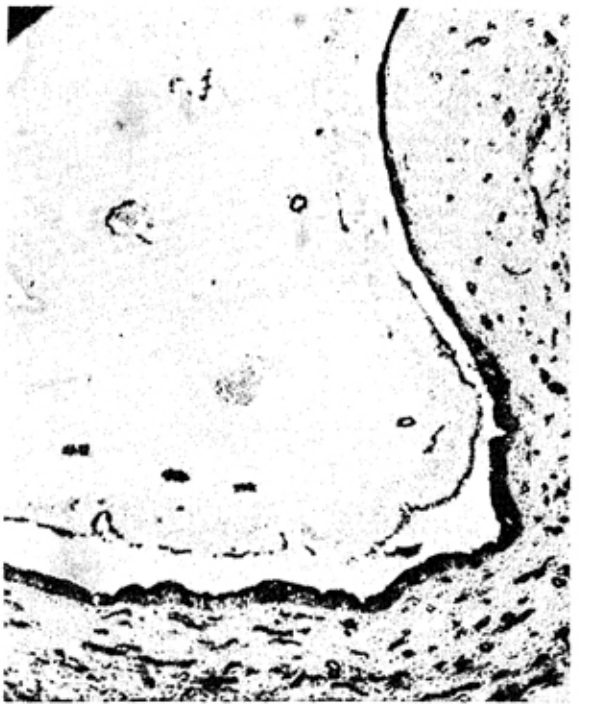


Fig. 38.—Frozen-cut section of chorion showing columnar arcade trophoblast, 178 days gestation. Cells loaded with a yellow-brown birefringent pigment. Unstained. X 274.

Fig. 39.—Arcade trophoblast of a cotyledon, 58 days gestation. Note vacuolated trophoblastic cells, hemotroph absorption, and delicate mesenchyme. Mallory's phloxine-methylene blue. X 249.





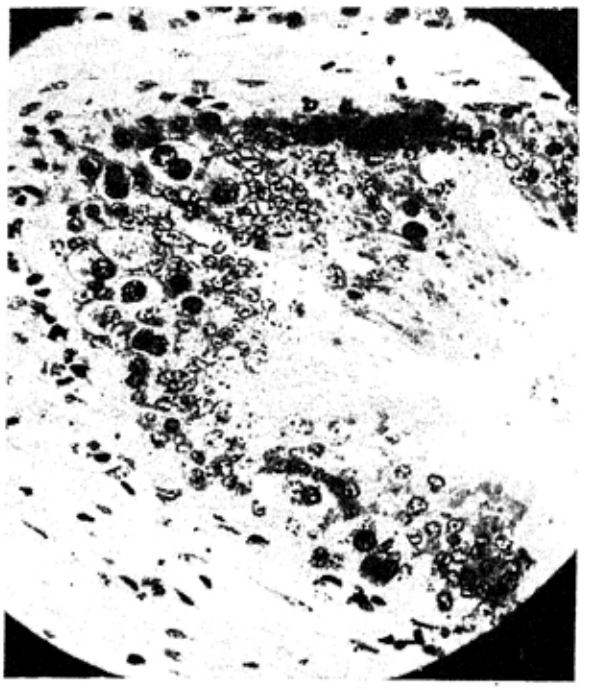


Fig. 40.—Irregular, columnar arcade trophoblast of the chorion, 79 days gestation. A large mass of hematroph is shown. Some erythrocytes are being absorbed. Mallory's phloxine-methylene blue. X 257. (Upper left)

Fig. 41.—Intercotyledonary chorion projecting into a uterine fold, 178 days gestation. Dark color indicates alkaline phosphatase activity. Note variability of uterine surface epithelium. Gomori's alkaline phosphatase. X 64. (Upper right)

Fig. 42.—Deep fold in the intercotyledonary chorion, 178 days gestation. Note giant cells, syncytial trophoblast, and histotroph. Mallory's phloxine-methylene blue. X 217. (Lower left)

fetal or amniotic side of the fused chorio-amnion (Fig. 43). The basement membrane of the trophoblast is a very thin reticular membrane. The general mesenchyme is negative for alkaline phosphatase, glycogen, and lipids. During gestation, a few glycogen deposits accumulate in the subtrophoblastic mesenchyme of the chorio-allantois at the bases or arcades of the villi.

The inner or fetal aspect of the avascular amnion is projected into high, branching folds. The tissue is generally covered by simple cuboidal ectodermal cells, however, there are patches of stratified squamous epithelium. These patches of amniotic epidermis are loaded, except in the germinal layer, with a Schiff's positive material which is completely removed by salivary digestion (Fig. 44.) Near the surface, the polyhydral cells of this amniotic epidermis tend to coalesce and disintegrate.

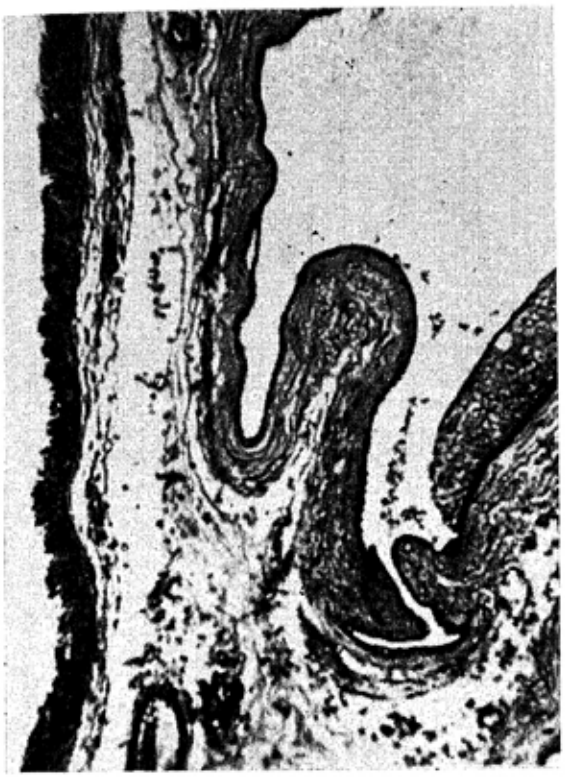


Fig. 43.—Intercotyledonary chorio-amnion, 79 days gestation. Note tall columnar trophoblastic cells, folds of amnion and patch of amniotic epidermis. Wilder's silver impregnation and Van Gieson's. X 80.

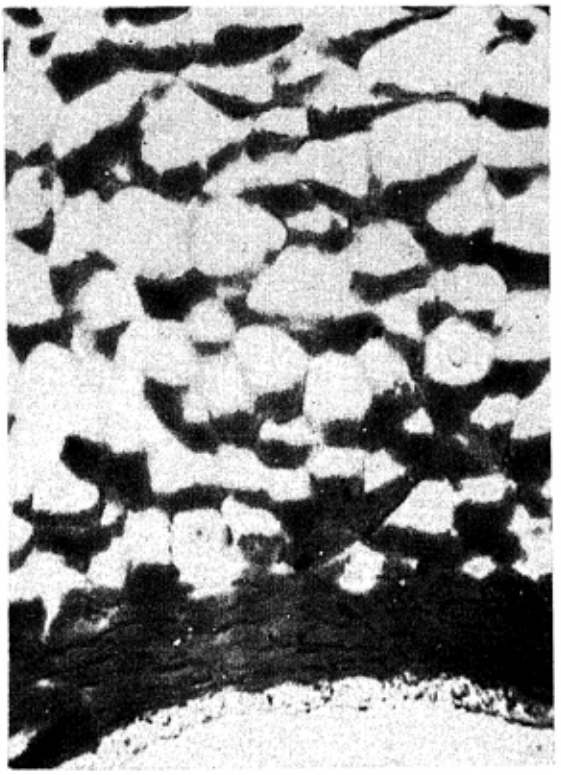


Fig. 44.—From patch of amniotic epidermis showing the stratified squamous epithelium, 102 days gestation. Darkened cytoplasm indicates glycogen; germinal epithelium is negative. Schiff's leucofuchsin. X 344.

The trophoblast of the villi is a heterogeneous tissue composed of large, discrete, often binucleated giant cells set in a syncytium (Fig. 37). The giant cells vary in diameter from 12.1 to 24.2 micra. Their nuclei range from 7.3 to 12.1 micra in diameter. The relative number of giant cells decreases slowly during gestation, and so, by late gestation the trophoblast of the villi is largely a syncytium. The nuclei of the giant cells show a few, coarse basophilic granules. The cytoplasm usually has a moderate amount of fine basophilia. A few giant cells show cytoplasmic vacuolation and nuclear degeneration at all times. The giant cells give a rather constant, heavy alkaline phosphatase reaction (Fig. 27). Another conspicuous feature of the giant cells is the moderate to heavy red staining of these cells with Schiff's reagent. This reactivity appeared to be unaltered by salivary digestion. The syncytial trophoblast of the villi is composed of a moderately acidophilic ground mass containing fine basophilic granules. Its nuclei range in diameter from 4.6 to 7.7 micra. These nuclei readily conform in shape to surrounding elements, such as, the cytoplasmic membrane of a giant cell. The syncytial trophoblast

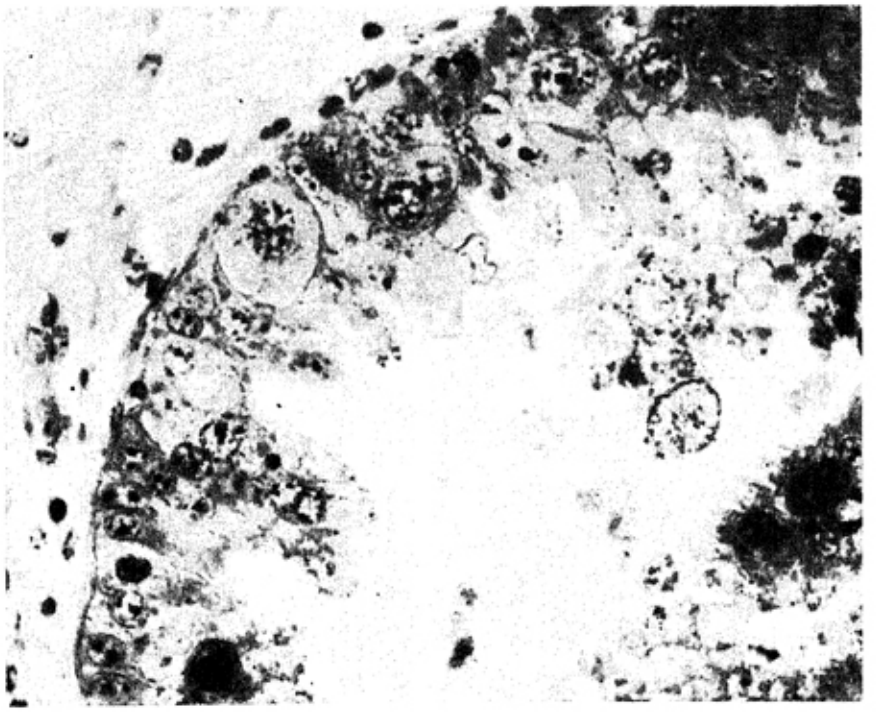


Fig. 45.—Small section of arcade trophoblast of the cotyledon, 98 days gestation. Several giant cells and histotroph are shown. Mallory's phloxine-methylene blue. X 367.

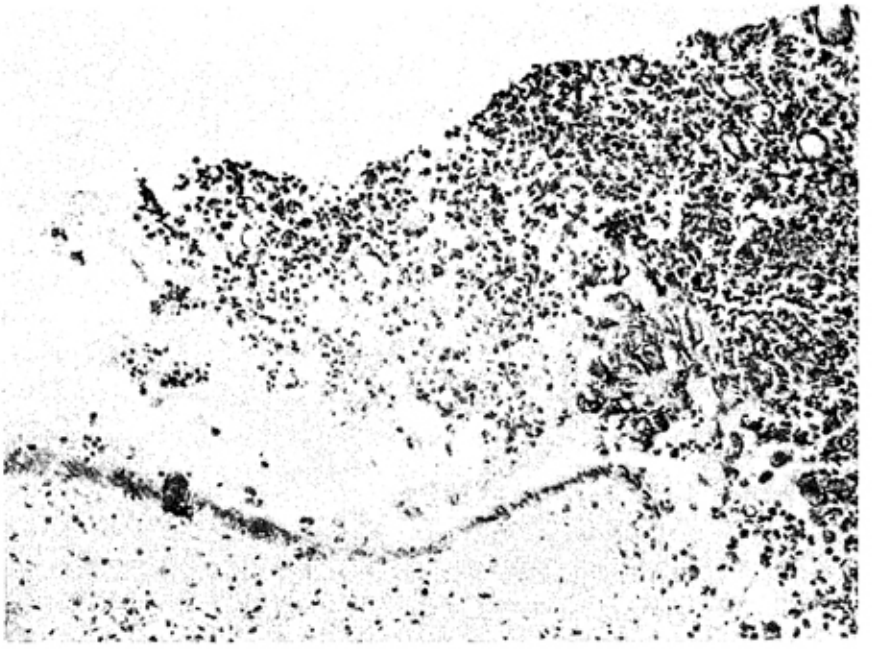


Fig. 46.—Uterine surface epithelium regenerating below the amorphous degenerating placentome, eight days postpartum. Dark stain in epithelial cytoplasm indicates glycogen. Schiff's leucofuchsin and Lillie's acid hemalum. X 97.

of the villi appeared negative for alkaline phosphatase, glycogen, and lipids.

The trophoblast between the bases of the villi, often referred to as "arcade trophoblast," differs from that on the villi. It has fewer giant cells and its syncytium is inconspicuous. The predominant cells are irregularly tall columnar cells (Fig. 40). The distal border of these cells is very serrated and their cytoplasm is often vacuolated. These columnar cells appear to absorb histolytic materials (Fig. 45), especially red blood corpuscles (Fig. 39), from the space between the distal ends of the caruncular septa and the arcade trophoblast. An acetone-insoluble, birefringent, yellow-brown pigment was seen in the columnar trophoblast of all placentomes except in the 58 day placenta (Fig. 38). The amount of pigment appeared to vary inversely with the amount of hemotroph seen in these cells, being light in early gestation and heavy in the 241 day placentomes. Extravasation of blood from the distal ends of the caruncular septa appeared to be inversely related to the size of the placentome. The columnar trophoblast of the arcades was negative for alkaline phosphatase activity. It gave a slight, fine granular Schiff's reaction in early gestation and a moderate reaction in latter pregnancy. The reaction appeared to be unaltered by exposure to saliva. Acetone-soluble lipids were not definitely identified in the arcade trophoblast.

Intratrophoblastic capillaries were seen in both the villi and arcades (Fig. 36). They were found in the inferior part of the trophoblast, were more numerous on the villi, and appeared most prominent in the smaller placentomes. They were usually dilated with red blood corpuscles. All the fetal erythrocytes which were seen in the placentas examined in this study appeared to be enucleated.

The trophoblast of the intercotyledonary chorion resembles that of the arcades, being pseudostratified tall columnar (30 to 55 micra) with a serrated distal border (Figs. 41, 43). Giant cells are not as conspicuous. Unlike in the arcade trophoblast, acetone-soluble lipid deposits were definitely identified in this columnar trophoblast of the intercotyledonary chorion. Pigment was seen and this is consistent with the evidence of embryotroph absorption by these cells (Fig. 42).

Within the placentome, a space which normally varies from 2.5 to 10 micra separates the villus from the crypt wall. This intracrypt space is narrower in the frozen-cut sections than in the tissues which were sectioned in paraffin. The syncytia bordering the 2 sides of the space have frayed distal edges, indicating a pulling apart of the tissues. (Fig. 29).

Moisture analyses on representative placentomes from Cows 15 and 17 showed an average moisture content of 90.2 per cent for the fetal part and 83.6 per cent for the maternal. The maternal part was 6.6 per cent higher in dry matter. The very high moisture content of the cotyledon is a reflection of the extremely loose appearance of the mesenchymal tissue of the chorio-allantois.

Two Postpartum Uteri.

In the 8-day postpartum uterus, the placentomes were a degenerate mass of cells showing much extravasation of blood from the collapsed vascular system. The mass had a high lipid content. Most of the cells of the area were negative for alkaline phosphatase and fuchsinophilia, however, a few large cells contained alkaline phosphatase and a glyco-protein. The surface epithelium was growing in from the intercaruncular area at the periphery of the placentome, cutting off much of the loose, degenerating mass (Fig. 46). Regression was not completed in the 25-day postpartum uterus. It still showed a moderate hypertrophy. The s. compactum was abnormally hypoplastic and negative for alkaline phosphatase activity (Fig. 47). Endometrial pigment was prominent, as was glandular and surface epithelial glycogen. Pigment deposits were especially numerous in the caruncle (Fig. 48). Many of these yellow-brown deposits appeared to be sudanophilic and many colorized leucofuchsin. The pigment was anisotrophic.

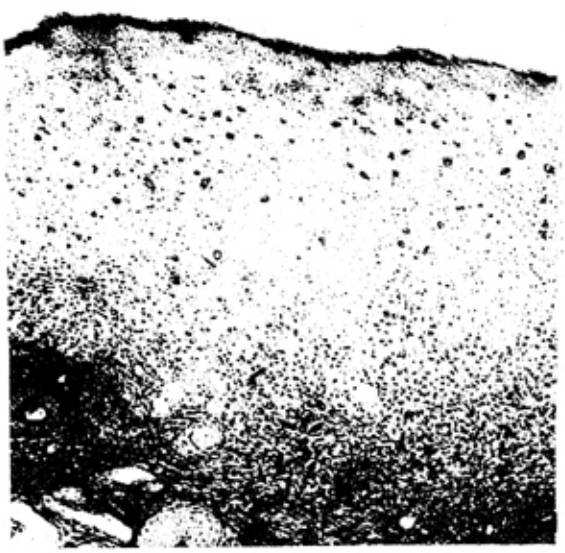


Fig. 47.—Caruncle at 25 days postpartum. Dark color indicates alkaline phosphatase activity. The stratum compactum is hypoplastic and alkaline phosphatase negative. Regression not completed. Gomori's alkaline phosphatase. X 62.

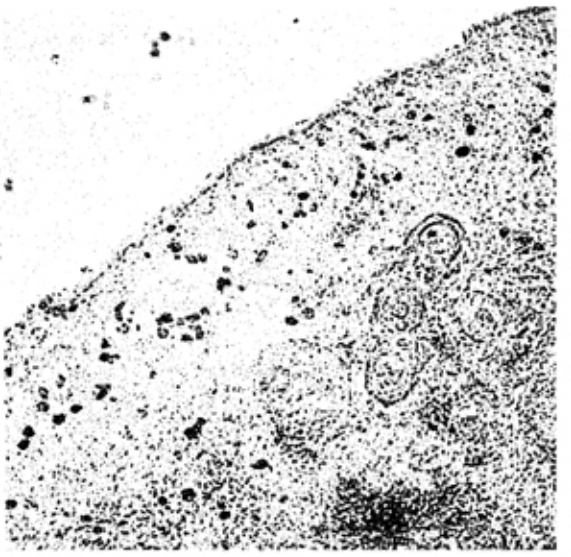


Fig. 48.—Frozen-cut section of caruncle at 25 days postpartum. Note concentration of the yellow-brown pigment granules in the stratum compactum. Sudan IV and hematoxylin. X 62.

DISCUSSION

Morphological and histochemical changes which occur in the oviduct and uterus during the estrous cycle are minor. This appears to be a reflection of the overlapping of cycles, as suggested by Asdell (1946a). The corpus luteum does not begin regression until late diestrus, and the genital tract shows both a luteal and a follicular influence during proestrus. Another confounding factor in the present study is, of course, the possibility of breed differences, age differences, and intraorgan variations. In the gravid uterus, gestational changes in the placentomes are equally difficult to evaluate. It is impossible to know the age of a placentome, for all placentomes do not form at the same time. Size of the placentome may not be an index of its age. For example, a recently established placentome with a favored blood supply could surpass in size an older placentome. Nevertheless, consistent trends can be interpreted with some confidence.

The oviduct is very active under follicular stimulation. Alkaline phosphatase, which is apparently involved in a number of solute transfer and metabolic functions (Moog, 1946), shows a high level of epithelial activity during estrus and decreased activity during diestrus. Basophilic cytoplasmic granules, which indicate ribonucleo-protein (Brachet, 1940), follow a similar trend. The absence of histochemically demonstrable epithelial glycogen and lipid during estrus and their accumulation during diestrus further indicates the cyclic activity of the tubal mucosa.

Sparseness of cilia in the mid-region of the oviduct, even during estrus, precludes their effectiveness in moving spermatozoa or ova through this portion of the tube. They are better described as stereocilia or cytoplasmic projections. Roark and Herman (1950) related the process of nuclear extrusion to cytoplasmic projections and to diestrous cellular regression. Casida and McKenzie (1932) noted a similar holocrine type of secretion in the tubal epithelial of the ewe. In the present study, a direct relationship was noted between the degree of pseudostratification, the number of goblet-like cells, and nuclear extrusions. All three phenomena, in addition to cytoplasmic projections, appear to indicate diestrous cellular regression in the tube.

The epithelial-lined pockets of the oviduct have been clearly figured, but not discussed, by Roark and Herman (1950), and Casida and McKenzie (1932). Serial sectioning indicates that these pockets are not artefacts. Since the pockets appear to open either anteriorly or posteriorly into the central lumen, it is difficult to ascribe any special physiological function to them. Their epithelial cells resemble those of the mucosal folds and they are, therefore, capable of adding to the secretions of the tube.

The myometrium increases in size under ovarian stimulation. There is both a hypertrophy (Cupps and Asdell, 1944) and a hyperplasia (Allen 1937) of the smooth muscle cells. Myometrial hypertrophy appears to occur during the luteal phase of the estrous cycle. This hypertrophy continues dur-

ing gestation. Enlargement of connective and vascular tissues, under continuous luteal stimulation, brings about a further increase in size of the myometrium. The transformation of argyrophilic fibers into collagenous fibers, which has been demonstrated in tissue cultures by Maximow (1928), is well illustrated in the myometrium. During gestation, the narrow reticular fibers, which represent an immature type of connective tissue (Maximow and Bloom, 1949), become replaced by wide collagenous fibers.

Progestational changes which were observed in the s. functionalis of the endometrium were essentially the same as those described by Asdell, *et al.* (1949). Stromal edema develops with the corpus luteum. When the corpus luteum regresses, the stroma atrophies. Burack, *et al.* (1942) observed in the rat that estrogen induced a hastening of the transformation of endometrial reticulum into collagen. In the present study, however, reticular fibers appeared to be slowly replaced by collagenous fibers during the cycle and during gestation. Collagen is evidently the hyaline material which Hammond (1927) observed to accumulate during the luteal phase.

The s. compactum is an area of prime importance in the formation of the placentome. The s. functionalis shows comparatively little hypertrophy or hyperplasia during gestation. Distention of the uterus during pregnancy stretches it into a rather thin, afibrillar stratum. Therefore, it is physically impossible for the large, definitive caruncle of pregnancy to have formed from this layer. Furthermore, the uninvaded, loose s. functionalis with its hypertrophied uterine glands can be seen below the largest of placentomes. The appearance of the s. compactum in the non-gravid uterus is indicative of its remarkable proliferative properties. It is an extremely hyperplastic area, and, as noted by Murphey (1924), its nuclei are essentially embryonic in character. Nuclei of this layer are rounded and the cells are rather discrete. Its dense connective tissue fibers are reticulum, that is, they represent a more immature type of tissue (Maximow, 1928) capable of considerable growth. The heavy alkaline phosphatase activity of the area in the cyclic uterus also suggests the high metabolic potentialities of this tissue. It is apparent that the maternal part of the placentome is an outgrowth from the s. compactum. This growth is evidently stimulated by contact with the trophoblast, but the mechanism of this influence is not indicated.

The postestrous accumulation of blood in the capillaries of the s. compactum, especially in the subepithelial stroma, has been reported by a number of investigators (Hammond, 1927; Kolster, 1903; Murphey, 1924). Weber, *et al.* (1948), observed that hemorrhagic material was associated with both caruncular and intercaruncular areas. Hansel and Asdell (1951) noted that coiling of endometrial arterioles served to reduce bleeding from endometrial capillaries and that caruncular arterioles were more tightly coiled than those in intercaruncular areas. In the present study, hemorrhage associated with localized disruption of the surface epithelium was most apparent in the

intercaruncular areas. Accumulations of blood were heavy at the margins of the caruncle where a dense, subepithelial capillary plexus is seen. Furthermore, the pigment granules of the stroma are concentrated in these same regions. The appearance of this yellow-brown pigment following metestrous hemorrhage indicates its hematogenous origin.

The histochemistry of the hematogenous stromal pigment suggests its lipoprotein nature. This lipoprotein may merely represent the end-product of degenerated red blood corpuscles in the process of being resorbed. However, it may have additional significance for Szego and Roberts (1946) have estimated that two-thirds of the circulating estrogen in cows' blood is found with plasmal protein. Hartroft (1951) has produced a ceroid-like substance by incubating erythrocytes with lipids. This pigment resembles that seen in the endometrial stroma of the cow. The hematogenous, endometrial pigment appears to be identical with that seen in the columnar trophoblast and in the subplacentome s. functionalis during early gestation.

Lipid and glycogen of the uterine epithelium in the non-gravida appear to be inversely related to luteal and alkaline phosphatase activity. Both of these metabolities are seen very little or not at all during much of diestrus. Alkaline phosphatase activity appears heavy during this proliferative phase. Then, when luteal activity begins to regress in late diestrus, lipid and glycogen start to accumulate in the uterine epithelium.

The uterine glands appear to continue active secretion during gestation. In late gestation, there may be decreased glandular activity as shown by some increase in lipid and a decrease in alkaline phosphatase. The isolated uterine gland epithelium seen in the lumina of some hypertrophied glands appears to be a sectioning artefact rather than histotroph for absorption by the intercaruncular columnar trophoblast, as suggested by Kolster (1903) and Jenkinson (1906).

No secretory difference was detected between the glandular and surface epithelium. However, more lipid and glycogen were noted in the surface epithelium. The glandular epithelium did not exhibit the fragility which was often seen in the surface epithelium. Pseudostratification, nuclear pyknosis, and other indications of cellular degeneration often characteristic of the surface epithelium were not noted in the glands. The significance of this surface epithelium fragility is conjectural. Melton, *et al.* (1951) in their careful study of implantation, noted that the uterine surface epithelium was eroded away by the trophoblast, preparatory to the embryo's implantation. Perhaps, the fragility enhances implantation.

Unfortunately, early placentation was not observed during this study. The average sized placentome on the 58th day of gestation contained all the elements seen in the definite placentome. The most conspicuous change in the average placentome up to 241 days of gestation was the formation of the peduncle and an increase in size. Increasing complexity in the branching of

villi aided in strengthening the placental union. The characteristic aging of connective tissue, replacement of reticular fibers with collagenous fibers, is seen in both the caruncles and the cotyledons.

Sections of the placentomes have been examined carefully in an attempt to define the relationship between the ectoderm of the chorion and the uterine caruncle. Wimsatt (1951) has recently stated that the caruncular crypts are lined by a simple cuboidal epithelium of maternal origin and that this covering of the septa persists except on the distal ends of the septa. Thus, the placental relationship in the cow would be mainly an epitheliochorial type with some syndesmochorial type where the septal stroma was exposed. Since Hammond (1927), Hallman (1925), and Melton, *et al.* (1951) observed that the trophoblast removed the uterine surface epithelium, it must by definition be regenerated on the crypt walls for an epitheliochorial type of placentation. Hammond (1927) does not believe this occurs, and there is some evidence for his belief.

The uterine surface epithelium next to the placentome appears degenerate and often desquamated. Morphologically, it does not resemble the low syncytial lining of the caruncular crypts. It differs histochemically in that it contains much less lipid and, unlike the crypt syncytium, it contains glycogen.

Since in the prepared sections the intracrypt space separates the villus from the crypt wall, it is logical to assume that the crypt lining syncytium is of maternal origin. However, the intracrypt space is an artefact caused by dehydration. The microscopic techniques used, shrink both the fetal and maternal tissues of the placentome. Since the villi contain less dry matter than the crypt walls, they shrink more and pull away from the crypt wall. Now, if the syncytial trophoblast adhered more firmly to the stroma of the crypt walls than it did to its homologous tissue, during dehydration it would separate leaving part of the syncytium on the villus and part on the crypt wall. When the two tissues are visualized as being together, as they are in the functioning placentome, it becomes apparent that the crypt lining syncytium could be a part of the trophoblast. Such a placental relationship would then be of the syndesmochorial type (trophoblast against the caruncular connective tissue), and would resemble closely that described by Assheton (1906) and Wimsatt (1950) for the sheep.

Two reactions of the crypt lining syncytium militate against this conclusion. Firstly, lipid was observed in the uterine syncytium, but not in the trophoblast on the villus. Secondly, the uterine syncytium formed a continuous alkaline phosphatase barrier between the crypt and the villus. The syncytial trophoblast of the villus was negative except for a few reactive spots on its frayed distal border. Therefore, because of these differences in histochemistry between the two syncytia, different origins may be indicated. It is interesting to note that in other species lipid deposits, which may be steroid

hormones, are localized in the trophoblast (Wimsatt, 1951; Wislocki and Bennett, 1943; Wislocki and Dempsey, 1946).

The function of the giant cells of the trophoblast remains in doubt. Wimsatt (1951) does not believe they form a syncytial trophoblast in the cow as they do in the sheep. Kolster (1903), Drieux and Thiery (1951), and Amoroso (1952) have figured giant cells within the uterine crypt lining. In the present study, large nuclei which resembled those of the giant cells were occasionally seen in the syncytium of the crypt; however, no discrete cellular membrane surrounded these nuclei. Amoroso (1952) in his Figure 15.42 does show discrete, binucleated giant cells in the crypt lining, and he considers the bovine placenta to be of the syndesmochorial type.

Marshall, in 1910, stated that it was doubtful if any anatomical structure had given rise to keener or more prolonged controversies than the placenta. The statement remains very applicable for the placenta of the cow.

The columnar trophoblast in the arcades of the cotyledon and in the intercotyledonary area is active in absorbing histotroph cast off from the endometrium. This function appears to be diminished in latter gestation as estimated from observation of the columnar cells. However, it is difficult to make any relative estimate for no data are available on changes in this absorptive area per unit of fetal weight.

The fuchsinophil in the patches of amniotic epidermis is glycogen as shown by its complete removal with salivary digestion. Bradfield (1951) has shown that glycogen tends to accumulate in areas which have a poor supply of glucose and oxygen. Morphologically, the mesenchyme of the chorio-amnion is avascular toward the fetal side, suggesting that the area is metabolically inactive. The accumulation of glycogen in the amniotic epidermis may reflect inactivity in this area.

In a study of the relation of the postpartum breeding interval to reproductive efficiency, Van Demark and Salisbury (1950) found a services per conception rate of 2.44 for cows bred between 21 and 40 days postpartum. When the first breeding was delayed until 81 to 100 days postpartum the services per conception rate was only 1.88. Microscopically, the uterus still showed the effects of gestation 25 days after parturition. Of particular significance was the hyaline, hypoplastic appearance of the s. compactum. The lack of alkaline phosphatase activity in this layer plus the abnormally high glycogen content of the surface epithelium suggests a quiescent state which may be unsuited for nourishing a free-living blastocyst or for allowing its implantation.

SUMMARY AND CONCLUSIONS

Reproductively normal oviducts and uteri from 8 non-pregnant, 8 pregnant, and 2 postpartum cows have been studied microscopically. Placentas of the pregnant animals were studied. In addition to the morphological examination, the tissues were observed histochemically for lipids, glycogen, alka-

line phosphatase, and type of connective tissue.

Cyclic changes in the oviducts during the estrous cycle indicate a high state of activity around the time of estrus, followed by decreasing activity during diestrus. The decreased metabolic activity is indicated by an accumulation of epithelial glycogen and lipid. The appearance of goblet-like cells and extruding nuclei in the mucosal epithelium during diestrus also reflect a regressive phase. Epithelial-lined pockets have been observed in the oviducts. They do not appear to have any special physiological function.

The non-gravid uterus is altered relatively little during the estrous cycle. This is especially apparent when gestational changes are compared with those seen in the non-gravida. There is no separate and distinct period of regression in the uterus during the estrous cycle. Progestational proliferation continues throughout diestrus, and there is very little evidence of uterine involution until proestrus.

The small amount of lipid and glycogen seen in the uterine glands was confined mainly to the gland necks. Amounts were minimal during the luteal phase, when alkaline phosphatase activity was high. The surface epithelium contained more lipid and glycogen than the glandular epithelium. The surface epithelium appeared degenerate and desquamated in many places during estrus and early postestrus.

Fragility of the surface epithelium coincides with hyperemia in the s. compactum. Blood may be trapped *in situ* and give rise to yellow-brown, lipoprotein pigment deposits.

The s. compactum is the very dense, cellular, superficial stratum of the endometrium. It is especially wide in the caruncle. The stromal cells of this area have remarkable proliferative properties for they form the connective tissue septa in the maternal caruncle of pregnancy.

Should conception take place, the proliferative activity which is initiated during diestrus in the non-gravida becomes exaggerated during gestation. The myometrium increases in size not only by hypertrophy and hyperplasia of the muscle cells, but also by growth in its connective tissue and vascular components. The s. functionalis becomes very edematous, and the uterine glands hypertrophy rapidly. The glands appear to secrete actively during gestation, with some decrease in activity being noted in late gestation. The intercaruncular surface epithelium shows localized desquamation in early pregnancy, but appears to be regenerated during gestation.

Placental attachment in the cow is localized in numerous placentomes. The placentome is composed of the fetal cotyledon with its villi, and the maternal caruncle which is formed into crypts. These crypts surround all villi except the short basal villi. The septa of the crypts are composed of connective tissue which is covered, except on the distal ends of the septa, with a low syncytium. The villi consists of a loose mesenchymal core covered by the ectodermal trophoblast.

The trophoblast is a heterologous tissue. That of the villi is an irregular, granular syncytium in which are numerous, discrete, often binucleated giant cells. The giant cells are especially conspicuous because of their normally heavy alkaline phosphatase and glycoprotein reactions. In the arcades, between the bases of the villi, the trophoblast is predominantly irregular, tall columnar cells which function in the absorption of histotroph. A few giant cells are seen here. The intercotyledonary trophoblast is essentially the same as the arcade trophoblast. Due to the absorption of hemotroph, a hematogenous pigment accumulates in the columnar trophoblast. Intratrophoblastic capillaries are seen on the villi and in the arcades.

The placental relationship between maternal and fetal vascular systems is of considerable teleological importance. However, the relationship remains in doubt because of the indefinite origin of the syncytium lining the caruncular crypts.

If the lining originates from uterine epithelium, the relationship is of the epitheliochorial type; that is, the trophoblast apposes the uterine epithelium. Evidence for this interpretation is: (1) the crypt lining syncytium contains lipid granules, but the syncytium on the villus is negative; (2) the syncytium of the crypt gives a positive reaction for alkaline phosphatase, but that of the villus is negative; (3) in prepared sections an intracrypt space separates the syncytium of the villus from that on the crypt wall, therefore the two syncytia appear to have different origins. The third reason can be disregarded since the intracrypt space is an artefact.

If the syncytium lining the crypts is the same as that of the trophoblast, the placental relationship is of the syndesmochorial type; that is, the trophoblast apposes the septal stroma of the crypt. Evidence for this interpretation is: (1) the uterine surface epithelium appears to be desquamated before implantation; (2) the surface epithelium next to the placentome does not morphologically or histochemically resemble the crypt lining syncytium; (3) frayed edges of the two histologically similar syncytia indicate a pulling apart due to dehydration.

Necrosis of the placenta occurs rapidly following parturition. However, regression was not completed by 25 days postpartum. This was especially apparent in the hypoplastic, alkaline phosphatase negative s. compactum of the endometrium.

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