THE

HISTOLOGY AND HISTOCHEMISTRY

OF

NORMAL ENDOMETRIUM

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THE HISTOLOGY AND HISTOCHEMISTRY OF NORMAL ENDOMETRIUM.

In the course of gynaecological practice at the Royal Samaritan Hospital, Glasgow, one has the invaluable opportunity to combine clinical observation and treatment of patients with a widening experience of the morphological and functional changes in tissues as a clinical pathologist. Some three thousand specimens are examined microscopically each year upon each of which a report is given to provide information useful to the clinician in establishing diagnosis and giving a basis for rational therapy.

At the present time, the outstanding advances in knowledge of the sex hormones and their actions have predominated in endometrial studies. However, clinical application of this knowledge is felt to be limited. Much research has been devoted to experimental animals and the analogies drawn from this material do not necessarily apply to the human. The variations in placentation in different animals serve to show how different may be the details of function of the endometrium. Again, although the method of simultaneously undertaking hormonal analysis and examination of the endometrium has become more reliable, it is now recognised that a given hormonal stimulus may have different manifestations depending upon the degree to which the mucosa has been prepared at the start of the stimulation and, more particularly, on the time which has elapsed since the preceding stimulus.

The endometrium presents very considerable variations in its reaction to the same stimulus in various locations. For example, it is noted that material from or near the isthmial portion of the mucosa is normally far less active than from elsewhere in the corporeal mucosa.

In clinical work, the present histological appearances are related, in time, to the date of the preceding menstrual period. Where these appearances do not coincide with that expected then hormonal imbalance is adduced as the reason. On the other hand, it can be postulated that there might be some inherent defect in endometrium itself which made its response to hormonal stimulation abnormal.

With the advent of modern histological techniques, it is now possible to apply these refinements where they have not previously been employed in routine examinations. These techniques are designed to show certain chemical constituents of tissue and one thought that their application to the histological study of endometrium might provide further knowledge of the physiological activity of that tissue.

In furtherance of this purpose, it was decided to select specimens of endometrial tissue obtained from women whose menstrual histories were normal and whose clinical conditions were in no way related to any upset of that function.

Some seven thousand specimens were reviewed and the routine histological picture assessed. Only specimens showing whole thickness of intact endometrium were used. Serial sections were cut from paraffin blocks and these were stained differentially so as to demonstrate the particular component required.

Before attempting to interpret the histochemical findings it was considered necessary to have as complete a record of the normal histological cyclical changes in endometrium as possible.

This study is therefore divided into several sections.

The first section comprises the embryological origin of endometrium, its development to maturity and the cyclical changes which occur in the course of a normal menstrual cycle. Personal observations are given at each phase and these are correlated with the standard descriptions and any recent development or finding in the literature corresponding to that phase. Only brief comparisons and criticisms are made in the course of this section.

Section two comprises the comparative histochemical changes of normal endometrium and, similarly, personal observations are correlated with the literature on each aspect with brief comment where necessary.

Section three was compiled to show the histological and histochemical appearances of endometrium prior to and subsequent to the reproductive era.

Section four comprises a critical investigation of the views or facts sited.

The illustrations are self-explanatory and are referred to in the subject matter.

Figures in parenthesis following surnames are the serial numbers of the references which are listed at the end of this study.

SECTION ONE.

CYTOLOGY OF THE NORMAL ENDOMETRIUM.

(a) Embryology.

The urogenital organs are mesodermal in origin (Gray) (1).

The mesoderm proper retains a dense packed cellular character. The mesenchyme consists of a loose tissue with a fluid matrix which allows cellular elements to alter their position by active migration.

The genito-urinary structures are differentiated very early in embryonic life. (Koff) (2) identified the Mullerian ducts in embryos only 11 mm. long considerably less than 8 weeks old. They appear as elongated infoldings of the epithelium of the posterior peritoneal cavity (coelome), on the lateral aspect of the Wolffian body. The edges of this infolding epithelium unite to form a tubular structure which is the Mullerian Duct. The upper end of this tube opens into the peritoneal cavity and becomes the fimbriated extremity. The lower end remains a solid mass of cells which burrows through the urogenital fold.

The Mullerian ducts also grow towards the midline approaching each other but remain distinct until they almost reach the urogenital sinus. The unfused transverse portions of these ducts combine with the fused caudal vertical portion to form the fundus and corpus of the uterus.

As differentiation proceeds, the wall of the uterus thickens and division into serous, muscular and mucosal layers gradually takes place. By the seventh month, the uterine glands appear (Hoffmann) (3).

The mucous membrane consists of an embryonic nucleated and highly cellular form of connective tissue in which run blood vessels and numerous lymphatic spaces. It contains many tube-like uterine glands which are lined with ciliated columnar epithelium and open into the cavity of the uterus (Gray) (1).

Novak (7) describes endometrium as a specialised type of connective tissue characterised by a remarkable lability.

Thus it is established that the mucous lining of the uterus is a columnar epithelium of mesodermal origin with a mesenchymal framework. A fuller study of these terms is now necessary.

Maximow and Bloom (4) state that the mesoderm keeps its epithelial character in the epithelium of the genital system. A large part of the embryonic mesoderm becomes the mesenchyme which gives rise to connective tissue. Between the epithelium and the underlying connective tissue there is usually a basement membrane, a condensation of the intercellular substance of the connective tissue at the surface of its contact with the epithelium.

These authors further describe the simple columnar citrated epithelium found in the uterus as consisting of tall prismatic cells adhering to one another by their lateral surfaces. In sections parallel to the surface is seen a mosaic much like that of other simple epithelia. In sections perpendicular to the surface the tall rectangles stand upright like fence palings. In many cases the oval nuclei are at approximately the same level. The free surface of the cells is provided with cilia. Epithelial cells sometimes cohere intimately by means of special structures. When the limits between cells cannot be detected, the epithelial sheet has the character of a syncitium. This is found, for instance, in the epithelium of the uterine mucous membrane at the beginning of pregnancy and in some areas of the ectoderm of the embryo (trophoblast).

Further, they state that the uterus is lined by a simple

Fig. 1. Showing presence of uterine glands at birth. H and E. 120 m.

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columnar epithelium. From fundus to vagina small groups of ciliated cells are scattered among the secreting cells. As far as the beginning of the cervical canal, the surface epithelium is substantially like that of the uterine glands which grow out from it in infancy. These are simple tubules, slightly branched in a zone adjacent to the myometrium.

In his textbook Stander (5) states that in the child the uterine glands are mere shallow depressions which according to Kundrat and Engelman do not appear until the third year; but the researches of Meyer, which were confirmed by Williams, show that they are frequently present at birth. Personal observation confirms this (Fig. 1).

Hoffmann (3), in a brief description of the interval from birth to puberty states that during childhood the reproductive organs gradually descend into the pelvis but their growth is very slow compared with the rest of the body. Except for a slight increase in size of the endometrial glands and some straightening of the convoluted uterine tubes there is very little change. The pubertal epoch occupies a period of years and is marked by a rapid development of the entire reproductive apparatus. The uterus begins to grow after the eighth year and does not attain full maturity for several years after the onset of puberty.

(b) Mature Endometrium.

The uterine glands are separated from one another by connective tissue, the stroma, which resembles mesenchyme. Its irregularly stellate cells have a large ovoid nucleus. The cell processes appear to anastomose throughout the tissue and adhere to the framework of reticular fibres, which are condensed as basement membranes under the epithelium. Elastic fibres are absent except in the walls of the arterioles. This is a ground substance at times rich in tissue mucoid; in it are lymphoid wandering cells and granular leucocytes. Maesophages are not uncommon but for some unknown reason there is no phagocytosis

Fig. 2. Regenerating Phase.

H and E. 120 m.

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of extravasated blood. This may perhaps be due to conditions similar to those responsible for the failure of blood to clot after it has been in contact with endometrial stroma. The number of uterine glands varies from one person to another and may be rapidly increased by budding and growth towards the surface from the basal zone. Such buds which do not reach the surface become dilated (cystic). The glands seem to vary in their proximity to one another during the menstrual cycle, owing largely to changes in the diameter of the glands and in the stromal ground substance.

(c) Historical.

It was not until 1873 that Kundrat and Englemann were among the first to study the microscopic changes in the endometrium during the menstrual cycle. Their specimens were obtained from cadavers. Sir John Williams supplemented their work. The observations of these men were probably the first clear exposition of the fact that menstruation is the culmination of a series of cyclic histologic changes. These views were far in advance of the times and were not generally accepted for 35 years until Hitschmann and Adler's (6) work appeared in 1908.

Since this date there have been many descriptions of endometrium at the various stages of the cycle that it is proposed, hereunder, to correlate the accepted appearances according to the classification of phases namely regenerating, proliferative, transitional, secretory and menstrual.

(d) Regenerating Phase (Figure 2).

Following menstrual desquamation which is uniform down to the basal arteries, the uterine cavity is lined by a raw surface which is quickly covered over, probably in a day or two, by proliferation of epithelium from the mouths of open uterine glands. Novak (8) describes creepers of flat epithelial cells arching out from the surviving gland elements and the surface being so quickly covered that examination of the

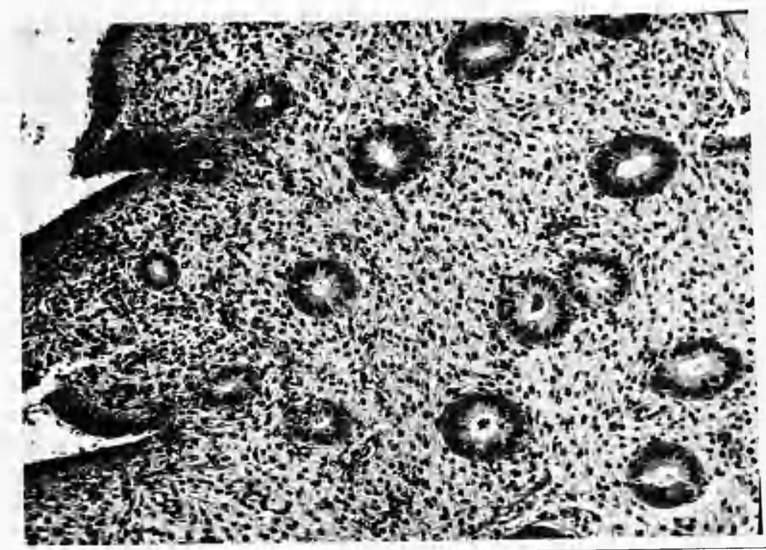

Fig. 3. Early Proliferative Phase.

H and E. 120 m.

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endometrium immediately after menstruation shows a completely epithelialised surface. Yet mitosès are rare at this stage suggesting that direct division of the cells must be the mode of regeneration. He further adduces another possibility that there may be a metaplastic conversion of stromal into epithelial cells; such a conversion does not seem impossible in view of the fact that uterine epithelium, like the stroma, is of mesodermal origin.

According to Schröder (9), re-epithelialisation is apparently accomplished by radial migration of the epithelial cells which line the wide-mouthed gland stumps. This has also been illustrated by Bartelmez (10) who has convincingly illustrated growth of surface epithelium from the open mouths of uterine glands.

(e) The Proliferative Phase.

This phase is described in two stages (1) early, (2) late.

(1) Early proliferation (Figure 3).

The glands are narrow and straight. Their course is almost a straight line from the basalis to the surface of the uterine cavity. They are also narrow; the epithelial cells of the opposite walls almost or actually touch each other. Hence, these glands appear to have very small cavities. The protoplasm of the individual cells take a red colour with eosin. The cell margins are sharp, especially where they are exposed to the cavity of the gland. The nuclei tend to be situated basally, in the deeper part of the cell. The nuclei may be round or oval or slender. With appropriate staining mitoses are visible in the glandular epithelium. In the glandular cells the Golgi apparatus is compact presenting as small rings of networks (12).

In the deeper part of the endometrium the stroma is quite dense. In the superficial layer, which has just been regenerated, the stroma is looser. In the basalis, the nuclei stain more deeply than in the superficial layer. The nuclei in the basalis are dense and small. In the superficial layer, the nuclei are rounder, more vesicular and longer. Mitotic figures

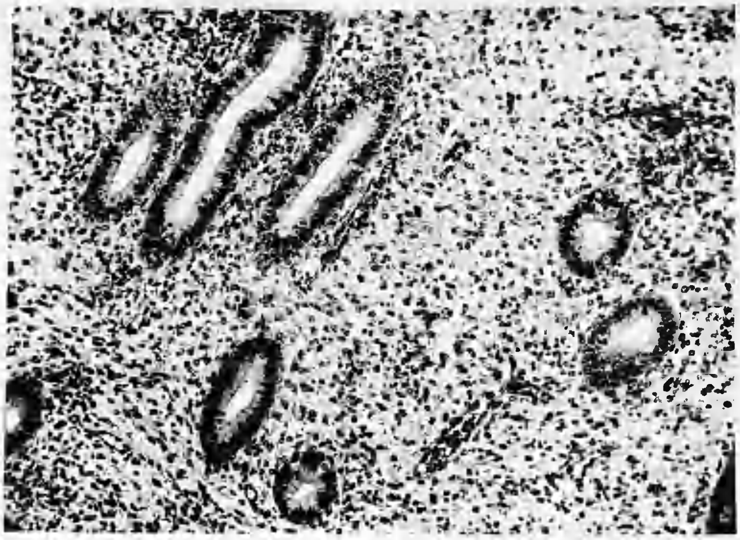

Fig. 4. Late Proliferative Phase.

H and E. 120 m.

To face page 9.

may be seen in the regenerating stroma.

There are numerous small blood vessels all through the stroma. In some places these vessels are as large as the glands, showing that the tissue is very vascular. There is no extravasated blood or lymphocytic infiltration in normal endometrium at this stage. Thus according to Wharton (11).

Hoffmann (3), on the other hand, describes the early proliferative phase as being poorly vascularised.

(2) Late Proliferative Phase (Figure 4).

Due to the continuing stimulation of oestrogen from the ripening Graafian Follicle, the proliferative changes become more marked. The total thickness of the endometrium increases. Changes begin to show in both the general configuration of the glands and also in the cytology of the individual cells. The glands are larger, their lumina are bigger.

There is now a definite space in most of these glands, whereas in the early proliferative stage many of the glands had no visible cavities. The glands are also not quite so straight; they show curves and infolding of the walls in places. Because of their increased size, they are closer together and occupy more of the stroma.

The individual gland cells have sharp cell membranes and one can distinguish separate cells in the gland. The surfaces of the cells that are exposed to the cavity of the gland are sharply delineated by a cell membrane that is everywhere smooth and intact. The protoplasm is acidophilic. There are occasional vacuoles in the protoplasm. The nuclei occasionally show a tendency to leave the base of the cell and many are now situated in the middle of the cell. The nuclei are round or oval and vesicular. Occasional vacuoles are found between the nucleus and the base of the cell.

The stroma is still quite dense, especially in the basalis. In the more superficial layer, the stroma cells are very evenly spaced, and have fairly uniform round or oval nuclei.

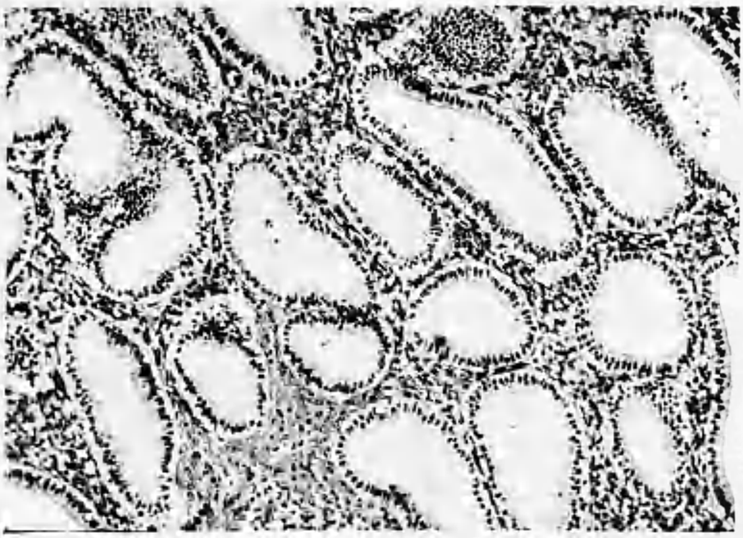

Fig. 5. Transitional Phase.

H and E. 120 m.

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These nuclei are larger than in the basalis and are fairly clear. The protoplasm of the stromal cells is ill-defined. In addition, there are many cells with long slender dark-staining nuclei, probably connective tissue.

The tissue is extremely vascular. The multiplicity of small vessels, engorged with red blood cells, suggests some rapidly growing tissue. These blood vessels are intact and there is no extravasation of either red or white blood cells (11).

Hoffmann (3) emphasises the marked mitotic activity of endometrium at this stage as do Maximow and Bloom (4).

On the question of mitosis, Ahrens and Prinz (13) made a study of this phenomenon. Mitoses were first seen on the sixth post-menstrual day: the highest number seen was in the early proliferative phase. In the late proliferative phase the number dropped to about half. The number dropped to about one fifteenth in the secretory and menstrual phases. None of these mitoses was abnormal. During the proliferative phase most of the mitoses were in the epithelium and in the secretory phase they were found mainly in the stroma.

One of their conclusions may be noted at this stage, namely, that a study of endometrial mitoses lends no proof to the theory of a continuous process of gradual transition from hyperplasia to adenocarcinoma.

About the fourteenth day of the menstrual cycle the brief phase of transition is reached. This is an extremely interesting phase and will be enlarged upon later but for the present section current descriptions may be summarised as follows:

(f) Transitional Phase (Figure 5).

The glands have now almost reached their greatest height. In the individual cells there is pronounced increase in size of the Golgi apparatus and the networks become looser (12). The surface epithelium is columnar (7). In the glandular epithelial cells, the nuclei have taken up a central position and large basal vacuoles are in evidence in the protoplasm.

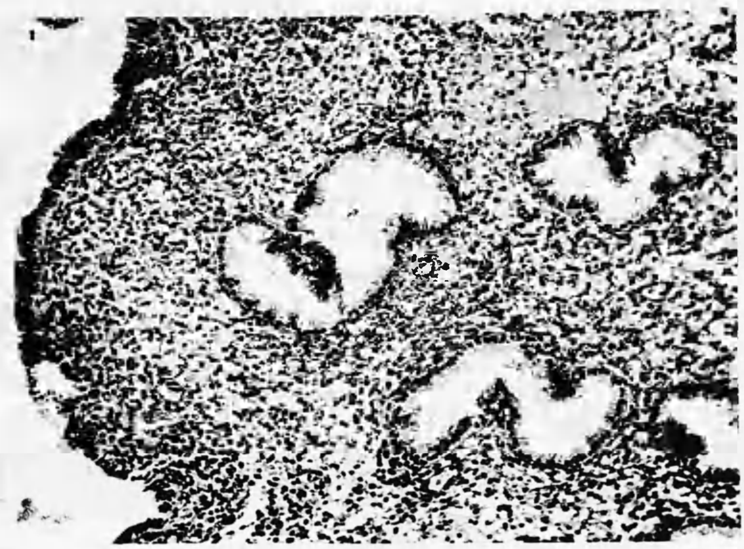

Fig. 6. Early Secretory Phase.

H and E. 120 m.

To face page 11.

The stroma is somewhat succulent. This condition of the endometrium only lasts for a short time, one or two days, often for an even shorter time (12).

Novak (7) comments that this stage of subnuclear vacuolation of the individual gland cells is commonly interpreted as representing the early stages of secretion or prosecretion, but there is still some doubt as to whether the vacuoles are indications of the early stages of progesterone activity or whether they can be produced by oestrogenic hormone alone.

Hoffmann (3), however, states categorically that within 48 hours after ovulation, the glandular nuclei are displaced towards the lumen side of the cell so that a clear space is left at the basal portion. This constitutes the earliest sign of secretory function. At this time, the corpus luteum is developing but has not yet attained full maturity.

(g) The Secretory Phase.

(1) Early Secretory (Figure 6).

On the fourteenth or fifteenth day of the cycle secretion begins. The epithelial cells become lower and broader, the protoplasm becomes paler and the nuclei return to the basal part of the cells (12).

The Golgi network is composed almost exclusively of long-meshed filamentous nets often cast upon the upper poles of the nuclei (12).

Vacuoles of secretion appear under the nucleus in the basal part of the cells lining the glands and push the nucleus upwards towards the centre of cell (Browne) (14). Later, the secretion pushes past the nucleus to the surface of the cell and in later stages is discharged into the lumen of the gland.

The stroma is more vascular and oedematous. Mitotic activity gradually ceases (3).

During this phase the thickening of the endometrium is due largely to the increase of secretion and oedema fluid. The glands become tortuous and irregularly sacculated especially in

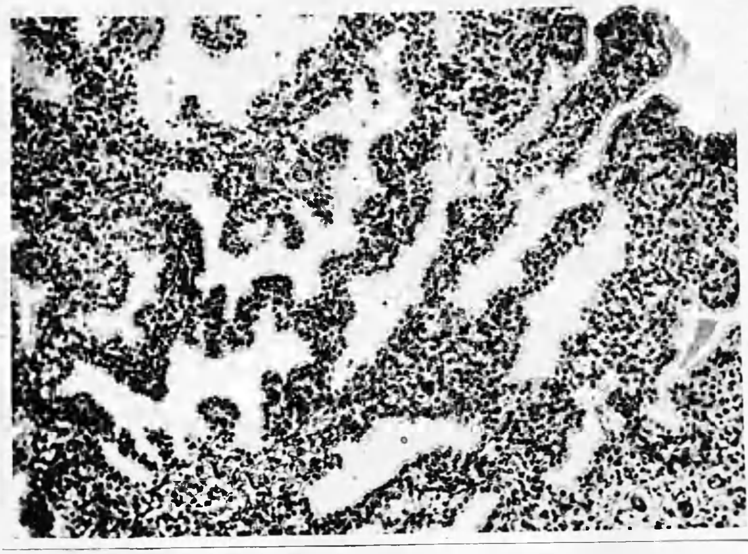

Fig. 7. Late Secretory Phase.

H and E. 120 m.

To face page 12.

the middle third (4).

In general, at this stage, the endometrium begins to differentiate into three layers, the basalis, the spongiosa and the compacta. Such progressive differentiation leads to a description of late secretory endometrium.

(2) Late Secretory Phase (Figure 7).

The three layers of endometrium at this phase may be described as follows:

The Basalis maintains its characteristic inactivity throughout the secretory phase. The basal glands may become more tortuous but do not share in the striking corkscrew contortions or fuzzy appearances of the secretory glands of the upper layers. This is an unexplained paradox, because the more superficial layers are derived directly from the basalis and all layers must receive common stimulation from the hormones and sympathetic nervous system (11).

The spongiosa constitutes the second or middle layer and is characterised by its appearance due to the large, dilated and tortuous uterine glands.

The compacta is the superficial layer. The glands become straighter and narrower in this area and the stroma constitutes a much larger portion of the tissue. This zone looks much denser than the spongiosa.

The glands have the characteristic corkscrew appearance. In the spongiosa, they wind about, are remarkably dilated and their walls are irregular and uneven. In the compacta, the glands are narrower and straighter but preserve these general features. The cavities of these glands are often filled with secretion whereas in the proliferative phase the glands were empty.

The striking feature is the raggedness of the surface of the cells that line the gland cavity. The regular, sharp, limiting membrane is gone in many places; the outer half or third of the cell has disappeared and left a frayed line of tissue. The nuclei seem exposed, in many places, to the cavity of the gland. The nuclei vary in their situations; some of them are basal, others in the middle third and others apparently lie uncovered in the peripheral part of the cell. In some places, the individual glands have sloughed off leaving the basement membrane or the basilar part of the cell exposed.

Equally striking changes are present in the stromal cells. The beginning of a typical decidual reaction may be seen. The stroma cells become larger; the protoplasm is clearly defined and sharply limited. The processes of the stroma cells extend out into the surrounding tissue. The nuclei become larger and more vesicular. The stroma becomes much looser and the cells further apart. These changes are noted, especially, in the spongiosa and the compacta. In the basalis, the stroma cells tend to retain their inactive features; they have dark-staining dense nuclei; the cell membranes are indistinct and the tissue is very dense. Even in the basalis, however, one sees evidence of some decidual change in some of the stroma cells (11).

The endometrium is very vascular in this stage. Every part of it is a network of blood vessels, all full of blood. There is no extravasation of blood into the stroma, however, and there is as yet no infiltration of white blood cells (11).

The late secretory endometrium is characteristically oedematous. This has been noted also in many mammals. It persists through the first few months of pregnancy (11).

Hoffmann (3) describes the strata as above but states further that the stroma of the functional layers, especially of the compacta, become progressively looser in texture. The cells take on much plasma, swell and become polygonal in shape. The nuclei are rounder and stain poorly. Towards the end of the cycle, the stroma becomes markedly oedematous and the cells often resemble decidual cells. The stroma of the spongiosa shows only a mild degree of oedema which is most pronounced around the blood vessels. The connective tissue of the basalis remains closely packed and takes no part in the process.

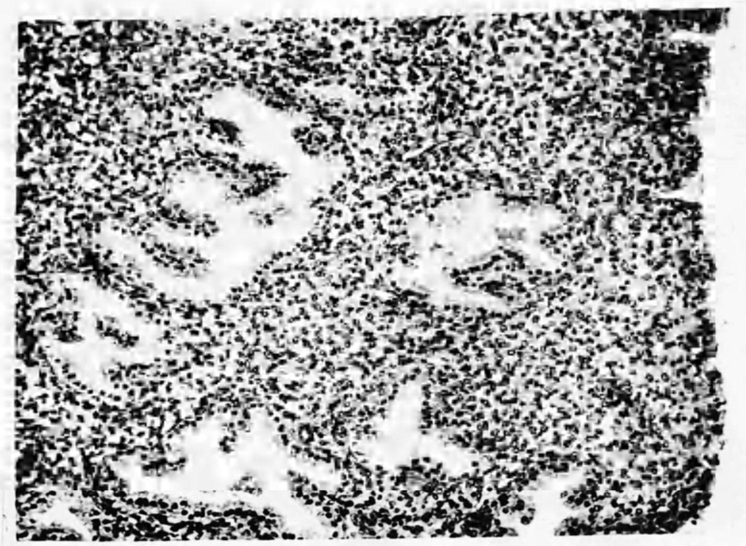

Fig. 8. Premenstrual Phase.

H and E. 120 m.

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Novak (7) in describing the secretory phase states that the gland cell in the basalis is often non-secretory in character, is responsive to oestrogen but not to progesterone. The surface epithelium remains tall and non-secretory.

In the latest edition of his book, Novak (14) has added a further comment upon this phase. There are marked individual variations in the degree and character of the histologic change characterising the secretory phase. In some cases, for example, the maximum of change may be represented by only a moderate degree of gland tortuosity and only comparatively slight stroma cell hypertrophy. In others, the glands are so tortuous and scalloped and the stroma cells so hypertrophied that the endometrium is indistinguishable from the very early decidua. These variations are due not only to quantitative differences in progesterone production, but also to the degree of responsiveness of the various endometrial elements to this hormone. For this reason, he does not believe that endometria can be as accurately dated as some investigators aver.

In an article on the uniformity of secretory endometrium, R.W. Moyes (15) concludes that reports in the literature concerning mixed endometrium and irregular ripening give an erroneous idea as to the frequency of these conditions. Most of the apparent variability of endometrium is due to faulty sampling or interpretive errors in small traumatised or poorly prepared tissue. If an adequate specimen is studied, the remarkably high degree of uniformity of secretory endometrium permits an accurate assessment of hormone-time relationships from a small biopsy.

(h) <u>The Endometrium just before Menstruation</u> (Figure 8).

Bartelmez (10, 16, 17) has made a careful study of the histological changes in the endometrium during the premenstrual phase and also during the stage of desquamation.

One of the distinguishing features of this short period is the infiltration of the stroma by white blood cells. This is probably the chief histological characteristic of impending

Fig. 9. Menstrual Phase.

H and E. 120 m.

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menstruation (Bartelmez 10, 16, 17, Kundrat and Englemann, Novak, 18, Novak and Tehinde 8). The white blood cells may be either leucocytes or lymphocytes. Coincidentally with this infiltration, the reticular framework of the stroma disappears, possibly as a result of digestive ferments released by the white blood cells. This infiltration is not a sign of infection and is not to be diagnosed as endometritis. Bartelmez (10, 16) has laid great stress on the vascular features of menstruation. The chief features are the extreme congestion and engorgement of all the blood vessels in the endometrium and the presence of spiral arterioles which extend to the surface. Markee's (19) remarkable studies emphasise this vascular change. The spiral arterioles become more tortuous. The oedema of the stroma is absorbed and the endometrium therefore becomes thinner.

Vesterdal-Jorgensen (12) states that pre-menstrually and menstrually in the epithelial gland cells the Golgi network is poorly stained and appears as if disintegrated into granules dispersed in the supranuclear part of the cells. Sporadically, compact forms and rings may be obtained. The looser structure of the network in the premenstrual phase may be an antefract depending on an osmosis with absorption of water into the protoplasm.

Holund (20) has been able to demonstrate an intimate relation between the size of the Golgi apparatus and the amount of secretion in the cells. He also describes the mitochondria as granular or filamentous. The form and density are very variable even in neighbouring cells. It has not been possible to demonstrate cyclical characteristics in these structures; in particular, no condensation is in evidence around the secreting vacuoles.

(i) Menstrual Phase (Figure 9).

It is only by a systematic chronological study of endometria removed on the various days of the cycle that the retrogressive changes characteristic of this phase can be demonstrated and traced. There was much difference of opinion as to whether the endometrial surface actually desquamated at menstruation up to the investigations of Schröder (21) in 1915 and later those of Novak and Tehinde (8) in 1924.

Even before desquamation begins its imminence is indicated by the degenerative changes in the upper layers of the endometrium as suggested by the poor staining and granular appearance of both the epithelial and stromal elements. With the onset of bleeding, tissue loss may be rather slight with perhaps only a small patch of the superficial layer being thrown off here and there (7). Bartelmez (16, 17, 22) has described the striking differences in tissue changes.

The blood vessels are widened and congested. This fact together with the thinness of their walls, most of them being small venules or capillaries, makes it easy to understand why both rhexis and diapedesis are probably factors in the bleeding. Small haematomas are often noted lifting the superficial layers and thus promoting further tissue loss so that usually on the second day of bleeding most of the surface has been lost (7).

The changes described by Bartelmez, however, may explain the prolongation of the flow with recurrence of bright bleeding on successive days.

The tissue loss proceeds in a fragmentary fashion with particles crumbling away here and there. In rare instances, however, the endometrium may be thrown off in the form of a more or less complete cast. By the third day desquamation has commonly reached its limit. The compacta and a variable thickness of spongiosa are usually lost and it is from the proliferative, non-secretory epithelium of the surviving stumps of the glands that the new surface epithelium is to be restored. Indeed, one often sees evidence of this epithelial regeneration even while desquamation is still going on with fragments of degenerated epithelium still lying on the surface (7).

The epithelium is restored with amazing rapidity, so that if one examines a uterus removed immediately after the cessation of menstruation the surface is already completely epithelialised. Mitoses are rarely seen at this stage so that it seems possible that direct rather than indirect reproduction of cells may be conceived. It has been suggested that there may be a metaplastic transformation of stromal into epithelial cells - a view which is possible because of the fact that both the uterine epithelium and stroma have a common mesodermal origin (1, 4, 7).

Describing the menstrual phase, Maximow and Bloom (4) state that by the third or fourth day the entire endometrium may present a raw wound surface. The superficial gland and stroma cells are normal histologically. Below the zone of extravasation the endometrium remains intact during menstruation although it does shrink down. Typical secretory glands may be recognised as such until the end of menstruation. The surviving zone is therefore wider than the basalis of many authors.

Wharton (11) describes the endometrium at the close of the menstrual period. The superficial part of the endometrium includes some of the stratum spongiosum with its coiled, irregular and dilated glands. Most of the endometrium, however, is the basalis, the rest of it having been sloughed off. The glandular epithelium shows very little trace of secretory activity although it has not entirely gone yet. The stroma is dense and there are many lymphocytes in the tissue. There is still some free blood in the stroma even in the basalis. The arteries are spiral and extend to the surface. The veins are not so prominent as in earlier stages. One can clearly see the process of repair, beginning in the superficial epithelium, which is spreading out over the raw surface from the dilated mouths of uterine glands. As a whole, the tissue looks quite healthy.

This section, therefore, might be placed first as well as last in the menstrual cycle, for it shows the beginning as well as the close of the cyclic changes. Tissue growth thus commences even before the menstrual discharges have stopped and continues until the corpus luteum ceases its activity.

A personal commentary upon the histological appearances of the normal endometrium.

In the prefacing remarks, it was stated that some seven thousand specimens of endometrial tissue constitute the basis of this study. This material was examined in the course of routine histological reporting for clinical purposes. There were two sources from which endometrial tissue could be obtained; these were either whole endometria in uteri removed at hysterectomy or material recovered by uterine curettage.

Only endometrium from patients whose menstrual history was absolutely normal, according to accepted standards, was utilised. Where there was any clinical complaint associated with menstruation then that specimen was rejected for present purposes. Furthermore, only whole thickness endometria were studied. If curettage had been imperfect resulting in fragmented or traumatised tissue then that specimen would also be rejected.

It seems clear, from a survey of the literature quoted in the previous section, that several authors have studied whole uterine sections for their descriptions of the histology. It could be argued that such a section would represent the natural lining of the uterus in its true setting. On the other hand, since the uterus had been removed, the clinical or potential pathological reasons must raise doubts as to whether the endometrium in such circumstances could ever be regarded as normal. Similarly, post-mortem specimens have also been excluded since the clinical condition occasioning death of the subject must inevitably influence the state of the endometrium and lead to false histological conclusions even although allowance may be made for supposed post-mortem change.

It has been observed that tissue fixation of whole uterine specimens is inevitably imperfect since the fixative cannot penetrate deep enough by total immersion nor can one be sure that retrograde injection of fixative through the uterine blood vessels would reach throughout the specimen. Numerous

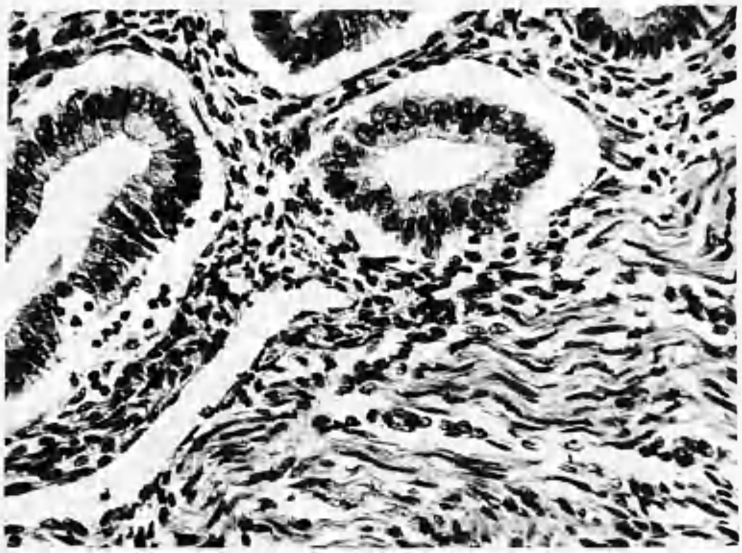

Fig. 10. Showing tissue shrinkage in
specimen of whole uterus.
H and E. 325 m.

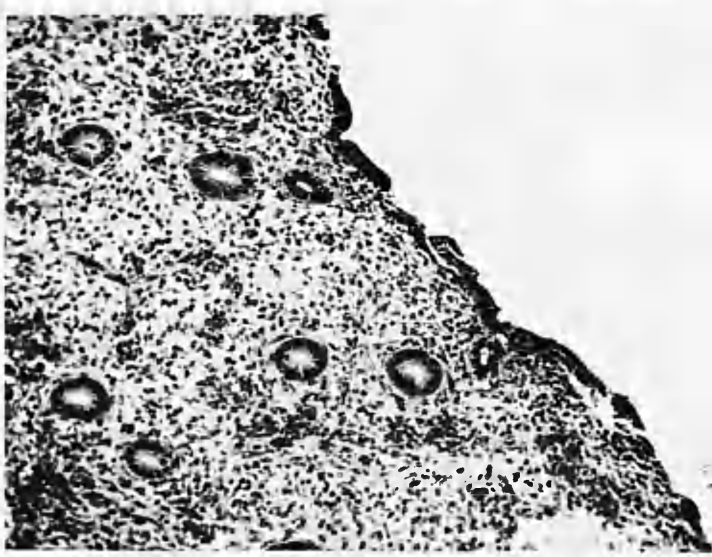

Fig. 11. Re-covering of endometrial
surface. H and E. 135m.

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artefracts, shrinkage and degenerative changes are unavoidable in such specimens (Figure 10).

Having described the source of the histological material, the tissue on recovery from the subject by careful curettage was immediately fixed in formol corrosive and subsequently sections, cut from paraffin blocks, were stained by haematoxylin and eosin.

The cyclic changes of the normal endometrium as described in the previous section were confirmed but it soon became evident that additional changes occur which do not appear to have been recorded.

For the purposes of description the whole thickness endometrium consists of (1) surface epithelium (2) gland epithelium and (3) stroma.

(1) Surface Epithelium.

During the reparative phase of the menstrual cycle the cells of the open gland mouth appeared to grow out over the surface of the denuded stroma. The individual cells were low cuboidal in shape and the dark staining nucleus was irregular in outline and basal in position. This method of re-covering of the surface agrees with the descriptions of Bartelmez (10) and Schröder (9).

On the other hand, many specimens showed an epithelial surface covering of flat immature cells apparently unconnected with an open gland and indeed separated from the adjacent glands by a raw stromal surface (Figure 11). Novak's (7) theory of metaplastic conversion of stromal into epithelial cells would therefore seem to apply to such cases but it will take some time to undertake a detailed examination of serial sections of such specimens before this fact can be definitely established.

There was no mitotic activity observed in the surface epithelium until about the sixth day of the cycle and thereafter it could be observed until the eleventh day. Such mitoses appeared to be normal. With the advent of mitoses, the shape of the surface epithelium cell became columnar. This columnar shape persisted until the twentieth day of the cycle when the cell reverted to a low cuboidal shape for the remainder of the cycle. From the sixth to the ninth days the nucleus was basal and from the tenth day to the end of the cycle it became mid cell in position.

From the twentieth to the twenty fifth days of the cycle, the surface epithelium was characterised by a basal vacuolation similar to that occurring in the gland cell during the transitional phase. The vacuole was a circumscribed clear area in the cell protoplasm but smaller than the vacuole of the gland cell. Novak (7) states that in the early proliferative phase under the influence of oestrogen the surface epithelium becomes taller and taller and at the time of ovulation it is columnar: in the secretory phase the surface epithelium remains tall and non-secretory.

Personal observation would agree on this description of the cell outline but also it seems that the surface cell, in addition to a modified cyclical change in shape, does show evidence of secretory activity in the secretory phase of the cycle. Further evidence of this will be adduced in the section of histochemistry.

(2) Gland Epithelium.

The shape of the gland cell was cuboidal from commencement of the regenerative phase until about the ninth day of the cycle. During this time the nucleus remained oval, well stained and basal in position. Over the next three days the gland cell becomes columnar in shape and the nucleus moves to a mid cell position. From the commencement of regeneration until this point normal mitotic figures may be seen in these cells. Thereafter no mitosis is seen in this situation for the remainder of the cycle.

From the thirteenth to nineteenth days the gland cell is tall columnar in outline but thereafter the cell outline gradually becomes less distinct and the general shape is low cuboidal. The nucleus remains mid cell until about the seventeenth

TABLE 1.

	Surface Epithelium				Gland Cell					Stroma					
										Round Cell					Spindle
Day	Mit	Cell	Nuc	Vac	Shape	Cell	Nuc	Vac	Mit	Cell	Nuc	Vac	Mit	Oed	
1															
2															
3															
4															
5	P	LC	IR		R	Cu	B		P	O	O				P
6	P	Co	B		R	Cu	B		P	O	O		P		P
7	P	Co	B		R	Cu	B		P	O	O		P		P
8	P	Co	B		R	Cu	B		P	O	O		P		p
9					O	Cu	B		P	O	O		P		P
10	P	Co	M		O	Co	M		P	O	O				
11	P	Co	M		O	Co	M		P	IR	IR		P		
12		Co	M		O	Co	M		P	O	O		P		
13		Co	M		IR	TC	M	P		O	R			P	P
14		Co	O		IR	TC	M	P		O	R	P		P	P
15		Co	O		IR	TC	M	P		O	IR	P		P	P
16		Co	O		IR	TC	B			O	R	P			P
17		Co	O		IR	Co	M	P		IR	R	P			P
18		Co	Sp		IR	TC	B	P		IR	R	P			P
19		Co	O		IR	TC	B	P		IR	R	P		P	P
20		LC	M		IR	TC	B	P		IR	R	P		P	P
21		LC	M	P	IR	LC	IR			IR	IR	P		P	P
22		LC	M	P	IR	LC	O	P		O	O				P
23		LC	M	P	IR	LC	O			O	O				P
24		LC	M		IR	LC	O			O	O				P
25		LC	M		IR	LC	O			O	O				P
26		LC	M		IR	LC	O			O	O				P
27		LC	M		IR	LC	O			O	O				P
28		LC	M		IR	LC	O			O	O				P

P. Present. LC. Low cuboidal. R. Round. Cu. Cuboidal.
Co. Columnar. IR. Irregular. B. Basal. O. Oval.
TC. Tall columnar. M. Mid cell

Sp. Spindle.

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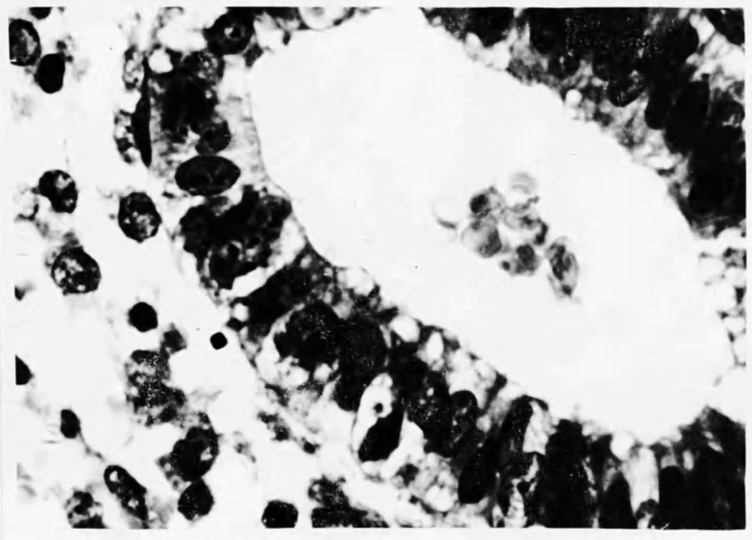

Fig. 12. Vacuoles pushing past nucleus.

H and E. 825 m.

To face page 21.

day and becomes basal again for the remainder of the cycle. The nucleus appears to adopt an irregular outline about the twenty-first day of the cycle and thereafter becomes oval and paler stained for the remainder of the cycle.

In addition to the foregoing description, the interesting phase of transition occurs any time from the thirteenth to the seventeenth day. It is characterised by the appearance of a clear circumscribed vacuole, unstained by haematoxylin and eosin, which is uniformly basal in position (Figure 5). The appearance of this phenomenon initially is of a sudden nature. Study of many specimens in the immediately preceding days has failed to show any sign of its advent yet it always occurs in the normal menstrual cycle.

However, having occurred within the interval stated, the phase is of short duration because within 24 hours and for the next five days the vacuoles can be seen to push past the gland nucleus into the superficial cytoplasm and finally erupting into the gland lumen (Figure 12). Indeed the material occupying the gland lumen in the secretory phase consists of many organic substances, which will be described in the histochemistry section, cell protoplasm, numerous vacuoles generated in the transitional phase and several degenerate gland cell nuclei.

The gland cells are arranged in a neat radial manner around the gland lumen until the stage of transition is reached. Not only the cells but the nucleus within show an ordered radial pattern. The lumen which was minimal in the phase of regeneration gradually increases in size until the phase of transition is reached when it may become irregular in outline and almost suggest a uniformly minor degree of cystic dilatation. From mid cycle to the end, the gland lumen assumes the irregular frayed and scalloped outline characteristic of the secretory phase. A synopsis of the tissue changes in normal endometrium is shown in Table 1.

(3) The Stroma.

The histological appearances of this structure are difficult to describe in view of the indistinct outline which most of the cells present.

It will be recalled that Maximow and Bloom (4) describe the stroma as resembling mesenchyme. Its irregularly stellate cells have a large ovoid nucleus. The cell processes appear to anastomose throughout the tissue and adhere to the framework of reticular fibres which are condensed as basement membranes under the epithelium. There are also lymphoid wandering cells and granular leucocytes. Macrophages are not uncommon.

The cytology of the stroma has been reviewed by von Numers (23). The stromal cells are reticulum cells. Normally collagen fibres are not found. (Personal observation refutes this as shown later.) Desquamation and regeneration of endometrium takes place without scar formation. The non-gravid decidua basalis shows two types of cells. These are elongated cells with spindle-shaped or rod-shaped nuclei rich in chromatin and rounded cells which have more protoplasm and nuclei which are round or oval in shape and stain less deeply. In the secretory phase there is an increase in the latter cells. A characteristic of the functional layer is the decidual cell: occasional mitotic figures are present. All evidence goes to show that the increase in the size of the decidua is attributable not to any increased cellular activity but to a passive storage.

The premenstrual decidual formation of the stroma has of late years often been mentioned in discussion of the problem of sterility, the point at issue being whether this is really necessary for the embedding of the ovum and whether it truly expresses the optimal reaction of the endometrium to the hormones. Holund (24) in agreement with others, holds the view that the decidua menstrualis is present in a more or less pronounced degree in these endometria which must be considered normal and fertile, but that absence of it does not necessarily mean a subnormal or "sterile" mucosa if height, form of glands and secretion are otherwise normal.

Novak (7) describes the stromal changes thus: in the postmenstrual phase the stroma forms a rather thin zone consisting of small round or oval cells compactly placed, with only a fine almost invisible network of supporting fibrillary tissue. Under high power magnification, one sees that each cell is made up almost entirely of nucleus, the surrounding cytoplasm being so scant as to be scarcely demonstrable. As the cycle advances, however, the stromal mouth of the glands becomes somewhat heavier and more vascular though no striking changes are seen in the individual cells, until the secretory phase is well advanced, becoming more conspicuous just before menstruation.

At this time the dark rather granular nucleus is seen to be surrounded by a well-marked cytoplasmic rim of varying width. At times the hypertrophy of the cell is so marked that it closely resembles the decidual cell of early pregnancy.

The decidua-like appearance of the premenstrual stroma is accentuated by the fact that considerable oedema and vascularity are often noted, giving the stroma a much looser texture than in the earlier phases. Many small vascular channels, chiefly capillaries and venules, become evident in the delicate partitions between the glands, while small tufts of spiral arterioles are seen in the deeper and middle portions of the spongiosa.

From personal observation, the presence and general characteristics of the two main types of stromal cells have been confirmed.

The rounded cells with the round or oval nucleus are in evidence at all stages of the cycle. From regeneration until about the tenth day of the cycle the cell and its nucleus remain rounded and the general stromal texture is loose with irregular intercellular spaces. About the end of this period these cells appear to be relatively smaller in size than the gland cells. During the succeeding two days the nuclei of these cells assume a granular appearance and an irregular outline. This seems to precede the transitional phase of the glandular epithelium. With the onset of this phase, the stromal cells once again resume their oval outlines and have round nuclei. On completion of the transitional phase the cell outline becomes irregular and remains so until the twenty-second day when they once again become rounded and are differentiated into the three zones of the progestational endometrial pattern. This outline remains oval until the phase of menstruation is reached.

Mitotic figures are noted in the stromal cells from the sixth until the twelfth days of the cycle. These mitoses appear to be normal. This timing of stromal mitosis appears in agreement with the description of Vesterdal-Jorgensen (12) and broadly with Ahrens and Prinz (13) although these authors have seen mitoses in the secretory phase of the cycle and more in stroma than in glandular epithelium.

Variations in size of the intercellular spaces of the stroma are noted at different stages. During the first week of the cycle the stroma texture may be described as loose. In the short period before the onset of the transitional stage there are more round and spindle shaped cells in the stroma and therefore the texture appears compact. At the onset of the transitional phase, the superficial stroma may be oedematous but very shortly areas of circumscribed vacuolations appear in the stroma. These vacuoles are similar in size, outline and staining qualities to those occupying the basal compartment of the transitional gland cells. The stromal vacuoles are scattered throughout the stroma and aggregates may be noted near blood vessels and also to gland walls.

It would be attractive to suppose that the stromal vacuoles migrate through the gland wall and become part of the gland secretion. Intensive study, however, of many specimens of this phase has failed to reveal any vacuole entering or pushing between gland cells and assuming a basal intracellular position or of a breach of the gland wall through which they might pass to enter the gland lumen.

Following the transitional phase, the stroma vacuoles

Fig. 13. Spindle cells supporting glands.

H and E. 220 m.

To face page 25.

continue to be present about the twenty-first day of the cycle. Thereafter the stroma begins to assume its decidua-like form: the vacuoles have disappeared and the intercellular spaces are fewer and smaller in size. The maximum pseudo-decidual reaction appears to occur during the three to four days preceding the onset of menstruation.

The spindle cells of the stroma do not appear to have invited much attention in the literature of endometrial histology. During the late proliferative phase, Wharton (11) notes in his description of the stroma that, in addition, there are many cells with long, slender, dark-staining nuclei, probably connective tissue. Novak (7) does not appear to mention them at all.

Von Numers (23) described the elongated cells with the spindle shaped or rod shaped nuclei rich in chromatin. These appeared not to show such cyclical variations as the rounded cell which became the prominent constituent of the three strata of the progestational endometrium.

Nevertheless the spindle cell is present throughout the menstrual cycle. From personal observation, it can be seen as a constituent of the basalis at all times but it is most noticable as the supporting structure of the epithelial gland cells. Glands do not appear to have this supporting structure throughout their length but certain sections in the stratum spongiosum are supported by these cells. The spindle cells are prominent in the late secretory phase when the glands are scalloped or infolded and these cells constitute the framework upon which the convolutions of glandular epithelium takes place (Figure 13). The spindle cells are infrequent supporters of the surface epithelium. Occasionally one may note an area of glandular endometrial tissue which is circumscribed by these spindle cells and which appears to restrict the dilatation of these glands lumina. This observation will be referred to again in a latersection.

In conclusion, it is interesting to refer to Maximow and Bloom's (4) description of loose connective tissue to determine whether some solution may be found to the origin of the endometrial stromal cells.

These authors described endometrial stroma as resembling mesenchyme. Its irregular stellate cells have a large ovoid nucleus. The cell processes appear to anastomose throughout the tissue and adhere to the framework of reticular fibres which are condensed as basement membrane under the epithelium. In the ground substance are lymphoid wandering cells and granular leucocytes. Macrophages are not uncommon which appear non-phagocytic to extravasated blood.

If now, these terms are studied further, mesenchyme or embryonic connective tissue gives rise, among other things, to connective tissue proper. In connective tissue, the intercellular substance always contains fibres; the substance forms the main mass of tissue and three parts can be distinguished in it. (1) collagenous fibres (2) elastic fibres and (3) the amorphous ground substance.

Reticular fibres are found at the boundary between connective tissue and other structures. In loose connective tissue elastic fibres are scarce.

These authors further state that alterations of the ground substance have been described with age, activity and certain pathological conditions. It is presumably the ground substance which appears to be affected by hormones such as relaxin, adrenocorticotropic hormone and cortisone. The whole range of activities influenced by the state of the ground substance is not yet known but it appears to be implicated in diffusion of nutrients in extracellular spaces. Associated with changes in the nature of the ground substance are certain connective tissue cells - fibroblasts and possibly others - containing granules which appear to be precursors of the ground substance polysaccharide.

Loose connective tissue contains the following cell types: fibroblasts, undifferentiated cells, macrophages, lymphoid wandering cells, mast cells, eosinophils, plasma cells, pigment cells and fat cells.

Fibroblasts are believed to be instrumental in the elaboration of the intercellular fibres. The fibroblasts are the long, flat elements which in profile appear as slender spindles. The large oval nucleus has a delicate outline and contains dust-like chromatin particles and one or more large nucleoli. Near the nucleus are a diplosome and a Golgi net. The cytoplasm rarely contains inclusions except occasional small fat droplets.

Undifferentiated mesenchymal cells are often smaller than fibroblasts but have the same general appearance. Many investigators believe with Marchand (25) that some connective tissue cells persist in the adult organism with the potencies of mesenchymal cells. The conviction that they are not common fibroblasts but are undifferentiated cells is gathered from numerous observations which show that under certain stimuli as in tissue cultures and inflammation they may develop into new cell types. They probably have much the same properties as the primitive reticular cells of the blood forming tissues. These primitive reticular cells, like the mesenchymal cells of the embryo, are endowed with the ability to turn into all types of blood and connective tissue cells.

In form, macrophages vary from flat round or oval cells to elongated spindle-shaped elements which sometimes have branched processes. The nucleus is smaller than that of the fibroblast and is irregular, oval or kidney-shaped. The cytoplasm has distinct ragged outlines and stains darkly. Near the nucleus is a distinct diplosome and a Golgi net.

The lymphoid cells are identical with the non-granular leucocytes of the blood.

Mast cells are found in the connective tissue. They are swollen, irregularly oval or flattened cells. The cytoplasm stains metachromatically and the relatively small nucleus is inconspicuous.

Maximow & Bloom (4) state that there is an accumulating mass of evidence that the mast cells elaborate an anticoagulant, identical with or much like heparin. This might well be the explanation of the fluid character of normal menses.

The eosinophile cells of connective tissue are eosinophile leucocytes which have migrated from blood vessels and have settled in the tissue.

Plasma cells have a round pale area adjacent to the nucleus the cytocentrum, and scattered round this are mitochondria and which finally degenerate. During degeneration, large spherical drops or crystals of a peculiar acidophile substance frequently accumulate in the cell body. When the cytoplasm disintegrates, these inclusions are set free and remain between the other elements as Russell's Bodies. (In this connection it is interesting to note that in the lamina propria of stomach mucosa, sometimes, cells with coarsely granular acidophile inclusions are found between the epithelial cells of the glands. These are Russell's Bodies).

All these types of cells may be seen in endometrial stroma and from the descriptions just given it would appear that the two types of cells quoted by von Numers (23) might possibly be identified.

His type one, namely the elongated cell with a spindle or rod shaped nucleus, could describe the fibroblasts of Maximow & Bloom. These would be responsible for the elaboration of the intercellular fibres. The process of collagen formation is identical in principle in the body of an embryo and in tissue culture. Silver impregnation methods (Maximow) (26, 27), give a clear insight into the morphology of this process. Delicate networks of branching and anastomosing fibrils appear on the surface and between the fibroblasts. The fibrils may follow the outlines of the cell bodies and their processes but they also extend far into the intercellular substance.

The type two cell (von Numers, 23), namely a rounded cell which has more protoplasm and a round or oval-shaped nucleus, might be supposed to arise from the undifferentiated mesenchymal cell.

Novak (7) describes the cyclic change of this round cell where, in the proliferative phase, the nucleus predominated in a scarcely demonstrable cytoplasm but that when the secretory phase is well advanced, the dark rather granular nucleus became surrounded by a well-marked cytoplasmic rim of varying width. The hypertrophy of the cell was so marked that it closely resembled the decidual cell of early pregnancy.

The potentiality of the undifferentiated mesenchymal cell has already been referred to and it is submitted that the progestational hypertrophy of the round stromal cell is yet another manifestation of the response of such mesenchymal cells to the special circumstances of pregnancy. It is an interesting observation, that, in the highly differentiated tissues constituting the adult female, there should exist a primitive tissue which regularly undergoes a morphological change, whereby a suitable nidus for the developing embryo is provided should pregnancy occur. The structures of the new development, the embryo, are thus brought into intimate contact with connective tissue perhaps no more differentiated than the embryo itself from which point the histological and physiological development can proceed on virtually equal terms.

(4) Unusual Cell Forms.

Before concluding this section of the histological appearances of cyclical endometrium reference must be made to certain structures observed during this study.

The endometrium usually responds to the hormonal stimulus of the ovarian secretions in a more or less well-defined pattern and the deviations found in functional disorders of menstruation have been studied extensively. Diagnosis for clinical purposes consists largely in establishing whether or not the endometrium is active and in determining the phase of its activity in relation to the clinical data of the patient.

The variations commonly seen in the endometrium are

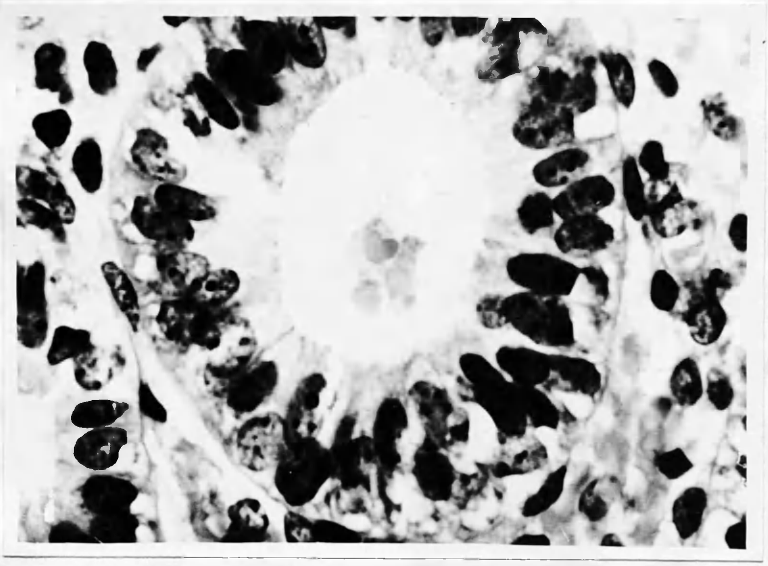

Fig. 14. Ciliated gland cells.

H and E. 825 m.

To face page 30.

reflections of the relative amounts of the ovarian hormones or of the response of the endometrium to them.

During the course of this study several unusual histological features have been noted on many occasions that, in view of the criteria adopted for the study, it must be concluded such features occur in normal endometrium. These will be described from personal observation and will be supplemented by the findings of others where the context is applicable.

In the study of functional uterine bleeding, Novak (7), Hoffmann (3) describe the work of a great many others upon the cytological appearance of hyperplastic endometrium. Novak describes what he calls the pathologic physiology of the condition and states further that opinion on this subject will be modified as knowledge grows. He has detailed gross characteristics of hyperplasia and also less marked proliferative features in endometrium associated with functional bleeding.

The following description might be entitled endometrial deviations associated with normal menstruation.

(5) <u>The ciliated cells of the endometrium.</u>

This phenomenon has been seen on several occasions in the present study. It was usually found to occur during the proliferative phase of the cycle although one example (figure 14) occurred during the transitional phase. Here subnuclear vacuolation was in evidence in addition to the tube-like appearance of the gland epithelium.

Novak (7) calls such tubal type epithelium metaplasia in a very broad sense. Normally the uterine epithelium is non-ciliated but a search for cilia by the fresh technique of Nylander, will at times reveal sparse distribution of cilia. Often the epithelium is of typical tubal variety with all three types of tubal epithelium represented, viz., the ciliated or non-secretory, the non-ciliated or secretory and the so-called peg cells.

According to Vesterdal- Jorgensen(12) normally, the endometrium contains ciliated cells as scattered insular areas

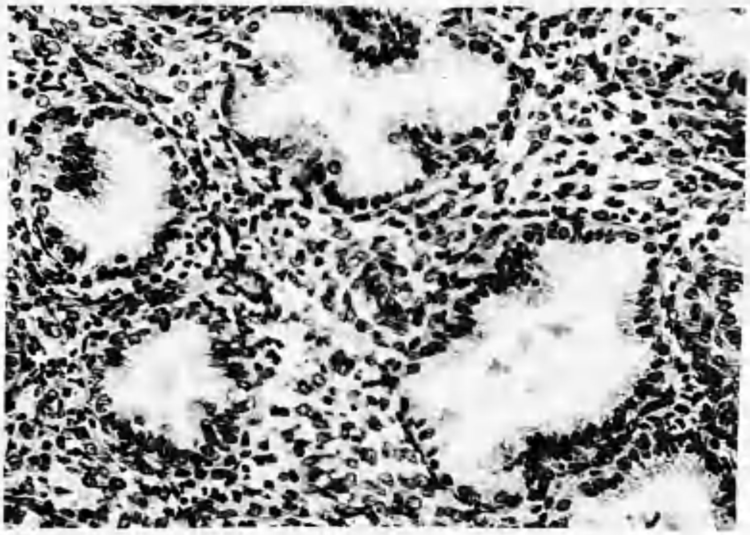

Fig. 15. Spindle stromal cells limiting gland convolutions.

H and E. 220 m.

Fig. 16. Clear Cells. H and E.

825 m.

To face page 31.

on the surface, whereas in the glands such cells occur only exceptionally.

Both writers note that the condition is mainly found in hyperplastic conditions.

Holund (20) found this metaplasia occurring only in the functional layer of the endometrium. He suggested that the presence of these cells in the endometrium was due to the protracted influence of oestrogen on the lining cells derived from the Mullerian Epithelium. Ciliated cells are also found to occur in non-hyperplastic endometria in the proliferative phase. This metaplasia has never been found in endometria of the normal secretory phase in which it has been reasonable to assume the presence of a lutein effect.

(6) The Limiting Stromal Basement Membrane (Figure 15).

Among the series it was frequently noted that the spindle-shaped stromal cell was often in evidence as a basement membrane to the glandular epithelium. As observed previously, the basement membrane is not a constant feature throughout the length of the uterine gland but seems to be mostly evident in the stratum spongiosum. Occasionally, areas may be seen where the gland convolutions are restricted by encircling spindle cells. This appearance may occasion atypical gland convolutions and involutions as described by Novak but in this series it appears to be compatible with normal menstruation.

(7) Clear Cells in Gland Epithelium (Figure 16).

In perhaps one per cent of specimens examined, clear cells were noted in the glandular epithelium. This figure should not be taken as accurate since this study is based on the histological appearances of the endometrium of several hundred normally menstruating women. The percentage incidence of one feature as opposed to another would be misleading as the present series has been deliberately selected from the in-patients of a gynaecological hospital and cannot represent a whole population figure although normality is the reason for selection.

These clear cells occurred singly, in twos or in small groups around the gland lumen. They were in evidence in both the proliferative and secretory phases of the cycle.

No mention of this phenomenon could be found in the standard text books of gynaecological histology.

Gundelach (28) on the occurrence of clear cells in the endometrium quotes Feyrter as claiming that "clear cells" in endometrium represent a peripheral endocrine system acting as an intermediary between hormones and end organs. The author's findings on 72 cases of normal endometrium give no support to Feyrter's theory. The relative frequency of clear cells was proliferative 0.6, secretory 0.4.

Hamperl (29) describes the "clear" ciliated epithelial cells of the mucous membrane of the human uterus. He associates the condition with hyperplasia of the endometrium. Interspread among the narrow columnar cells of the endometrium were found clear, broad cells with large nuclei arranged singly, in small groups or in sheets. These clear cells were bulbous, tapering somewhat towards the base and the free surface; they appeared to push aside the adjoining darker, non-ciliated cells. Within these clear cells a ciliary vesicle forms which travels towards the free edge of the cell, breaks open and displays the cilia which now form a fringe all over the free surface of the cell: this fringe may be discharged from the cell by apocrine secretion in the form of a globule bearing cilia all over its surface. Evidence is adduced that all ciliated cells of the hyperplastic endometrium are clear cells. Not all clear cells are ciliated: clear cells are of three types (1) ciliated (2) migrating lymphocytes and (3) special non-ciliated clear cells.

Müller (30) made an extensive study of the morphology and the possible function of clear epithelial cells in the human endometrium. He mentions that similar clear cells have been observed by other authors in the intestines, the pancreas and the gall-bladder. They are usually arranged in small groups and

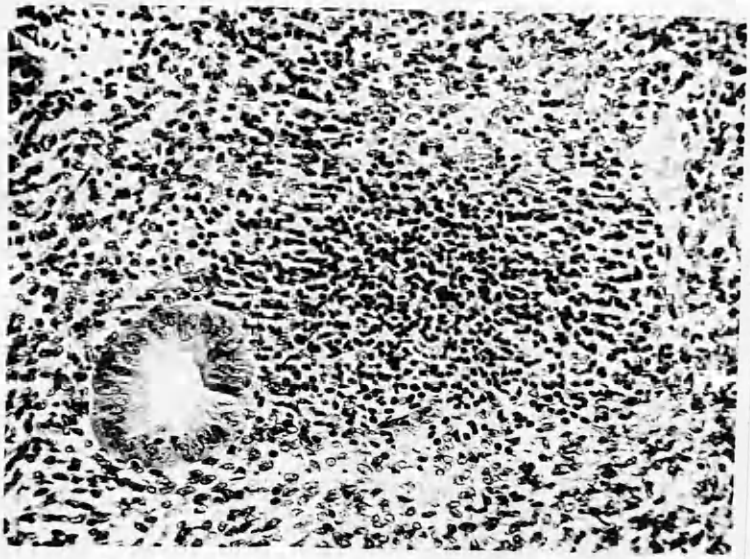

Fig. 17. Lymphoid Follicle.

H and E. 225 m.

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appear to be triangular or flask-shaped in sections; their protoplasm is clear and their nucleus is small and circular: according to Feyrter, the clear cells occurring in the pancreas constitute a diffuse disseminated endocrine organ.

The author emphasises the great variations in both the size of these cells and the shape of their nuclei and tries to correlate these variations with the functional state of the endometrium in which they occur. "One would feel inclined to think that the corpus luteum hormone ... exerts its influence not only on the secreting cell and its nucleus but also on the clear cell, being responsible for increase in size of the cell and its nucleus." It is thought, however, that the follicle hormone may be partly responsible for these changes in the clear cells.

The author demonstrated that there was no connection between the clear cells and the nervous system. Referring to the clinical experience that atrophy of the ovaries and menopausal symptoms not infrequently supervene some time after hysterectomy, the author suggests that the clear cells may constitute an endocrine organ, forming one link in the hypophysis-ovary-thyroid chain.

Lymphoid Follicles (Figure 17).

Circumscribed aggregates of small lymphocytes have frequently been noted in stroma during the present study. These may be in evidence at any phase of the menstrual cycle and are usually situated in the basalis. There is no histological evidence of giant cell formation in association with these groups of cells neither is there any clinical association of tuberculous disease elsewhere in the subject from whom the specimen was obtained. The significance of this phenomenon is not known.

This concludes the section of cytology of the normal endometrium.

SECTION 2.

THE HISTOCHEMISTRY OF THE CYTOLOGICAL CHANGES IN NORMAL ENDOMETRIUM.

The present study is concerned with the histochemical changes in endometria studied concurrently with the routine histological reporting of endometrial tissue for clinical purposes.

The cytological changes in the previous section were described from sections stained with haematoxylin and eosin. These had been prepared from paraffin embedded blocks after formol corrosive fixation. It had been found that formol-corrosive gave the clearest cytological definition and is the routine preparation employed by the laboratory technicians of the department in which this clinical reporting and histochemical study is made.

Extra sections were cut from the same blocks after H. & E. check for suitability. By this method simultaneous chemical study and comparisons could be made by differential staining.

The methods employed will be explained in each subsection as will any special technique for which formol corrosive fixation would not be suitable.

The application of modern histological techniques is concerned with the interpretation in chemical terms of the processes of staining and the obtaining of information in regard to the production, metabolism and functions of substances present in cells.

The functions of this branch of histology - histochemical physiology - has been well expressed by Dempsey & Weslocki (31) - "Physiological histochemistry has as its chief aim the morphological

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TABLE 2.

PERIODIC ACID - SCHIFF.

Day.	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Gland Cell.	P	P		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P			P		
Gland Lumen.												P		P	P	P	P	P	P	P	P	P	P	
Stroma Cell.	P	P					P	P	P	P	P		P	P	P	P	P	P	P	P	P	P	P	P
Surface Epith.	P		P	P			P	P	P	P			P	P	P	P								

P. Present.

localisation within tissues of the chemical components which are involved in physiological processes and the correlation of any changes in this chemical machinery with the different functional states of the tissues or organism. In other words it concerns itself with the ultimate description of physiological, biochemical and histological phenomena in terms of the others."

Much work in this field has been done in relation to the trophoblast and pregnancy but the present study is confined to the non-pregnant uterus.

Refinements in histological technique have been applied to routine examinations in the course of this study and it is considered that the results of each method should be described under the title of the substance studied and at the same time the literature of the subject correlated and any conclusions drawn in consequence.

(a) Periodic-Acid-Schiff Positive Material with particular reference to Mucin and Glycogen.

Staining technique: periodic acid oxidation - Schiff (McManus) (32). Many substances give a positive reaction when stained by this method but of those reasonably thought to be associated with the endometrium may be compound lipids glycogen and mucus. If sections are stained by P.A.S. then the presence of material compounded of these substances can easily be demonstrated. Indeed the P.A.S. technique is commonly used for exhibiting mucins as a method by itself and the lipid and glycogen fractions can be differentiated by other methods which will be detailed later in this section. P.A.S. positive material may be shown in endometria at all stages of the menstrual cycle. (Table 2).

(1) Regenerating phase: The positive material is seen on the free surface and superficial cytoplasm of the cells of the surface epithelium. The basement membrane of these cells also shows the material.

The gland cells likewise show positive material on their borders presenting to the lumen and granules of the material are present in the superficial cytoplasm. The bases of the cells have positive material along the fixed edges.

There may be some amorphous material in the gland lumina. This also stains P.A.S. positive.

The stroma shows a fairly uniform positive staining reaction. This substance appears to be granular in the cytoplasm of the typical stromal round cell.

(2) Late proliferative: The positive material stains in the surface epithelium as before but the layer in the basement membrane is thinner. The gland cells show the presence of the material in the superficial cytoplasm. The lumina are empty.
The staining of the stroma cytoplasm appears paler: this is due to wider arreolar spaces as the cytoplasm of the round stroma cells is more distinct and the P.A.S. positive material is seen to be granular. There are also small inclusions which stain positively. The cytoplasm of the spindle stromal cell stains more intensely than the round cell.

(3) Transitional phase: The surface epithelial cells show uniform staining of the cytoplasm. There is now a wider area of "oedematous" stroma beneath this, the ground substance of which is uniformly P.A.S. positive. The material stained is still granular but the inclusion bodies seem larger than in the late proliferative phase. The gland cells show the characteristic subnuclear vacuolation. The superficial cytoplasm is granular and P.A.S. positive: the most intense staining is in the free border of these cells. The subnuclear vacuoles remain unstained. Some of the lumina contain small granulations of material which is strongly positive.

The stroma of the stratum compactum has already been described but in the spongiosum there are areas of ground substance in the vicinity of gland convolutions which stain P.A.S. positive more intensely than elsewhere.

(4) Secretory phase: The surface epithelium cell shows P.A.S. positive material in the superficial cytoplasm. Where small vacuoles arise in these cells they may be subnuclear or alongside

Fig. 18. P.A.S. positive material
in lumina of glands.
P.A.S. 120 m.

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the nucleus but do not stain positively.

The stroma is less intensely stained at this stage but there are still comparatively large areas of stained ground substance in the stratum spongiosum.

The gland cells show characteristic fraying of the edge. This edge stains intensely. The superficial cytoplasm contains granular substance which also stains intensely. The vacuoles, which are still unstained now push past the nucleus and emerge into the lumen.

The outstanding feature of this phase is the large amounts of strongly stained material in the lumen of the gland (Figure 18). The greatest amount is seen in the lumina of the stratum spongiosum with a thin wisp in the narrow parts of the glands in the stratum compactium. The secretory activity diminishes in the deeper layers - no stained material is seen in the lumen - and the superficial cytoplasm of the basal gland cells stains pale P.A.S. positive.

Menstrual phase: The appearance of the endometrium in this phase is one of a paler staining with P.A.S. material. The surface epithelium cytoplasm stains positive but less so than in the secretory phase.

The gland cells are almost completely exhausted of P.A.S. material.

The decidual stromal cells have their outlines demonstrated by intense P.A.S. positive material but the intracellular cytoplasm appears to be devoid of this substance.

The foregoing description does not differentiate the constituents of the substance produced in endometrium. At the outset it was explained that the P.A.S. reaction is nowadays regarded as almost specific for the mucins. It may therefore be assumed to have shown the abundant production of mucins which take place in endometrium. There now remains the object to demonstrate the presence of glycogen and fats in the endometrium during the phases.

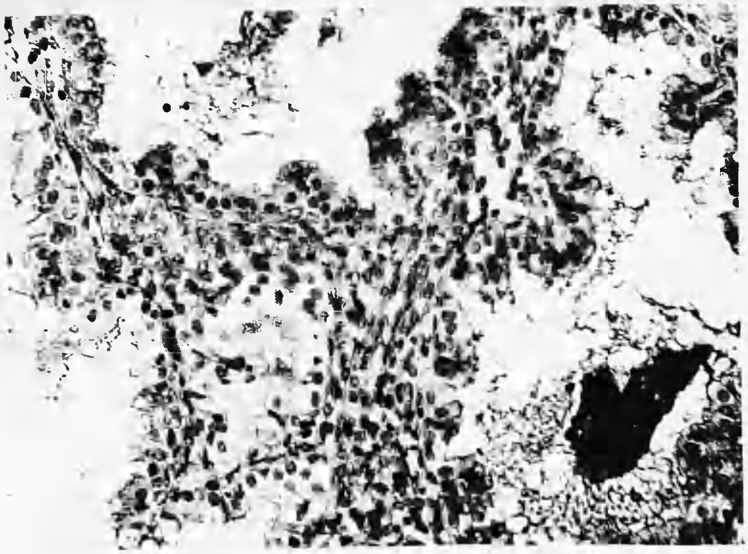

Fig. 19 A.
P.A.S. positive
material.
260 m.

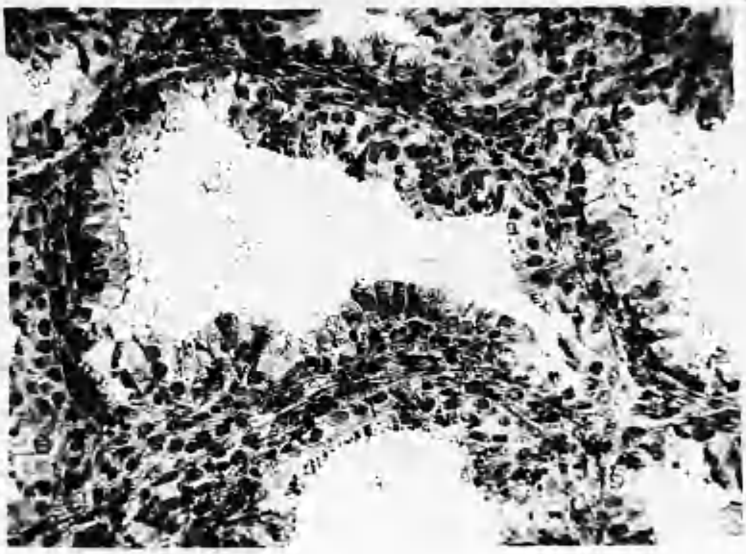

Fig. 19 B.
Best's
Carmine.
260 m.

Fig. 19 C.
Diastase
treated.
260 m.

To face page 38.

(b) Glycogen (Figure 19).

In order to demonstrate this substance, sections cut from the same block as the above specimens were stained by Best's Carmine Technique (1906) which is specific for glycogen. This specificity is determined by staining a control section after diastase digestion. The absence of stained material following enzyme digestion which was previously carmine positive is proof that the substance is glycogen.

Regenerating phase: No glycogen could be demonstrated in the surface epithelium, gland cells or stroma.

Proliferative phase: Glycogen appeared to be absent from all constituents in this phase.

Transitional phase: A little glycogen is present during this stage. It appears to be confined to the gland cells and is predominantly basal in position. It can also encircle the subnuclear vacuole and appear as small granules in the superficial cytoplasm. A small quantity is also present in the lumen.

The surface epithelium and stroma appear to be devoid of glycogen.

Secretory phase: The strongest glycogen reaction is seen at this time and is shown in the cytoplasm of the cells of the surface epithelium and the glands. Glycogen is mostly present in the basal cytoplasm of these cells but may extend beyond the nuclei to the superficial cytoplasm. Where this occurs the glycogen presents either as globules or as minute granules. In the glands these may be extruded into the lumen. Small granules are also seen in the aveolar spaces of the stroma.

Menstrual phase: Glycogen has largely disappeared from the tissue and presents only as small granules in the areolar spaces in the stroma.

These then are the personal observations of the presence of glycogen in endometrium.

According to Vesterdal-Jorgensen (12) large amounts of glycogen are always demonstrable in the normal secretory phase and used to be regarded as a criterion of this phase, but glycogen cannot be said to be specific because it has been proved to be present at any stage of the cycle even if it is but inconstantly present in the menstrual and proliferative phases. McKay et al (33) found that most of the biochemical alterations were due to changes in the glandular and surface epithelium whereas the stroma contributes only slightly. Glycogen content was low in the proliferative phase and increases in the secretory phase. Dezza (34) found very little glycogen in the proliferative phase but conspicuous quantities in the progestational phase. The finding of the present study would appear to be in accord with these statements.

Rossmann (35) is inclined to regard glycogen as a simple nutriment and as an indicator of a disproportion between intake and consumption or of reduced oxidation analagous to the evaluation of the substance in other localities.

The source of glycogen is glucose which is carried to the region by the blood stream. Synthesis of the glycogen is accomplished from a hexose phosphate which is split by alkaline phosphatase. Atkinson and Engle (36) have shown that in the glands and surface epithelium of the human endometrium alkaline phosphatase activity is high during the proliferative phase, is reduced during the secretory phase and disappears in the premenstrual phase.

These findings correspond to the way in which, morphologically, it is found that at the end of the first week glycogen begins to accumulate at the basement membrane. During the second week the amount increases and the substance begins to appear basally in the cells and at ovulation in mid cycle it is found in the basal part of the cell below the nucleus which has moved upwards (compare with fats later). Following ovulation the discharge into the lumen begins through the fringed surfaces and here hydrolysis takes place and to an increasing extent with increasing secretion of glycogen.

TABLE 3.

FATS - Sudan Black B.

Day.	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Gland Cell.	P		P						P		P	P		P	P	P	P	P				P		
Gland Lumen.									P	P	P	P	P	P	P	P	P	P	P	P	P		P	P
Stroma Cell.	P											P						P				P		
Surface Epith.													P	P	P									

P. Present.

To face page 40.

Hisaw and Greep (37) have shown that glycogen is retained basally in the cells in cases of prolonged oestrin administration and only small amounts of glycogen are carried to the lumina of the glands. When progesterone was given there was increased elimination of glycogen.

Hughes (38) investigating the glycogen content of endometrium in infertility, concluded that an adequate carbohydrate metabolism in the endometrium is essential for pregnancy. Observations of the glycogen in primitive trophoblast before embedding seems to show that glycogen is necessary for gestation to continue.

Ferin (39) found that in ovariectomised subjects to whom folliculin had previously been given that a single dose of progesterone administered intramuscularly produced basal vacuole formation in the endometrium within 24 hours. Glycogen was found first as fine granules in the surface epithelium and in the necks of the glands. Within a few hours granules were also found in the depths of the glands.

Falconer (40) studying endometria in the late secretory phase of the cycle where typical appearances were seen in the glands but not proper co-ordination in the stromal cells concluded that an incomplete stromal cell reaction indicates that the storage of glycogen is insufficient.

Finally, de Senarclens (41) investigated the time of appearance during the menstrual cycle of glycogen and mucus in the glands of the endometrium. The biopsy specimens were correlated with ovarian changes. Glycogen was present in small amounts on the sixth day of the cycle. It appeared in large amounts on the two days following ovulation and this coincided with the appearance of mucus. The presence of large amounts of glycogen and mucus indicated progesterone action. The progesterone may be extrogenital, for example, from the suprarenal, or it may originate from the theca interna cells which sometimes undergo an epitheloid instead of a fibroblastic reaction.

(c) Fats (Table 3).

According to Dempsey and Wislocki (31) Sudan Black makes

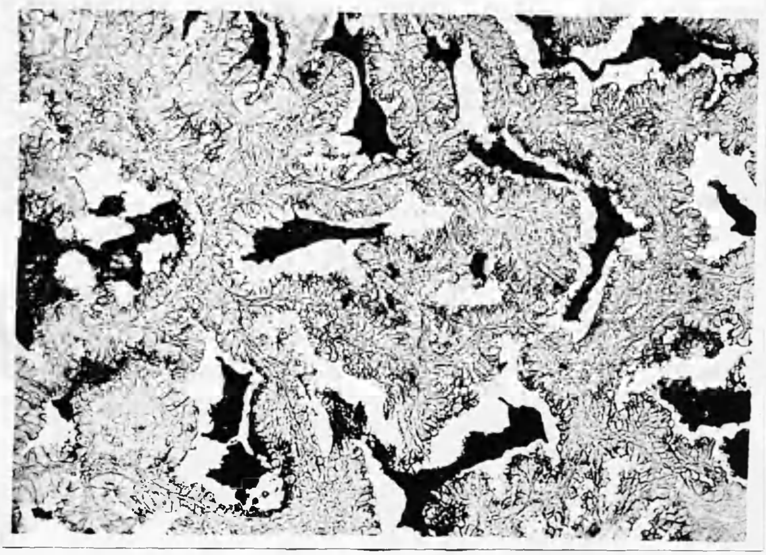

Fig. 20. Showing fat in lumina.
Secretory phase.
Sudan Black B. 120 m.

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fat more visible than Sudan Red and this method of staining was employed in the present study.

The many samples stained showed a uniform appearance at each stage of the menstrual cycle.

The free border of the surface epithelium always showed an intense fat stain. During the regenerating and proliferative phases the superficial cytoplasm of the cells of the glandular epithelium showed the presence of fat droplets. In the transitional and secretory phases there was intense fat staining of the free surfaces of the fat cells. Fat secretion appeared in the lumen first in the transitional phase and the quantity increased as the secretory phase progressed (Figure 20). The premenstrual endometrium appeared to be empty of fat. At all stages there was a greyness of the stromal cytoplasm which might have been due to fat droplets but this is uncertain. The dark stained material in the lumen appeared to correspond in position to the P.A.S. positive substance.

The term mucin is used to describe an intracellular secretion formed in cells. It is compounded of mucopolysaccharide and mucoproteins. The term lipid describes all naturally occurring fats and fat-like substances.

Lipids, glycogen and mucoid substance have for a long time been known to be present in the products of gland cells. Lipids are found in the gland cells even in the newborn and in children. According to some investigators these substances present no special cyclical characteristics even though there is a tendency to an increase in the secretory phase.

Gillmann (42) studied the fat changes in endometrium. Staining with scharlach does not show any fats in the glands during the proliferative phase whereas there are a few droplets situated at the poles of the nuclei in the stroma cells. In the progesterone phase a remarkable accumulation of fats is seen in the basal one-third to one-half of the glandular cells below the nuclei. The fat occurred in fine droplets. On the first day of menstruation there is even more fat so that the whole cell may be occupied. During the early part of pregnancy basal fat is constantly present in the cells. Its presence in this location can be demonstrated till the middle of pregnancy. Fat in the basal part of the cells occurs on the administration of progesterone and is believed to denote an optimal lutein effect. By excessive dosage of oestrogens, associated with a deficiency of progesterone, an abundance of fat is seen in the stroma while the glands are empty.

The findings of the present study, even although a different stain were used, do not correspond with those of Gillman. It has been observed that fine fat droplets may occur in the cytoplasm of stromal cells at all stages of the cycle, otherwise fine droplets were seen only in the superficial cytoplasm of the gland cells until the secretory phase was reached. Thereafter the intense stain of the secretion in the lumen was noticed to increase in quantity to the immediate premenstrual phase. At menstruation, the endometrium and gland lumina were devoid of fat-stained substance.

(d) The Secretion.

The endometrial secretion is viscous. The substances which have been demonstrated previously have been of a relatively simple composition of fat, glycogen and mucin. From the earliest days of endometrial histology the secretion has been described as a mucous substance which is only seen near the surface of the cells from which it is discharged into the lumen of the gland where it can be stained. The mucin which has given its name to the secretory phase has up till now only been demonstrated in the lumen of the glands by mucicarmine. Development of the Periodic Acid-Schiff technique, however, has allowed greater histochemical differentiation and the location of the P.A.S. positive substance has been detailed in a previous section. It has been shown also that in addition to mucin, glycogen and fat are also present in the material shed into the lumina of endometrial glands.

Basophile substance in endometrium, presumably identical

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RIBONUCLEIC ACID. TABLE 4.

Day.	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Gland Cell.	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Gland Lumen.											P	P	P	P	P	P	P	P		P			P	P
Stroma Cell.	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Surface Epith.												P	P	P	P			P	P	P				

P. Present.

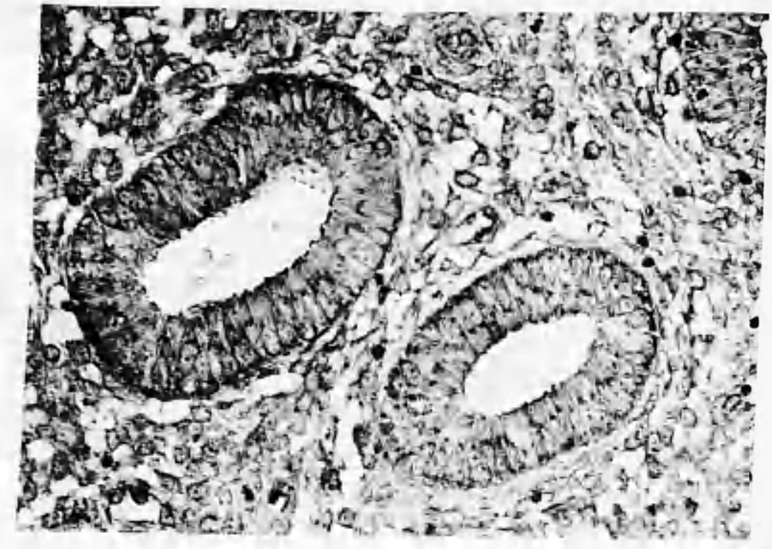

Fig. 21a. Showing presence of R.N.A.
M.G.P. stain. 350 m.

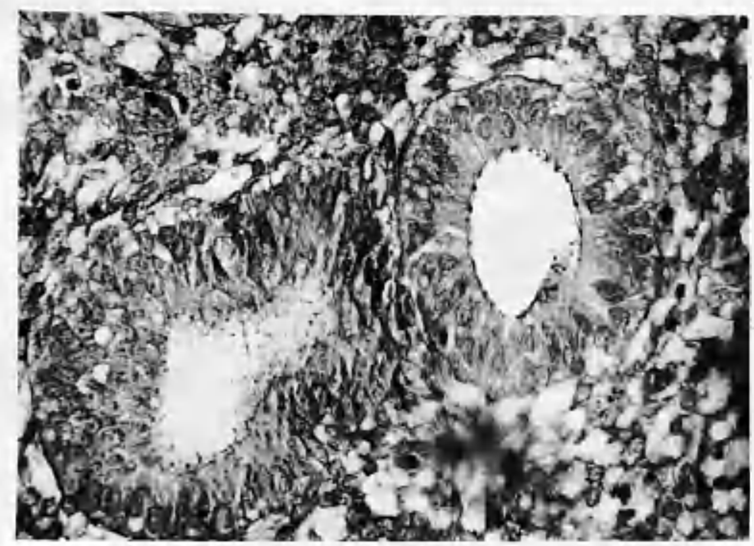

Fig. 21b. R.N.A. treated ribonuclease.
M.G.P. stain. 350 m.

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with ribonucleic acid has been studied by Dempsey and Wislocki (44, 45). Nucleic acids are abundant in cells in which rapid protein synthesis is taking place in the production of the main bulk of cytoplasmic protein for growth or for secretion. In such cells, ribonucleic acid is particularly abundant in the cytoplasm which is intensely basophilic and the nucleolus is also large and prominent (43).

(e) Ribonucleic Acid in Endometrium (Figure 21). (Table 4).

In the course of this study sections were stained by the Methyl-Green Pyronin method and also control sections were treated with ribonuclease. The ribonuclease treated section was compared with the M.G.P. stain and the absence of a structure which was present in the M.G.P. slide indicated that this structure was composed of ribonucleic acid.

In the regenerating phase ribonucleic acid is present throughout the cytoplasm of the surface epithelial cell. In the gland cells, the substance is seen basally and on the upper pole of the nucleus. The stromal reaction is fainter and granular. Treatment with ribonuclease removes the material.

During early proliferation, the cytoplasm of the surface epithelium stains more intensely. The gland cell shows a similar reaction but now the entire cytoplasm stains evenly. The granular stromal reaction is more distinct. Treatment with ribonuclease again removes the material.

The surface epithelium cells, in the transitional phase, show a clear space basally whereas the rest of the cytoplasm shows the presence of ribonucleic acid. The gland cell cytoplasm stains superficially to the nucleus but the subnuclear vacuole remains unaffected. Ribonuclease removes the material but it is noted that the spindle stromal cell cytoplasm appears to retain some stained properties.

During the secretory phase, the surface epithelium stains as before. The gland cell cytoplasm is slightly paler and there is stained secretion in the lumina. The stroma shows no advance on its previous appearance. Treatment with ribonuclease removes most of the material but some stained substance remains in the lumen.

The premenstrual endometrium shows clear subnuclear areas in the surface epithelium. The gland cell is not so intensely stained and that in the superficial cytoplasm only. The upper pole of the nucleus shows a clear area. The lumina of the glands show abundant stained secretion. Treatment with ribonuclease removes all the previously stained cellular elements, but most of the material in the gland lumina retains some stain.

Atkinson et al (46) state that the ribonucleic acid content of the cytoplasm of cells provides an index of the rate of protein synthesis. Basophilia of the cytoplasm of the human uterine glands has been shown to be due to the presence of ribonucleic acid. It is very pronounced during the proliferative phase of the cycle and can be made to disappear completely by treating the sections with ribonuclease. Cytoplasmic staining during the early secretory phase is completely destroyed by ribonuclease but a slight residual basophilia is apparent in the gland secretion and can be demonstrated to be due to mucin. In the advanced secretory phase there is a decided reduction in the amount and staining power of the basophilia which, however, persists in the gland secretion, giving a slight to moderate reaction for mucin.

These findings appear to correspond with those of the present study although no mention is made of the activity of the stroma. It would appear that there is a morphological difference in the cytoplasm of the spindle stromal cell and that a substance other than ribonucleic acid is retained after treatment with ribonuclease.

Comparison of the ribonucleic acid sections with those stained P.A.S. shows that the volume of material secreted contains ribonucleic acid in addition to other materials unaffected by ribonuclease. The impression gained is that the amount of

Fig 22a. Alkaline Phosphatase.

Proliferative phase.

Gomori. 135 m.

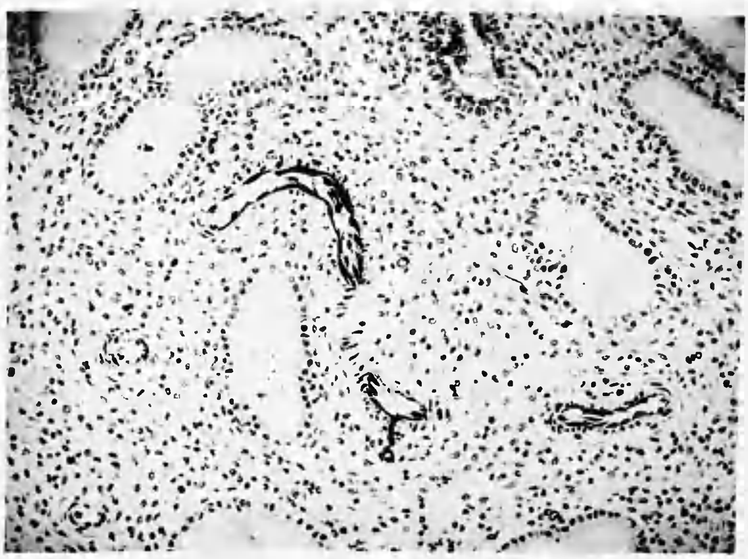

Fig. 22b. Alkaline Phosphatase.

Late secretory phase.

Gomori. 135 m.

To face page 45.

ribonucleic acid increases in amount in endometriam in the locations shown until the late secretory phase when it is apparently totally expelled into the gland lumen.

In a similar study McKay et al (33) report that ribonucleoprotein, which is known to be associated with protein synthesis, is present in increasing amounts reaching a peak in the late proliferative phase and decreasing in the progestational phase. They also observed that most of the biochemical alterations were due to changes in the glandular and surface epithelium whereas the stroma and blood vessels contributed only slightly.

The present study has so far confirmed that endometrial cyclical changes result in the production of a secretion which is compounded of mucins in which are intimately admixed glycogen and ribonucleic acid.

During the investigation of the glycogen fraction mention was made of the activity of alkaline phosphatase but it is now necessary to describe the histochemical appearances of this substance.

(f) Alkaline Phosphatase (Figure 22).

In order to conduct this part of the investigation it was not possible to use formol-corrosive fixed sections of endometrial tissue as heretofore. Gomori (1941) introduced a technique whereby the enzyme is identified by its action on a substrate containing organic phosphate, in the presence of calcium ions, to form calcium phosphate in situ. The calcium phosphate so formed may then be directly demonstrated by treating with cobalt nitrate and ammonium sulphide.

Fresh endometrium was fixed in cold acetone.

The findings were as follows:

In the early proliferative phase of the cycle alkaline phosphatase was present in the entire cytoplasm of the gland cells and the surface epithelium cells. The late proliferative phase showed the substance in the same position but staining more intensely. On the sixteenth day of the cycle alkaline phosphatase appeared to be abundant in the same situation but in a slightly later phase it was found in the superficial cytoplasm of the gland and surface epithelium cells, in the gland lumina as an irregular amorphous mass and also lining the blood vessels. In the late secretory and premenstrual stage alkaline phosphatase could only be found in the blood vessels. The stroma did not appear to share in this activity at any stage.

The description just given appears to correspond with the investigations of others on whose work a brief description can now be given.

Botella Llusia (47) employing Gomori's method established that in the first week of the cycle, alkaline phosphatase is present in the endometrial glands throughout the thickness of the cells, making them stand out against the stroma. In the second week, deposits are similar but as the glands reach greater development, coloration is more intense. In the third week, the deposits begin to disappear leaving the cellular protoplasm free, but on the other hand, the enzyme is deposited in the gland lumen. In the fourth week, the enzyme has totally disappeared from the glands, but may be present in the walls of the endometrial vessels in the endothelium of new vessels, a fact that explains its presence in pre-menstrual endometrium.

Using this method for microscopical study, the secretory phase of the menstrual cycle is characterised in the endometrium by depletion of alkaline phosphatase in the glands, its deposit in their lumen and its presence in the vessel walls. If none is observed on the 24th-26th days of the cycle in the vessels, it is inferred that ovulation has not occurred. Lutein insufficiency is indicated by the late beginning of secretory characteristics.

Hedberg (48) states that phosphatases are enzymes capable of hydrolysing phosphoric esters and that phosphatase activity is determined by oestrogenic influence. Since the folliculin secretion curve (judged by urinary excretion) has a second peak in the second half of the secretory phase, the

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TABLE 5.

METACHROMASIA.

Day.	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Gland Cell.	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
Gland Lumen.														P	P	P	P	P	P	P	P	P	P	P
Stroma Cell.	P	P	P			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Surface Epith.	P				P					P	P	P	P	P	P									

P. Present.

decrease in phosphatase activity in this phase is attributed to the inhibitory effect of progesterone. It is suggested that there is a functional relation between phosphatase activity and proliferation.

Ober (49) studied alkaline phosphatase activity in the endometrium and ovary during the menstrual cycle and in the myometrium during parturition. The enzyme appears in the endometrium and ovary at a definite point in the cycle and its estimation appears to offer a valuable method of diagnosis in disturbance of function. In addition to the findings as above, this worker states that a positive reaction is given by the endothelium of capillaries but not by that of the larger vessels. It was absent from the endometria of cases treated with progesterone. Surprisingly, where no alkaline phosphatase was found in uterine muscle fibres during pregnancy it appeared in varying quantities at the onset of parturition. Its presence appears to be of short duration, no ferment being found in one uterus two days after delivery. The author wonders whether this activity is an index of the strength of uterine contractions and whether it is a necessary factor in producing the violent efforts made by that organ during parturition.

Finally, Atkinson and Engle (36) showed, in the ovariectomised monkey, that the administration of oestrogens is associated with the formation of alkaline phosphatase and that if progesterone is then given the amount of the enzyme is decreased. Besides taking part in glycogen metabolism, alkaline phosphatase is concerned in the metabolism of nucleo-proteins, which indicate protein synthesis during the period of growth stimulation of the uterine glands.

Opportunity was also taken to study metachromatic change in serial paraffin sections of the endometrium.

(g) <u>Metachromasia</u> (Table 5).

Certain tissue components in the presence of certain dyes of the coal-tar group will stain a colour other than the basic colour of the dye. Mucin will stain red with toluidine blue while the rest of the tissue stains in shades of blue. (50).

Everson Pearce (51) defines metachromasia histochemically as "the staining of a tissue component so that the absorption spectrum of the resulting tissue dye complex differs sufficiently from that of the original dye and from the ordinary tissue complexes, to give a marked contrast in colour."

The findings of a study of sections were as follows: In the regenerating phase a contrasting reddish-violet substance was noted throughout the cytoplasm of the surface epithelium cell. This substance had a granular appearance in the superficial cytoplasm of the gland cell but stained intensely in the areas just superficial and basal to the nucleus.

The cytoplasm of the round stromal cell also showed granular staining.

During the proliferative phase the substance moved to the superficial cytoplasm of the surface epithelium, became granular throughout the whole cytoplasm of the gland cell and maintained its granular appearance in the stromal cytoplasm.

In the transitional phase, the substance appeared as droplets in the superficial cytoplasm of the surface epithelium and similarly in the gland cell. The subnuclear vacuole of the gland cell had a few minute stained granules adhering to its surface. The stromal cytoplasm showed granular staining.

During the secretory phase droplets were seen in the superficial cytoplasm of the surface epithelial and gland cells. The gland lumen was partly filled with a pink staining amorphous secretion. The intercellular stromal substance stained granular.

Premenstrually and menstrually the surface epithelial and gland cells appeared to be devoid of the stained material. A small residue of secretion may be present in the gland lumina. There are droplets in the cytoplasm of the decidua-like cells. The intercellular substance of the stroma also showed droplets.

These appearances seem to be at variance with those described by Vesterdal-Jorgensen (12) whose description states that in normal endometria, metchromasia is never seen in the glands post-menstrually or in the early phase of proliferation. During the latter part of the proliferative phase accumulations of secretion are in evidence in the basal part of certain of the cells of the surface and at the necks of the glands. These accumulations increase in the transitional phase, when the secretion occupies the vacuoles below the nuclei which are visible in preparations stained by the ordinary routine methods (that is, the metachromatic substances are found in the same localities as are the lipids and glycogen). In the secretory phase the gland cells are filled with the secretion at first basally; later the secretion is present throughout the cell to its apex and is thence discharged into the lumen some days before the onset of menstruation. Thus, premenstrually and menstrually the glands are empty whilst the secretion is piled up in the lumina or may occasionally be absent.

He concludes by saying that unfortunately this method has proved to exhibit vagaries in the staining results. However, in the present study, the results as given have shown a constancy throughout the great number of specimens examined.

Where metachromatic sections have been compared with identical sections stained Periodic Acid-Schiff, Best's carmine, Best's carmine diastase treated and Sudan Black, it is seen that metachromatic substance is sited in those localiites in tissues where mucin, glycogen, ribonucleic acid and lipids are detected. These latter substances can be fractionally differentiated by these stains and therefore it can be said that metachromatic substance is compounded of mucin, glycogen, ribonucleic acid and lipids.

Vesterdal-Jorgensen (12) concludes from a comparative table of reaction results that the substance demonstrated is a mucopolysaccharide present in the cell before discharge into the lumen.

Bartelmez and Bensley (52), Wislocki, Dempsey and Fawsett

(53) have described metachromatic substances in the lumina of the glands. Wislocki and Dempsey (54) had not been able to demonstrate an intracellular precursor and consider that it exists in some neutral form which is activated only on discharge into the lumen.

Bensley (55) and Sylvèn (56) have investigated the presence of metachromatic substances in endometrial stroma.

Davidson (43) quotes Lison and Matsaars (57) by stating that metachromactic staining is usually due either to the presence of the sulphuric esters of polymeric carbohydrates or to polyphosphates of the hexametaphosphate type.

McKay (58) states that intercellular stromal metachromasia was demonstrated during the proliferative phase. It was diminished or absent during the early secretory phase and re-appeared between the pre-decidual cells and the late stages of secretion. There appeared to be a reciprocal relation between intercellular stromal metachromasia and oedema of the stroma. Mast cells were present during the proliferative phase; they were fewer in number or disappeared in the early stages of secretion and re-appeared in moderate numbers in later stages. Metachromatic granules, which were probably of a ribonucleoprotein nature, occurred in predecidual cells of the late secretory endometrium. It is suggested that the secretion of the proliferative phase contains ribonucleoprotein, but during the secretory phase, mucus is an additional constituent.

The differential stains and their effects as described to this point, represent the main body of this study but in order to confirm or elaborate certain points made previously additional stains were used in certain cases.

(h) Frozen sections stained by Sudan III.

The object of this investigation was to assess the reliability of the Sudan Black method as described previously. Fresh frozen sections were employed thereby preventing simultaneous comparison by reason of the method of fixation.

The results, however, showed that fat substances appeared to be absent in the proliferative phase, appeared in the superficial

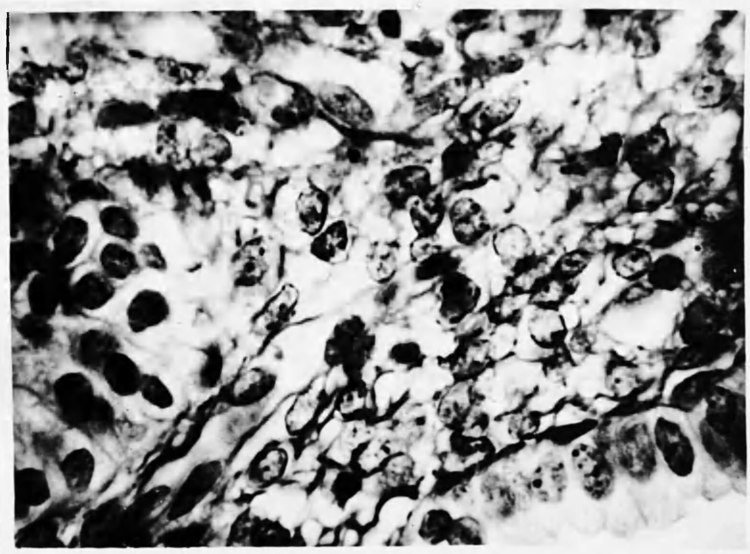

Fig. 23. Collagen fibres in stroma.

Van Gieson. 825 m.

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cytoplasm of the gland cells in the transitional and secretory phases with increasing amounts in the gland lumina until the point of menstruation. Minute histology is extremely difficult with frozen sections but the impression gained was that the sub-nuclear vacuoles in the transitional phase did not contain fatty substance.

(i) Secretory Granules.

Maximow and Bloom (4) show a plate depicting the first stage in the elaboration of secretion granules in the pelvic gland of Triton taeniatus. The method employed was fixation of tissue in Champy fluid and staining with Altmann's method and aurantia.

This method was employed in selected specimens of endometrium but close examination including oil immersion failed to provide a convincing demonstration of similar secretory granules in the gland cells.

(j) Stromal supporting Tissue - Collagen fibres (Figure 23).

In the course of the main study, comparative sections were stained by Van Gieson's method in order to show the presence of collagen fibres in stromal ground substance.

Collagen was found in all stages of the menstrual cycle as a fine open fibrillary network in interglandular spaces, adjacent to gland cells and also around the endometrial capillaries. Where gland cells were enshrouded by the spindle stromal cells, the cytoplasm of these cells gave a typical collagen stain.

This completes the decription of the histochemical changes observed in the endometrium throughout the phases of the normal menstrual cycle.

SECTION 3.

THE HISTOLOGICAL AND HISTOCHEMICAL APPEARANCES OF ENDOMETRIUM OUTWITH THE REPRODUCTIVE ERA.

1. The Infantile Endometrium (Figure 1).

Specimens of post-mortem uteri and adnexae were kindly supplied by the Pathology Department of the Royal Maternity Hospital. These were obtained from cases of stillbirth and neonatal deaths.

The histological appearances as seen by haematoxylin and eosin stain show that the uterus is lined by a simple cuboidal epithelium with large oval granular nuclei. Glands are present and show as branched tubules extending from one third to one half the depth of the endometrium. The surface epithelium cells and those lining the glands appear to be of identical origin. There is a condensation of ground substance separating these cells from the cells of the stroma. The stromal cells are either round or spindle shaped of which the round cells appear to be in the majority. The spindle cells form a lining to the ground substance beneath the surface epithelium. Mitoses are present in the cells of the surface epithelium, glands and stroma.

In all the specimens of infantile uteri examined endometrial glands were present.

Meyer and Williams state that uterine glands are frequently present at birth while Kundrat and Englemann were of the opinion that they did not appear until the third year.

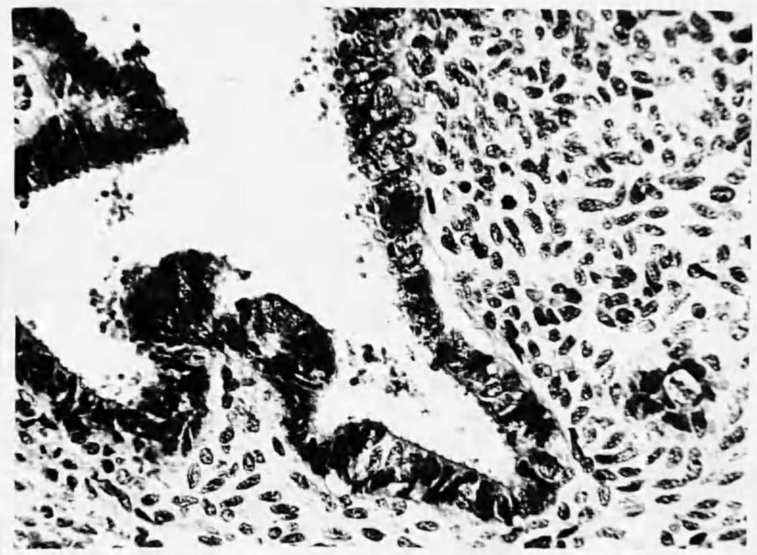

Fig. 24. Gland bud in infant with vacuole cells pushing into stroma. H and E. 325 m.

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These findings are quoted by Stander (5) who himself, has described the uterine glands as mere shallow depressions.

The present study shows that there is more endometrial development at this stage than has been, perhaps, supposed.

While the cells of the surface epithelium and glands appear to be of the same origin there appears to be greater activity in the cells of the tip of the gland crypt. These cells show subnuclear vacuolation reminiscent of that occurring in the transitional and early secretory phases of mature endometrium (Figure 24). When ovarian and endometrial development were compared it was noted that ripening Graafian follicles were present in infantile ovaries. It is evidence that a ripening follicle produces subnuclear vacuolation in the surface epithelial and gland cells of the infantile endometrium.

In such specimens where no ripening Graafian follicle could be seen, the subnuclear vacuolation appeared to be confined to the tips of the gland crypts.

2. The Histochemical Appearances.

(a) Periodic Acid-Schiff Reaction:

P.A.S. positive material is present in basement membrane beneath the surface epithelium. The stromal ground substance shows a faint positive reaction.

Where the ovary shows ripe Graafian follicles, the endometrium shows a strong positive P.A.S. reaction in the superficial cytoplasm of the surface epithelium, gland cells and also in secretion in the gland lumina and uterine cavity.

(b) Fats stained Sudan Black.

Fatty material could not be demonstrated in the surface epithelial, gland or stromal cells of the infantile endometrium where the ovaries contained only primordial follicles.

Where ripening follicles are present, then fatty substance is stained on the free surface of the gland cells and also in the secretion in the lumen.

(c) Metachromasia.

Where the ovaries appear to be inactive then a purple stain is evident in the cytoplasm of the stromal cells and also in the cytoplasm of the surface epithelium. If ripening Graafian follicles are present then the infantile endometrium shows the same purple stain in stromal and epithelial cells but a strong positive reaction in secretion in the lumina and cavity of the uterus.

(d) Methyl Green-Pyronin Stain (Ribo-nucleic acid).

There is a strongly positive reaction in the cytoplasm of the surface epithelium and the gland cells suggesting the presence of ribonucleic acid. However, treatment with ribonuclease of a control section showed that the stained material remained virtually unaltered suggesting that this substance is not ribonucleic acid. It was also apparent that the secretion present in the lumina and uterine cavity did not stain positive for ribonucleic acid.

Conclusions derived from these findings will be deferred until the critical review at the end of the study.

It had been hoped to include specimens of endometria from girls in the intermediate age groups from infancy to puberty. The pathology department of the Royal Hospital for Sick Children do not normally retain block specimens of post-mortem uteri or adnexae but have promised to provide these when they become available. However, the inability to describe the histology and histochemistry of the range in the present study is, perhaps, of no great moment when it is recalled that Hoffmann (3) states that during the phase there is very little change except for a slight increase in size of the endometrial glands.

There now remains the post-menopausal era.

1. Post-menopausal and Senile Changes in Endometrium.

Novak (7) describes how with withdrawal of oestrogen at the menopause the endometrium becomes thin; the surface and gland epithelium becomes low cuboidal in type and the glands become very narrow. Sometimes they are cystic possibly because of blockage by the contracting stroma which becomes increasingly fibrotic with the years. Terminal menstrual cycles are sometimes ovulatory but very often anovulatory and the latter endometrium often shows a hyperplastic pattern. Hyperplasia represents an exaggerated growth response in the endometrium, affecting both epithelial and stromal elements. With the onset of the menopause the growth stimulus is removed. The stromal cells regress and may become fibrotic in appearance. The epithelial cells no longer exhibit mitotic evidence of active growth. However, the endometrium of the post-menopausal woman is subject to the influence of post-menopausal oestrogen stimulation probably from the adrenal cortex. After the menopause the endometrium may show a mixed pattern of localised areas of typical and active endometrium in a generally senile retrogressive pattern. More rarely a secretory pattern may be seen but apparently never more than one year after the menopause (Novak & Richardson) (59).

McBride (60) studied the endometrial appearances of post-menopausal women in whom bleeding had not occurred since the cessation of menstruation.

He found the proportions of endometrial patterns to be (1) simple atrophy 31.5 per cent (2) active hyperplasia 12.6 per cent (3) inactive cystic gland pattern in 42.7 per cent. In the majority of cases this was in polyp formation.

Endometrial atrophy: the gland epithelium was low cuboidal in type and inactive in appearance.

Inactive cystic gland pattern existed in two varieties (1) simple polyp and (2) diffuse. The cells lining the gland were flattened and inactive in appearance. The lumina frequently contained amorphous material of a mucoid nature and degenerating epithelial cells were also present in many cases. The stroma had a loose almost myxomatous appearance with small spindle cells.

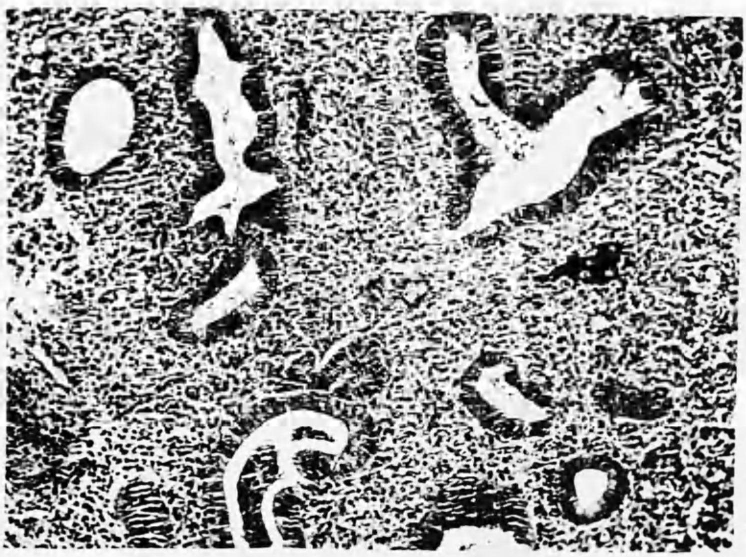

Fig. 25. Endometrium one year after menopause. P.A.S. 135 m.

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Active hyperplasia: the glands were lined by columnar epithelium with basophil cytoplasm and large nuclei. There was some enlargement of the stromal cells and the cells of both glands and stroma showed frequent mitotic figures. In some cases the hyperplasia took the form of an almost oedematous arrangement of the glands.
Proliferative endometrium: pattern similar to that of the first half of the menstrual cycle of a woman in the reproductive era mitotic figures were present in this endometrium.
Fibro-adenomatous polyp: the stroma was abundant and had a fibrous structure. The cells were spindle shaped and the matrix was homogeneous and hyalinised.
Secretory endometrium: this was similar to that of the secretory phase of the menstrual cycle. The glands were serrated and there was prominent subnuclear vacuolation.

McBride concluded that atrophy of the endometrium associated by a varying incidence of cystic dilated glands is present in about three quarters of all post-menopausal women who have had no bleeding since the menopause. Endometrial polypi, composed essentially of cystic glands are found in about 15 per cent of all cases.

A somewhat similar histological description has been given by Davies and Williams (61).

Routine examination of histological specimens is in accordance with the findings of these authors and this present study continues with the examination of sections by differential histochemical staining.

2. <u>The Histochemistry of the Post-menopausal and Senile Endometrium.</u>

It will be appreciated from the foregoing description of the normal histology of post-menopausal endometrium that a variable pattern is to be expected. For the purposes of this study random sections from asymptomatic patients were stained differentially with the following results:

(a) <u>Within one year of the menopause.</u> (Figure 25).

Where a predominantly proliferative pattern of the

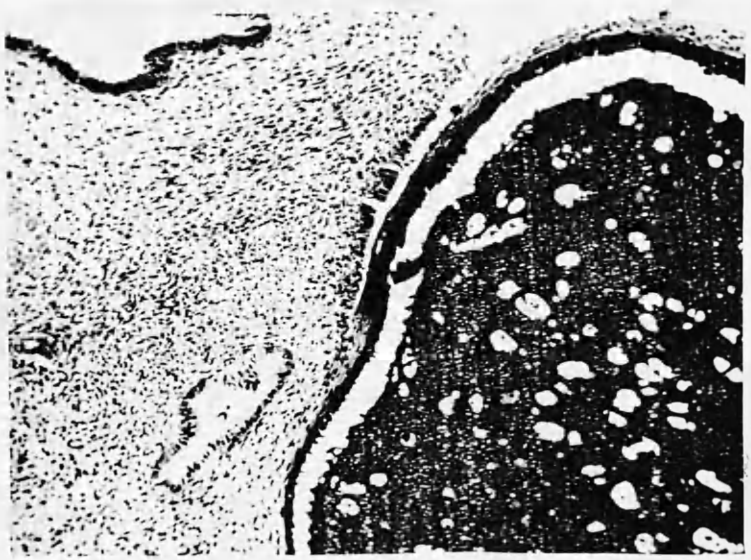

Fig. 26. Endometrium five years after menopause. P.A.S. 135 m.

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endometrium was seen, the Periodic Acid-Schiff reaction showed a mild positive result in the glands cells where no lumen secretion was present. Occasional glands showed a strongly positive secretion and the superficial cytoplasm of the cells of this gland also stained positively. Metachromasia was present in gland cells only.

The stroma generally was fibrotic and the ground substance stained weakly positive. However, certain areas, apparently unrelated to active glands, showed accumulations of positively stained amorphous material. Fat stain produced a negative result in gland, stroma or secretion. Ribonucleic acid was present in the cytoplasm of gland cells and was removed by ribonuclease digestion. Van Gieson stain revealed an increase of collagen fibres in the stroma.

Where the histological pattern was atrophic: the Periodic Acid-Schiff reaction was weak and only stained the stroma ground substance. Metachromasia was present in the gland cells only. Fatty substances were detected only in the denuded and fibrotic stromal surface. Rubonucleic acid was present in the cytoplasm of the gland and stromal cells. It was removed entirely by ribonuclease digestion. There was a diffuse increase in the collagen fibre content of the stroma.

(b) Five Years after the Menopause (Figure 26).

The histological pattern was that of a fibrous polyp with cystic hyperplasia in an atrophic endometrium. Metachromasia was present in the cytoplasm of gland and stroma cells. The lumina of cystic glands contained a strongly positive secretion. The Periodic Acid-Schiff reaction was strongly positive in the cells of dilated glands and the secretion was strongly positive. The cells of some undilated glands stained weakly others strongly. The stroma cytoplasm gave a positive reaction and there was an occasional accumulation of positive material in the stromal ground substance. Fatty substance was present on the free surface of the cells of certain glands. The cystic glands and their secretion gave a negative reaction to Sudan Black stain.

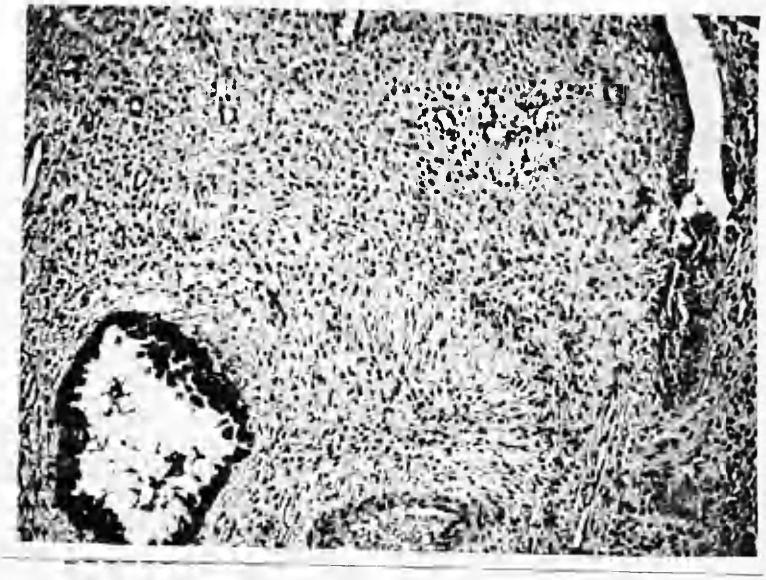

Fig. 37. Endometrium ten years after menopause. P.A.S. 135 m.

Fig. 38. Endometrium thirteen years after menopause. P.A.S. 135 m.

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Ribonucleic acid was present in the cytoplasm of the cells of undilated glands and weakly present in the stroma. Neither the cells of cystic glands nor the secretion showed the presence of ribonucleic acid. Treatment with ribonuclease did not remove all the stained material. Van Gieson's stain showed increased collagen fibres in the stroma especially around glands showing cystic dilatation.

(c) Ten Years after the Menopause (Figure 27).

The histological pattern showed cystic hypertrophy in an atrophic endometrium and the differential staining was similar to that of the five year interval.

(d) Thirteen Years after the Menopause (Figure 28).

The endometrium was atrophic. Metachromasia was present in the cytoplasm of gland cells and less so in stroma. The Periodic Acid-Schiff reaction was positive for some glands in which a little secretion was present. The surface of the tissue was coated with a substance giving a strongly positive reaction. Fatty substances were not detected. Ribonucleic acid appeared to be present to a certain extent in the gland cell cytoplasm but ribonuclease did not remove the stain. Van Gieson's stain showed a moderate and uniform amount of collagen fibres in the stroma.

It may be concluded that the post-menopausal endometrium does not respond uniformly to such ovarian hormones as are available. Furthermore what may appear to be a proliferative type of gland can actually be secretory in activity. The production of fatty substances in the endometrium ceases with the menopause. The production of ribonucleic acid appears gradually to diminish with the passage of time. The post-menopausal endometrium seems to be capable of secreting varying amounts of P.A.S. positive material. Fatty substances are absent and ribonucleic acid activity diminishing. The secretion therefore may be a mucopolysaccharide.

However, with such a variable histological pattern it will take some considerable research and time to establish the many interesting histochemical aspects of this phase in endometrial tissue.

To conclude this study reference is made to the work of McKay et al (62) on the histochemical changes in atrophic post-menopausal endometrium.

These workers state that in atrophic endometrium there is a general decrease in the histochemical constituents of the epithelial cells, the one exception being an increase in non-specific esterase activity. Cystic hyperplasia is the result of prolonged oestrogen stimulation and the histochemical reactions of the epithelium lining the glands are very variable. Ribonucleoprotein may be abundant or undetectable. Alkaline and acid phosphatase activity is usually present. Glycogen is found in patchy distribution. Glycoprotein is abundant in the secretions. Non-specific esterase activity is seldom seen in the epithelium but is abundant in the macrophages. In adenomatous hyperplasia the same histochemical variations are found.

SECTION 4.

COMMENTARY.

At the outset of this study it was stated that the purpose was to learn something more of the physiological activity of endometrium by an application of modern histological techniques.

It would be improper for a clinician to attempt a minute study of the various substances detected as this is the province of the histochemist. Likewise, the physiologist and the histopathologist have, each in his own way, contributed a vast amount of learning to the chemical and functional activities of tissues and organs. Nevertheless, there is a borderland between these various fields of research in which the clinician may be able to apply, in his own fashion and to his own requirements, some of the fruits of these researches in order to gain information which may be turned to good account in his own specialty. It was Bacon, in one of his Essays, who praised the advantages of travel. This was the spirit in which the present investigation was carried out and must be the rightful justification for its purpose.

The result has been most rewarding from a personal point of view and it is hoped that the presentation of the facts and findings has been adequately expressed.

Opinion has been given on the origins of certain endometrial cells which, from a survey of the literature, do not seem to have been identified.

There has been built up a picture of the quality of endometrial chemical change and the location in which it occurs. It has been shown that the surface epithelium plays an active part in the physiology of endometrium and there is a better understanding of the roles of gland epithelium and stroma.

Several additional histological patterns compatible with normality have been described.

There has been no attempt to correlate the histological findings of this study with the biochemistry of ovarian hormones. Being mindful of the criteria by which the investigation was conducted, one must assume that, since the menstrual histories of the subjects from whom the specimens were obtained were normal, therefore, the levels of oestrogen and progesterone must also have been normal. Where other research workers have undertaken the description of endometrial histochemical change as the result of exhibiting ovarian hormones then such descriptions have been included in the preceding sections.

As a result of this personal study it is possible to advance a theory of endometrial development.

The mesodermal epithelium which constituted the surface epithelium of the infantile endometrium appears to have remarkable capacity for change and re-constitution.

It has been shown that endometrial glands appear to be derived from it by their budding and downgrowth into the stroma. During the mature epoch, it is the ultimate downgrowth namely the glands of the basalis that maintain the cyclical regeneration of endometrium and its re-covering with surface epithelium. The gland cell of the basalis would, therefore, be the oldest tissue represented in endometrium which, having originally grown downwards as Mullerian epithelium, thereafter, continually, grows backwards towards the surface in order to prepare circumstances for the continuation of possible pregnancy in this mature period.

It has been noted that the gland buds in the newborn have a subnuclear vacuole (the position being relative to the surface as visualised) in the cell edge which presents to the stroma. This appearance is reminiscent of that seen in the surface epithelium of mature endometrium during the secretory phase.

Furthermore, the subnuclear vacuole seen in the newborn bears comparison with that which is seen in the transitional phase of mature endometrium. It will be recalled that this phase is immediately succeeded by an early secretory phase where the gland lumina are widely dilated and relatively circular in outline after which the typical scalloping of the secretory phase is seen.

The vacuole has resisted all attempts, so far, to disclose its chemical constituents despite the many methods employed.

One cannot help wondering if this vacuole stage is connected, in some way, with altering the consistency and activity of stroma in order to permit increased gland activity. This would explain the cell appearances of the gland bud in the infantile uterus and it may be the mechanism whereby, in mature endometrium, the gland, which was virtually a straight-walled tube during the proliferative phase, can now become the coiled and convoluted tube of the secretory phase.

The shedding of these vacuoles has been described during the secretory phase and also the presence of similar sized vacuoles in stroma. That the vacuoles might have passed from stroma to gland cell thence to lumen has been mentioned in section one but there has been no direct evidence of this. The presence of vacuoles in stromal intercellular spaces cannot be accounted for by this means and it can only be concluded that stroma vacuoles, coincident at the transitional phase, are derived from the disintegration of plasma cells. However, the vacuole of the gland cell could be responsible for stromal change as adduced above and, having fulfilled its purpose, the vacuole is discharged during the secretory phase into the gland lumen.

It would appear that the stroma itself can sometimes resist the action particularly by the positioning of the spindle stromal cells. Where these cells encircle a developing gland structure then the gland cannot produce its typical secretory configuration but must become infolded instead of scalloped.

It has not been possible to detect how the subnuclear vacuoles have been created but the transitional phase is characteristic of the normal menstrual cycle. Whether the effect is attributed to proliferative or to secretory activity is still uncertain.

Novak (7) wonders whether this effect is the result of oestrogen activity alone. Hoffmann (3) declares firmly that the condition is seen after ovulation. Ferin (39) administered folliculin to a subject for a time and then within twenty four hours of giving progesterone noted the appearance of subnuclear vacuoles. Furthermore, de Senarclens (41) states that the appearance of glycogen and mucus in endometrium denotes progesterone activity and since one has been able to demonstrate the presence of these substances in the transitional phase it must, therefore, be concluded that the transitional stage is a manifestation of secretory activity and a necessary precursor of the fully developed secretory phase.

The present study has shown that a form of subnuclear vacuolation exists in the infant endometrial gland cell where there was a ripening Graafian follicle in the ovary. It must be assumed that the infant primes its endometrium by secreting oestrogen from the developing follicle and that the oestrogen level is augmented from the mother's hormonal output before the child is born. Since the child does not appear to reach the phase of ovulation and progesterone secretion, the progesterone must be supplied by the mother.

The conflict of opinions regarding the development of the infantile endometrium may be due to whether the foetus can elaborate oestrogen or not. It would appear that the failure to secrete oestrogen retards glandular development perhaps until later in the child's life but that the initial priming coupled with a progesterone effect of maternal origin can induce the infantile mucosa to develop a much modified secretory endometrium. What has been regarded as oestrin-withdrawal bleeding in girl babies after birth may turn out to be a progesterone withdrawal and that this bleeding, in certain cases, can be similar to what would occur as a normal menstruation from a mature endometrium.

The transitional phase, then, may be assumed to denote a progestational effect in endometrium but cannot be said to prove that ovulation has, in fact, occurred. The reason for this is the evidence of the infantile endometrium but, logically, in mature endometrium it has been shown that progesterone is necessary for the transitional phase to occur and, therefore, extrusion of an ovum must have occurred to permit a corpus luteum to develop, secrete progesterone and evoke the normal secretory response of endometrium. The most that can be said, quae endometrium, is that the appearance of the transitional stage is presumptive proof of ovulation.

The various substances elaborated in or stored by endometrium have been described in the previous two sections. It is appreciated that the findings are highly subjective and one is conscious of the pitfalls of artefracts arising as a result of instituting staining reactions. Endometrium is one of the most delicate structures of the body and its cells quickly exhibit changes after removal from the uterus which must become more pronounced during fixation. Indeed, Bartelmez and Bensley (52) and Bartelmez (24) have described the blebs and herniations in the surfaces of the secreting cells as artefracts supposedly produced by hydrolysis of the substances which are to be secreted by the cell so that only tentative conclusions can be drawn from them as to cellular activity.

Nevertheless, having made allowance for this with regard to the cell outline as seen by microscopy, it is felt that such changes cannot affect the substances which it was desired to detect.

It is now possible to measure the effects of hormone activity by these histological methods and perfection of technique should allow the adoption of some of these methods for routine histological reporting.

Having established the normal pattern of change, one now hopes to go forward and apply this experience to the alterations of chemical activity which must exist in the wide variety of functional and pathological conditions associated with the practice of gynaecology.

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