Barbara S. Apgar, MD, MS
Clinical Professor of Family Medicine
University of Michigan Medical Center
Ann Arbor, Michigan

The Normal Transformation Zone of the Cervix

Colposcopy involves the pattern recognition of different types of epithelia and blood vessels that penetrate the epithelium from the underlying stroma. To the colposcopist, these patterns indicate whether the cervix is normal or abnormal. Every cervix exhibits a different pattern depending on its maturity and clinical evidence of precancerous or cancerous changes. Because each cervix is unique and undergoes transformation throughout the lifespan of the woman, the colposcopist will need to be aware of the subtleties and peculiarities of each stage of the transformation process. This chapter will describe the normal and abnormal findings on the cervix in an area called the transformation zone.

It is important to understand the basic terminology of colposcopic findings. There are two primary types of epithelia that define the dynamic architecture of the ectocervix and endocervix. The stratified squamous epithelium usually resides on the portio or ectocervix and is contiguous with the vaginal epithelium. The columnar epithelium lines the endocervical canal and is contiguous with the endometrium. Squamous metaplasia, a hybrid of squamous and columnar epithelium, is a transient epithelial process that may occur during the life of the woman, most notably during late fetal life, at puberty and during the first pregnancy. Metaplasia is defined as a transformation from one mature cell type to a second mature type and on the cervix usually involves a conversion from a columnar type cell to a stratified squamous cell (1), thus the term "transformation zone". Squamous metaplasia is the key to understanding the evolution of the transformation zone of the cervix.

The transformation zone is a physical space or "zone" on the cervix that includes both mature and immature cervical epithelium. Histologically, it is a zone of metaplastic
or transitional epithelium. The dynamic area where the stratified squamous and columnar cells meet is called the squamocolumnar junction (SCJ). This is an area of great importance to the colposcopist because it is here that malignant transformation and integration of the human papillomavirus (HPV) can occur. It is also the area that must be identified if the colposcopy is "satisfactory". As the normal process of squamous metaplasia evolves and the cervical architecture is changed or transformed, the colposcopic pattern can be further altered if HPV is introduced into this area of greatest vulnerability. Observing the relationship of the abnormal epithelium to the blood vessels as the developing neoplasia evolves allows recognition of the abnormal colposcopic patterns subsequently induced.

The 2003 International Federation for Cervical Pathology and Colposcopy Classification adopted the following terminology for cervical pathology.\(^{(2)}\)

I. Normal colposcopic findings.
   - Original squamous epithelium.
   - Columnar epithelium.
   - Transformation zone.

II. Abnormal colposcopic findings.
   - Flat acetowhite epithelium.
   - Dense acetowhite epithelium.
   - Fine mosaic.
   - Coarse mosaic.
   - Fine punctuation.
   - Coarse punctuation.
   - Iodine partial positivity.
   - Iodine negativity.
   - Atypical vessels.

III. Colposcopic features suggestive of invasive cancer.

IV. Unsatisfactory colposcopy.
   - Squamocolumnar junction not visible.
   - Severe inflammation, severe atrophy, trauma.
Cervix not visible.

V. Miscellaneous findings.
Condylomata.
Keratosis.
Erosion.
Inflammation.
Atrophy.
Deciduosis.
Polyps.

The Origin of the Cervical Epithelium

At birth, the female vagina and cervix are lined by two original types of epithelia:

1. The original stratified squamous epithelium that lines the vagina and most of the portio or the external surface area of the cervix.

2. The original columnar epithelium lining the endocervix.

The vagina is originally lined by columnar epithelium derived from the fusion of the mullerian ducts that is gradually replaced by the upward growth of a core of stratified squamous epithelium originating in the urogenital sinus. Eventually, this core forms the vagina. The vagina and the portio of the cervix thus become covered with stratified squamous epithelium. The original columnar epithelium lines the endocervical canal and communicates with the endometrium at the internal os.

Normal Colposcopic Findings

Columnar Epithelium

The original columnar epithelium consists of a single layer of tall columnar cells with round to oval basal nuclei, which are mucus-secreting and may exhibit cilia. The columnar cells extend from the internal os, through the endocervical canal to the margin of the original squamous cells or to the area of squamous metaplasia. The columnar epithelium lines both the surface and the underlying "glands" of the endocervix. The
columnar epithelium is creased into numerous folds or papillae representing an invagination of the endocervical cells into the cervical stroma to a depth of approximately 5-8 mm. (1) The “glands” are actually deep infoldings or crypts of the columnar epithelium and not true glands because they lack ductal structures. By convention however they have historically been called glands. The subepithelial capillary network is well developed and, because the epithelium is only one layer and conceals the vessels poorly, the deep pink or red color of the epithelium is exhibited. Because of this, the columnar epithelium may exhibit contract bleeding. Within each papillus or fold, coiled vessels intertwine in a network of loops. The terminal capillary loop is easily seen with the colposcope. After dilute acetic acid is applied to columnar epithelium, the mucus is washed off the surface and the clefts or folds swell, demonstrating a grape like appearance to the epithelium and accentuating the infoldings.

Ectropion is a term used to describe columnar epithelium that is present on the ectocervix. The ectropion is a normal physiologic finding and should not be misconstrued as an abnormal finding. In the past, the term eversion was frequently used to describe the ectropion and should no longer be used. In the prepubertal years, the columnar epithelium remains in the endocervix. At puberty under the influence of estrogen, the endocervical or columnar epithelium will evert out onto the ectocervix with subsequent positioning of the SCJ on the portio. (3)

After puberty, lactobacilli in the vagina convert glycogen in the squamous cells to lactic acid thus producing a decrease in the vaginal pH. Exposure of the columnar cells to the acidic pH produces chemical denaturation at the tips of the clefts. The destruction of the villus tips initiates an inflammatory or reparative process. This metaplastic process will be ongoing so that eventually the columnar epithelium will actually be replaced by stratified squamous epithelium thus dramatically altering the appearance of the transformation zone.

**Stratified Squamous Epithelium**

In the newborn, the original squamous epithelium is fully mature but rapidly
becomes atrophic as the maternal hormones are withdrawn shortly after birth. The squamous epithelium, under the influence of endogenous estrogen, again matures at the time of menarche. Under the influence of estrogen, the squamous epithelium undergoes constant remodeling, characterized by epithelial proliferation, maturation and desquamation of the various epithelial layers. At menopause, it again becomes atrophic as the endogenous estrogen declines.

The stratified squamous epithelium is divided into four layers or horizontal zones that are constantly replaced or remodeled under the influence of estrogen. The basal and superficial layers are the most distinctly identified. The following zones are present in the epithelium. (3)

1. The basal layer whose primary function is epithelial regeneration.
2. The parabasal layer where squamous differentiation is seen.
3. The intermediate layer where cells do not divide.
4. The superficial layer that is the most differentiated zone and serves as a protective surface over the more vulnerable basal layers.

There is a great deal of variation in the maturation of the various layers. The upper layer of mature epithelium contains cells with abundant glycogen that produce the characteristic iodine staining when Lugol’s solution is used. The basal and parabasal cells have estrogen and epithelial growth factor receptors. Estrogen stimulates DNA synthesis and epithelial growth factor produces differentiation of the cells. (1) In women with adequate endogenous estrogen, the epithelium is constantly proliferating and undergoing maturation. Colposcopically the original squamous epithelium is smooth, pink and featureless. The stromal vessels penetrate the basal layer of the epithelium and supply the epithelial cells with oxygen and nutrients. The network vessels of the epithelium can be seen with the green filter and have a delicate and reticular pattern. The original squamous epithelium does not stain acetowhite but does stain a deep mahogany brown when Lugol's solution is applied indicating the presence of cellular glycogen.
Squamocolumnar Junction

The interface between the original columnar and original squamous epithelium is called the squamocolumnar junction (SCJ). The original SCJ becomes defined during menarche and separates the original glycogenated squamous epithelium of the vagina and ectocervix from the original single layered columnar epithelium of the endocervix. At the time of puberty, the original SCJ is usually located closer to the distal fornix than the external os. The original SCJ is the embryologically determined caudal extent of columnar epithelium. The original SCJ is stationary and not usually visible colposcopically. Eventually as the transformation zone matures, the area of the original SCJ becomes a squamo-squamo junction. At puberty, significant changes in the physical appearance of the SCJ begin by a process called squamous metaplasia or squamous transformation.

Although the transformation begins at the original SCJ, the leading edge of the transformation, the new or colposcopically visible SCJ, abuts against unaltered columnar epithelium. The transformation zone, where the columnar epithelium is actually replaced by the stratified squamous epithelium, is the area bound by the original SCJ and the new SCJ. After puberty, the new or colposcopically visible SCJ has a variable location that will evolve closer to the external os where it resides in the majority of reproductive-aged women. The new SCJ represents an area of the most immature cell proliferation and because it is where HPV-associated disease begins to develop, it must be carefully assessed at each colposcopy.

This process of columnar epithelial cell conversion to squamous epithelium occurs in a continuously dynamic fashion until one mature type of epithelium is replaced by another mature type of epithelium.(3) Gradually, the columnar epithelium that comprises the "ectropion" is replaced by immature squamous epithelium, causing the new SCJ to move centripetally toward the internal os.(3) Finally, when metaplasia is completed and the newly formed squamous epithelium is mature enough to resemble the original squamous epithelium, the transformation zone is at its largest dimension. The process is completed in postmenopausal women with the SCJ located in the endocervical canal. Once the process of squamous metaplasia is completed, no
remnants of the metaplasia may remain. Pregnancy and use of oral contraceptive pills can alter the progression of the metaplastic process by hastening the transformation of columnar to squamous epithelium.

The colposcopic features of the transformation zone

The transformation zone varies among individuals and exhibits specific landmarks for the colposcopist. As the metaplastic epithelium matures and the transformation zone evolves, the newly formed squamous epithelium more closely resembles the original squamous epithelium. The original SCJ may be difficult to define but the new SCJ is readily visualized in most reproductive aged women. The new SCJ is visible to the colposcopist as the definable line between the typically beefy red columnar epithelium and the smooth, pink, mature squamous epithelium. There are landmarks on the transformation zone that help differentiate where the metaplastic area ends and the mature squamous epithelium begins.

There are four types of transformation zones: (2)
1. The transformation zone is completely ectocervical and fully visible, and may be small or large.
2. The transformation zone has an endocervical component that is fully visible and may have an ectocervical component that may be small or large.
3. The transformation zone has an endocervical component that is not fully visible and may have an ectocervical component that may be small or large.
4. The transformation zone extends caudally onto the upper vagina called the congenital transformation zone; fine mosaic may be present; may partially stain with Lugol’s.

The transformation zone exhibits many landmarks for the colposcopist:
1. Nabothisan cysts or follicles.
2. Islands of columnar epithelium.
5. Squamous metaplasia.
The area of immature squamous metaplasia cephalad to the new SCJ will continue to undergo change and exhibit a variable appearance. The columnar villi in this immature region may exhibit opaque acetowhite tips as the metaplastic cells begin to proliferate and the gland crypts and papillae converge and fuse. Patches of metaplastic epithelium can be seen on the portio interspersed with unaltered columnar epithelium. These immature areas have the potential to undergo neoplastic transformation and must be identified at the time of colposcopy. The metaplastic epithelium may stain lightly acetowhite and partially take up Lugol's resulting in a partially brown mahogany appearance. The surface of the metaplasia is typically smooth with fine, uniform vessels.

**Nabothian cysts**

Nabothian cysts are formed when the openings of the endocervical infoldings or clefts become blocked or obliterated by the squamous metaplastic process. The secretions produced by the mucus-secreting columnar cells accumulate because there is no outlet. The accumulated mucus forms cystic structures called nabothian cysts that distend the openings of the clefts and compress the dilated vessels. The overlying squamous epithelium becomes stretched which accentuates the vessels. Nabothian cysts may be a yellow, white or blue in color. Puncture of the cysts allows the mucus to escape. If the cysts are multiple, they may present a confusing colposcopic picture. The vascular pattern over the nabothian cyst displays arborization and should not be confused with the atypical vessel pattern of invasive disease. No treatment of nabothian cysts is necessary.

**Gland openings (ostia)**

Small endocervical gland crypts can be identified in areas of squamous metaplasia. They represent persistent endocervical gland crypts on the squamous epithelial surface. They are usually surrounded by a thin rim of metaplasia or can be identified by mucus extruding from the crypt opening.

**The mechanism of the development of the transformation zone**

There are several proposed histogenetic mechanisms by which the columnar
epithelium is replaced by squamous epithelium (2) but the one most generally accepted
is reserve cell proliferation. The process of squamous transformation depends on the
vaginal milieu with the initial stimulus being the low or acidic pH of the vagina produced
at puberty. The low pH of the vagina is the main stimulus to squamous metaplasia. (4)
The process may also be initiated by trauma including laser, cryotherapy or loop
excision, chronic irritation, or cervical infection. These factors stimulate a repair and
remodeling process.

It is theorized that the process of squamous metaplasia depends upon the
appearance of undifferentiated subcolumnar cells (reserve cells) beneath the columnar
epithelium. The basic feature of these cells is their potential to transform or differentiate
although their origin is debatable. During the metaplastic process, the metaplastic cells
take up glycogen. They also have some cytologic similarities to the parabasal cells of
the mature squamous epithelium. Theoretically, the process occurs as follows (1) (3):

1. A single row of reserve cells forms under the columnar epithelium.
2. Maturation and stratification of the reserve cells occur as the cells gain
more cytoplasm the size or the nucleus decreases.
3. The columnar cells degenerate and slough as the stratification continues
and the cells become more differentiated.
4. Adjacent clefts of the evolving epithelium fuse.
5. Maturation of the stratified layers of reserve cells into mature squamous
cells after glycogen is acquired.

Metaplastic cells are distinguished from mature squamous cells by lack of
surface maturation and glycogen. Metaplastic epithelium therefore does not take up the
Lugol's solution. Areas of squamous metaplasia may be covered with a single layer of
squamous epithelium.

Colposcopically, the immature squamous metaplasia abuts against unaltered
columnar epithelium or is distributed randomly. The maturation process is not
synchronous or continuous as one section of the cervix may differentiate faster than the
others. The process is especially rapid during fetal development, at puberty and at the
time of the first pregnancy. Squamous metaplasia has a random distribution on the ectocervix that produces a patchy, uneven distribution on the ectocervix reflecting the differing rates of transformation and maturation. The translucent bridges or tongues of immature metaplasia may intersperse with mature columnar epithelium giving the epithelium a textured appearance.

The colposcopic examination may reveal islands of squamous metaplastic epithelium on the tips of the columnar epithelium. These islands develop delicate bridges that fuse with neighboring islands, eventually leading to the complete obliteration of the columnar epithelium. Immature squamous epithelium (metaplastic cells) exhibits cell cohesion and organization resembling both mature columnar and squamous epithelium. (3) Immature metaplastic epithelium can resemble cervical intraepithelial neoplasia (CIN). Grossly, squamous metaplasia may exhibit fine mosaic or punctuation patterns but so can low-grade CIN. However, histologically, there is no nuclear atypia and the cellular stratification remains organized. As the metaplasia progresses and the cervical architecture is transformed, the pattern can be further altered if HPV is introduced into the area of metaplasia.

The Abnormal Transformation Zone

The abnormal TZ displays evidence of abnormal epithelial and vessel patterns reflecting disorganization or a derangement of the normal architecture. The area of the TZ predetermines the atypical or abnormal extent of cervical intraepithelial neoplasia (CIN). The dysplasia or CIN develops within the TZ in well over 95 percent of women. The lesions expand centrifugally by mechanically displacing and eventually replacing the immature metaplasia with CIN. Colposcopically, the cellular transformation from metaplasia to atypia, then to intraepithelial neoplasia then invasion results in characteristic findings such as leukoplakia, acetowhite epithelium, abnormal blood vessels and ulcerations.

The difference between the normal and atypical TZ may depend on:(5) (6)
1. The color of the colposcopic lesion before and after the application of vinegar or 3-5 percent acetic acid.

2. The sharpness of the border between the transformation zone and the original squamous epithelium.

3. The vessel pattern.

4. The depth or surface contour of the lesional tissue.

5. Uptake or rejection of iodine solution.

The normal transformation zone, as previously described, is identified as a physical space or zone on the cervix containing mature stratified squamous epithelium, squamous metaplasia, gland opening, nabothean cysts and vessels. The abnormal transformation zone displays evidence of abnormal epithelial patterns reflecting disorganization or a derangement of the normal cervical architecture. The cellular hallmark of the abnormal transformation zone is the transition to a dedifferentiated cellular state and the evolution of dedifferentiated cells, called basaloid cells, which are characterized by nuclear atypia and enlargement and reduction of cytoplasm (increased nuclear-cytoplasmic ratio). (5)

There have been several theories on how the abnormal transformation zone develops. Two older theories were proposed to explain the origin and evolution of the abnormal transformation zone. One theory proposed that cervical intraepithelial neoplasia (CIN) is multicellular in origin and spreads in a vertical manner up the width of the epithelium. The theory proposed by Burghardt presumed that CIN arises in predetermined areas of abnormal cells. The primary lesion would expand by transformation of normal cells in adjacent areas or by the coalescence of multiple abnormal areas. (7) The other theory presumed that CIN is unicellular in origin and begins as a single cell or small group of cells. This theory suggested that the CIN spreads horizontally along the basement membrane by mechanically lifting the adjacent normal cells and endocervical epithelium. (5) (8) Wright et al further postulate in light of what we know about low and high lesions that is likely that low-grade CIN is most likely multicellular with multiple types of HPV DNA in contrast to high-grade CIN that is
aneuploid and more likely to be one cell type of HPV DNA.\textit{(5)}

It is possible that CIN develops in one of three sites of cells: the basal cells of the stratified squamous epithelium, the basal cells of the transformation zone or reserve cells. \textit{(5)} The most probable area of the origin of CIN is in the basal cells of the transformation zone where squamous metaplasia occurs. \textit{(5)} Most if not all of CIN begins at the SCJ where one edge borders the endocervical epithelium or areas of squamous metaplasia. \textit{(5) (9)} Some authors believe that a small percentage of CIN arises in areas of squamous metaplasia beyond the SCJ (called skip lesions). The area bordering the SCJ or slightly distal to the SCJ is usually higher grade than lesions residing on the periphery of the portio. The area of the transformation zone determines the extent of CIN. The can therefore be assumed that CIN develops within the transformation zone in the majority of women. The lesions expand centrifugally by mechanically displacing and eventually replacing the normal squamous epithelium.

As HPV DNA is incorporated into the metaplastic cells, the cells become potentially neoplastic. Instead of forming the normal stratified squamous epithelium, the cells develop into blocks of tissue that exhibit pleomorphism, nuclear atypia and disorganization. These abnormal cells stimulate the capillary endothelial cells of adjacent capillaries thereby altering the vascular network. The blood vessels become compressed and tortuous and extend to the surface of the epithelium where they can be recognized by their characteristic colposcopic appearance. The epithelial and vascular abnormalities of the atypical transformation zone may occur to a greater or lesser extent and may change (regress or progress) on consecutive examinations.

\textbf{Leukoplakia}

Leukoplakia (white patch) is characterized by the presence of keratin overlying the surface epithelium. Normal glycogen-producing squamous epithelium does not exhibit keratinization. The layer of keratin in leukoplakia does not however predict the underlying condition of the epithelium. It is a nonspecific colposcopic finding and needs further evaluation to discover the etiology.

To the histologist, leukoplakia appears as: \textit{(5)}:
Hyperkeratosis: cornification without the presence of nuclei.
Parakeratosis: Retention of pyknotic nuclei in the keratin layer.

Hyperkeratosis usually indicates a more dense, thick and roughened epithelium than leukoplakia alone. Parakeratosis indicates a less dense and more delicate form of leukoplakia. The two types of leukoplakia may occur in acanthotic, dysplastic epithelium or invasive carcinoma.(5).

Clinically, the leukoplakia is colposcopically visible with or without magnification prior to the application of vinegar. Leukoplakia may reside both inside and outside the transformation zone. Seen with the naked eye, it creates the impression that it can be wiped off but actually it is not easily removed. It typically has the appearance of an elevated white plaque. Because the epithelium is keratinized, the light does not effectively transmit through the epithelial layers but rather is transmitted back giving the impression that the tissue is opaque.

The origins of leukoplakia include:(5)
1. Idiopathic
2. Epithelial trauma, e.g. prolapse
3. Estrogen (exogenous or endogenous)
5. Invasive cancer.

It is recommended that leukoplakia be biopsied because the keratotic layer may obscure the accurate identification of abnormal or atypical vessels that may indicate a neoplastic process. (6).

**Acetowhite epithelium**

The acetowhite reaction is the transient white coloration that occurs after the application of 3-5 percent acetic acid or vinegar to the cervical epithelium. (10) The exact mechanism of how the acetowhite reaction occurs is unclear. It may arise as a temporary protein change in cells with enlarged nuclear content or result from temporary dehydration of the cells. Because the reaction is transient, additional vinegar will need to be applied during the colposcopic examination. This colposcopic finding on
the epithelium resulting from the acetowhite reaction is called acetowhite epithelium. The application of dilute acetic acid or vinegar to squamous metaplasia and the atypical transformation zone results in a white or gray color that has varying degrees of opacity. Not only will epithelial lesions become more distinct with application of dilute acetic acid but mucus will be easier to remove from the surface epithelium. Once the mucus is removed, the details of the transformation zone become more apparent.\(^{11}\)

Columnar epithelium exhibits a distinctive acetowhite reaction when dilute acetic acid is applied. The red to pink color of the epithelium is converted to a whitish-pink color. The dilute acetic acid produces accentuation of the clefts or infoldings as well as edema and swelling of the epithelium. Glandular epithelium that is acetowhite can represent squamous metaplasia or possible a glandular epithelial abnormality. Mature squamous epithelium does not exhibit an acetowhite reaction.

Atypical structures in the transformation zone are also altered by application of dilute acetic acid. The lesional tissue may be identified as gray to pearly white depending on the severity of the lesions. The abnormal vessels become more pronounced as the acetowhite reaction fades allowing mosaic and punctuation patterns to be easily visualized. The more severe lesions will turn white more rapidly while those of lesser severity will take longer to convert. It must be recognized that the atypical vessels of invasive disease may be masked by dilute acetic acid so the vessels should be viewed with and without the green filter before the acetic acid is applied. \(^{12}\)

The acetowhite reaction may be observed in normal and abnormal tissue. Condylomata will turn pearly white with the application of dilute acetic acid. Precancerous lesions will turn varying degrees of white depending on the grade of severity of the lesion. Lesser grade lesions tend to exhibit a mild, sometimes translucent acetowhiteness compared with higher grade lesions that are usually denser and more opaque.

The colposcopist will learn to differentiate various grades of acetowhiteness and correlate this appearance with clinical significance. It is not true that all acetowhite tissue requires biopsy. The more experienced colposcopist will learn to be discriminating in deciding whether acetowhite findings should be biopsied.
The Iodine Test

The iodine test (dilute iodine or Lugol's solution) depends on the interaction between iodine and the glycogen in the epithelial cells. (13) The iodine allows the borders or margins between the normal and abnormal tissue to become more distinct. If the epithelium contains glycogen, it will take up the iodine to produce an intense mahogany color called positive iodine staining. Columnar epithelium and immature squamous metaplasia do not stain and the epithelium will retain its pink to red color. Maturing metaplasia may partially take up the iodine and stain a variegated pattern or tortoise-shell appearance. The iodine reacts with dysplastic tissue to produce a mustard yellow color due to poor glycogen content of the dysplastic epithelium, called iodine rejection. The iodine may also produce a weak reaction in areas of decreased estrogen such as atrophy. In postmenopausal women, the squamous epithelium is atrophic with scant intracytoplasmic glycogen (3) and therefore does not stain deep brown.

Vessel Abnormalities

Intraepithelial capillaries in the transformation can be seen during colposcopic examination. The pattern of these capillaries is seen in normal epithelium, cervical intraepithelial neoplasia and invasive carcinoma. The most common vascular abnormalities are mosaic, punctuation and atypical vessels. The abnormal vessel patterns of mosaic and punctuation are hallmarks of precancerous lesions whereas atypical vessels are hallmarks of invasive disease. Mosaic and punctuation result from remodeling of the original vascular structures of columnar epithelium. (4) Adolf Stafl's work on angiogenesis has furthered understanding of the morphology of the vessels in the cervical epithelium.

The green filter is useful for examining vascular patterns and should be used before and after the application of dilute acetic acid. The mosaic and punctuation vascular patterns may be seen singly or in combination. There are good diagnostic criteria to distinguish between the two but it is not always possible to grade a given lesion by the presence of one or the other. Mosaic and punctuation may be found in the
same lesion. The abnormal patterns are always identified against a background of acetowhite epithelium. (14) The coarser patterns tend to appear more dramatically and intensify as the acetowhite reaction fades. The finer patterns tend to evolve with application of acetic acid but fade more quickly without being intensified by time. The finer mosaic and punctuation exhibit a smaller intercapillary distance between the vessels. When a coarse punctuation or mosaic pattern is present, the suspicion of a high-grade lesion should be considered and a thorough inspection for atypical vessels should be performed.

**Punctuation** refers to single-looped capillaries coursing through the stromal papillae and penetrating the epithelium. As they rise perpendicular or obliquely toward the surface, they appear as red dots on the surface. They appear as small vascular dots that represent the tips of capillary loops located within a field of acetowhite epithelium. By compression of these unconnected vessels as the metaplastic cells fill in all the clefts of the columnar epithelium, punctuation develops.(4) If compression is severe, the columnar epithelium disappears and the intercapillary distance is increased. Colposcopy identifies the stippled appearance of the punctuation pattern as fine or coarse punctuation. Fine punctuation describes the appearance of looped capillaries that have a narrow caliber, are closely spaced and form a regular pattern. The intercapillary distance is minimal giving the appearance of delicate stippling. The dots are usually pink to red and lie in the same plane even after the application of dilute acetic acid. Coarse punctuation refers to wider spaced, larger and more pronounced capillaries. After dilute acetic acid is applied, coarse punctuation stands out from the plane of the surface epithelium. The punctuation may even appear as small papillae. The dots are redder than in fine punctuation.

**Mosaic** represents a network of capillaries lying perpendicular in stromal ridges like grout between tiles. The mosaic vessel pattern results when these capillaries form partitions between blocks of proliferating neoplastic epithelium. The vessels surround the blocks of atypical epithelium in basket-like structures. (4) The branching of the vessels is irregular as the atypical epithelium proliferates. Colposcopically, mosaic appears as acetowhite tiles bordered by vessels that represent confluent arborizing
capillary loops located within a field of acetowhite epithelium. Like punctation, the mosaic pattern may not be distinct before the application of acetic acid. The mosaic pattern demonstrates a network of red lines that can be fine or coarse. Fine mosaic is characterized by thin pink lines that remain in the same plane as the surface epithelium. Adjoining areas of fine mosaic may become contiguous producing a network of pink lines in an area of acetowhite epithelium. Fine mosaic may also be seen in non-glycogenated acanthotic epithelium called the congenital transformation zone. Coarse mosaic exhibits a greater irregular cobblestone effect. The red lines are more intense and the fissures are larger and more widely spaced. This gives the appearance that the acetowhite tissue is above the plane of the surface epithelium. The pattern will be intensified with time after dilute acetic acid is applied as the surrounding areas of abnormal epithelium become more acetowhite.

Immature squamous metaplasia may also exhibit mosaic and punctation. As the metaplastic epithelium matures, the capillaries of the columnar epithelium may be compressed vertically producing the characteristic vessel patterns. The pattern is exaggerated in abnormal immature metaplasia because the vessels are in greater contrast to the acetowhite epithelium. The capillaries are delicate however compared to high grade CIN. Immature metaplasia is highly vulnerable to HPV. The colposcopist should carefully inspect immature squamous metaplasia. If it is not possible to reliably determine whether the area is truly immature squamous metaplasia or whether it represents CIN, a directed biopsy may need to be performed.

Atypical Vessels

As the intraepithelial neoplastic process progresses, the epithelium thickens and the mosaic or punctation pattern may be more difficult to identify as the vessels are further compressed. Without new vessel formation however, the intraepithelial lesion can not continue to proliferate. The neovascularization process is stimulated by tumor angiogenic factor (TAF). TAF stimulates new vessels to form under and parallel to the surface epithelium. The differences between punctation and mosaic patterns of precancerous lesions (abnormal vessels) and the atypical vessels of neoplasia are usually apparent. As the intraepithelial neoplasia progresses, the abnormal vessels
become irregular, wider and display abrupt changes in caliper. The vessel pattern is completely lost as the vessels take on a non-branching pattern and can be seen running parallel or making acute angles to the surface epithelium. The intercapillary distance is increased as the neoplasia continues to proliferate. Instead of displaying arborization as benign vessels do, atypical vessels do not decrease in caliper as they branch but remain straighter and non-branching. The atypical vessels may bleed easily. Use of a green filter will screen out the red color and make the vascular pattern appear blacker so they are easier to visualize and differentiate. Atypical vessels should be biopsied to rule out the presence of invasion.

Benign and atypical blood vessels can be differentiated by the following characteristics:

1. Benign blood vessels
   ~ Regular branching of vessels (arborization)
   ~ Gradual reduction in caliper
   ~ Normal intercapillary distance
   ~ No abrupt change in direction
   ~ Do not appear in acetowhite areas
   ~ Usually diffuse

2. Atypical blood vessels
   ~ Irregular and haphazard arrangement
   ~ Abrupt acute angles
   ~ Increased intercapillary distance
   ~ Bizarre shapes
     • corkscrew
     • hairpins
     • commas
     • star-bursts