Chapter 7

Colposcopic assessment of cervical intraepithelial neoplasia

- The colposcopic diagnosis of cervical neoplasia depends on the recognition of four main features: intensity (colour tone) of acetowhitening, margins and surface contour of acetowhite areas, vascular features and colour changes after iodine application.
- The occurrence of abnormal features in a localised area in the transformation zone increases the probability of diagnosis of a neoplastic lesion.
- Considerable skill may be required to differentiate between low-grade CIN, immature squamous metaplasia and inflammatory lesions.
- Biopsy should be directed whenever in doubt.
- The observation of well-demarcated, dense, opaque, acetowhite area(s) in the transformation zone close to or abutting the squamocolumnar junction is the hallmark of colposcopic diagnosis of CIN.
- Low-grade CIN is often seen as thin, smooth acetowhite lesions with well-demarcated, but irregular, feathery or digitating or angular margins.
- High-grade CIN are associated with thick, dense, dull, opaque or greyish-white acetowhite areas with well-demarcated, regular margins, which sometimes may be raised and rolled out. They may be more extensive and complex lesions extending into the endocervical canal. The surface contour of the acetowhite areas associated with high-grade CIN lesions tend to be less smooth, or irregular and nodular. Visualization of one or more borders within an acetowhite lesion or an acetowhite lesion with varying colour intensity is associated with high-grade lesions.
- Abnormal vascular features such as punctation and mosaics are significant only if these are seen confined to acetowhite areas.
- Vascular features, such as fine punctation and/or fine mosaics in acetowhite areas, may be associated with low-grade CIN.
- Coarse punctation and/or coarse mosaics in acetowhite areas tend to occur in high-grade lesions.
- CIN lesions do not contain glycogen and thus do not stain with iodine and remain mustard or saffron yellow areas.
- Using a scoring system such as Reid's colposcopic index may guide colposcopic interpretation and diagnosis.

The colposcopic diagnosis of cervical neoplasia requires an understanding and recognition of four main features: colour tone and intensity of acetowhitening, margins and surface contour of acetowhite areas, vascular pattern and iodine staining. Colposcopy with directed biopsy is described as the reference investigation or 'gold standard' for the diagnosis of cervical precancer (Singer & Monaghan, 2000). Colposcopy has a reported sensitivity ranging from 87% to 99% to diagnose cervical neoplasia, but its specificity is lower, between 23% and 87% (Mitchell *et al.*, 1998; Belinson *et al.*, 2001).

The colposcopic features of cervical intraepithelial neoplasia (CIN) are described in this chapter to equip the student with the skills to distinguish the colposcopic findings associated with high-grade CIN (CIN 2-3) from those of low-grade lesions (CIN 1). Although the appearance of a single abnormal feature alone is not a strong indicator that a lesion is present, the occurrence of abnormal features together in a localized area in the transformation zone increases the probability of a lesion. It will become obvious during colposcopic practice that considerable skills are required to differentiate between low-grade lesions, immature squamous metaplasia and certain inflammatory conditions. The student is encouraged to obtain biopsies whenever in doubt, and to review the histopathological findings with the pathologist. Close collaboration with pathologists is obligatory and useful in improving one's diagnostic skills. At the end of this chapter, a system that enables the colposcopist to score abnormalities is presented. This system is useful as a basis for the choice of which area(s) to select for biopsy. It is essential to biopsy the 'worst' area(s) - that is, the area(s) with the most severe changes in features.

The colposcopic findings of an abnormal or atypical transformation zone can involve the whole transformation zone but more commonly affect only a portion of it and there may be multiple distinct lesions. There is usually a distinct demarcation between normal and abnormal epithelium.

The colposcopic features that differentiate an abnormal transformation zone from the normal include the following: colour tone of acetowhite areas; surface pattern of acetowhite areas; borderline between acetowhite areas and the rest of the epithelium; vascular features and colour changes after application of iodine.

After application of normal saline solution

Following application of saline, abnormal epithelium may appear much darker than the normal epithelium.

Vasculature

Using the green (or blue) filter and higher-power magnification when necessary, the best opportunity to evaluate any abnormal vasculature patterns is before the application of acetic acid, the effect of which may obscure some or all of the changes, especially in an

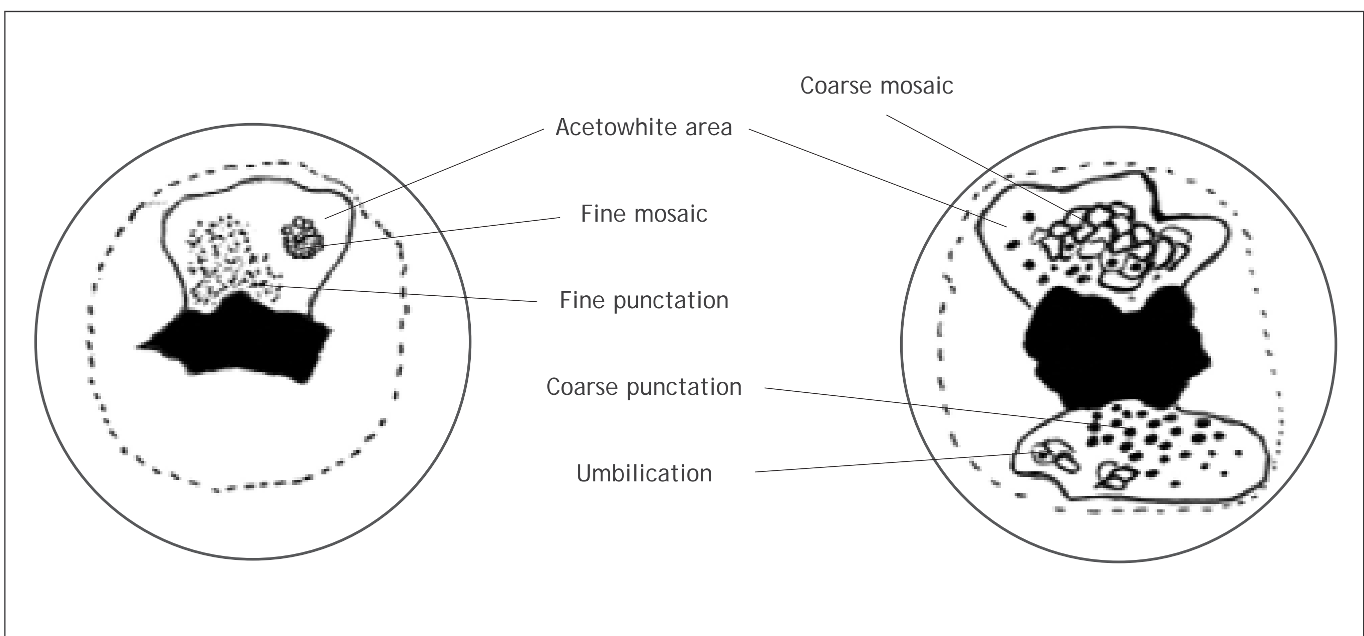


FIGURE 7.1: A schematic representation of punctation and mosaics

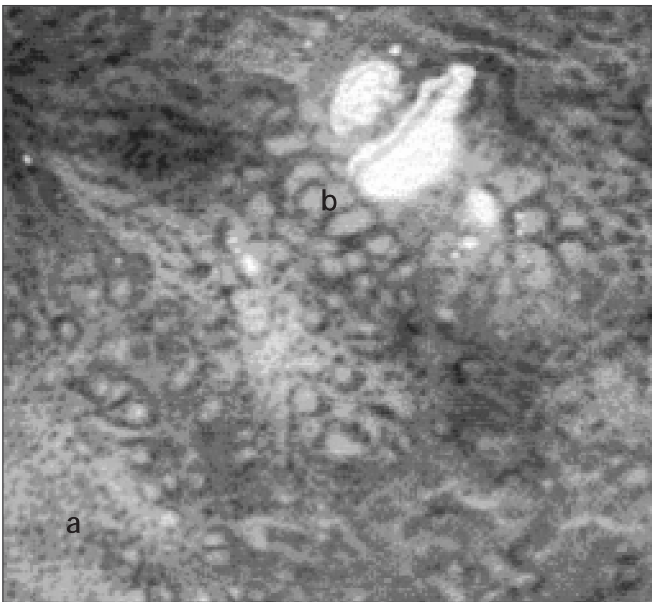


FIGURE 7.2a: Fine punctation (a) and coarse mosaic (b) seen after application of normal saline

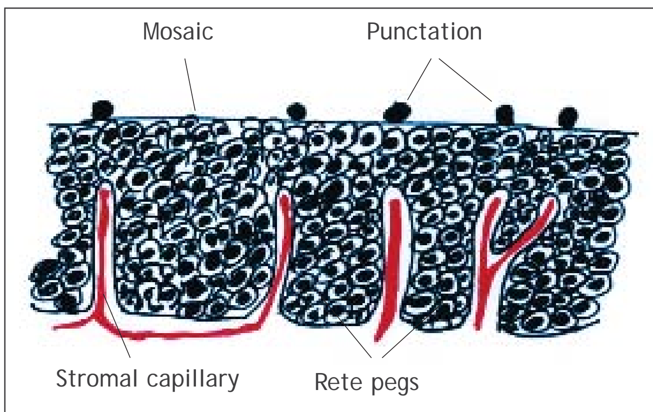


FIGURE 7.2b: Schematic diagram to show the rete pegs and the stromal capillaries which on end-on view appear as punctations

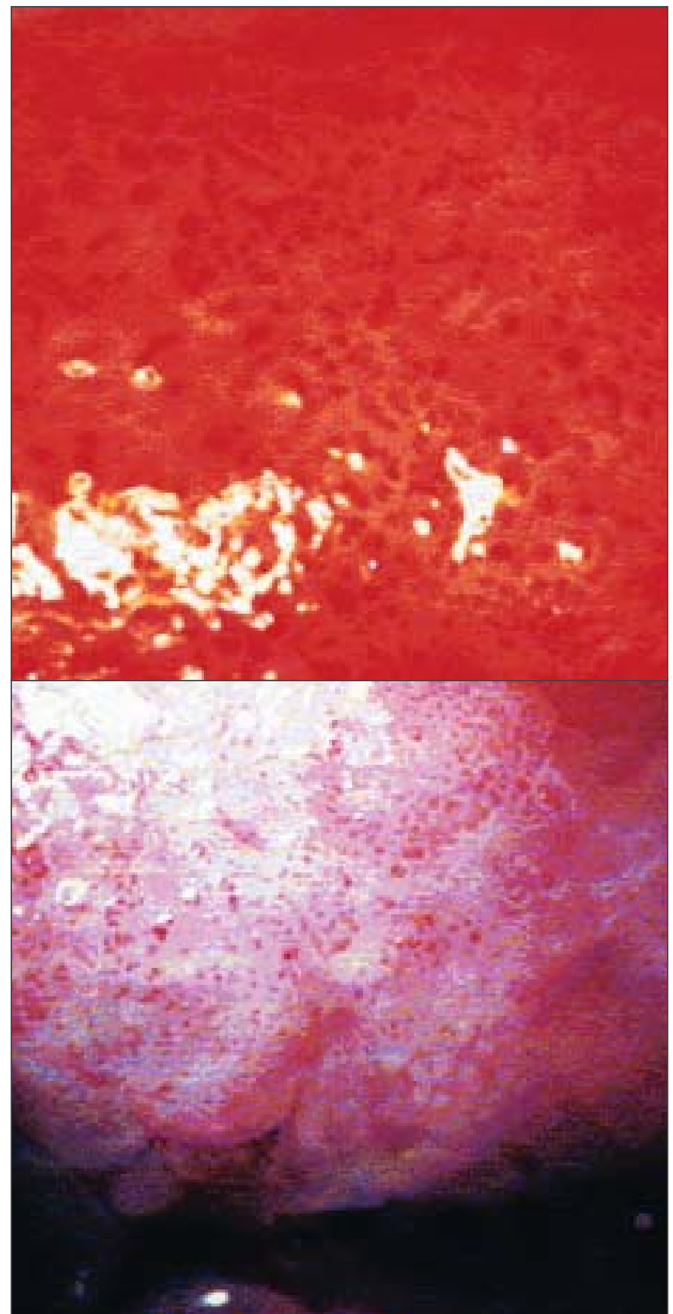


FIGURE 7.3: Coarse punctation before and after application of acetic acid

acetowhite area. The abnormalities of interest are punctation, mosaics and atypical vessels.

Capillaries: The afferent and efferent capillaries within the villi (Figure 6.4) of columnar epithelium become compressed during the normal metaplastic process and are not incorporated within the newly formed squamous epithelium. Instead, they form a fine network below the basement membrane. When CIN develops as a result of HPV infection and atypical metaplasia, the afferent and efferent capillary system may be trapped (incorporated) into the diseased dysplastic epithelium through several elongated stromal papillae (Figures 2.3 and 2.4), and a thin layer of epithelium may remain on top of these vessels. This forms the basis of the punctate and mosaic blood vessel patterns (Figures 7.1, 7.2 and 7.3). The terminating vessels in the stromal papillae underlying the thin epithelium appear as black points in a stippling pattern in an end-on view under the colposcope, making what are called punctate areas (Figures 7.1, 7.2 and 7.3). The inter-connecting blood vessels in the stromal papillae surrounding the rete pegs of the epithelium, running parallel to the surface, are observed colposcopically as cobbled areas of mosaic pattern (Figures 7.1 and 7.2). In mosaic areas, the epithelium

appears as individual small, large, round, polygonal, regular or irregular blocks. Punctation and mosaic areas may be classified as either fine or coarse. Coarse changes tend to be associated with more severe degrees of abnormality. When both punctation and mosaic patterns are found to coexist, the same evaluation criteria for colposcopic prediction of disease are used as when they exist separately.

Vessels exhibiting punctation and mosaics are usually more strikingly obvious than the normal stromal vessels because these vessels penetrate into the epithelium and are thus closer to the surface. When acetic acid is applied, these abnormal vascular patterns seen to be confined to the acetowhite areas.

Fine punctation refers to looped capillaries - viewed end-on - that appear to be of fine calibre and located close to one another, producing a delicate stippling effect (Figures 7.1 and 7.2a). *Fine mosaics* are a network of fine-calibre blood vessels that appear in close proximity to one another, as a mosaic pattern, when viewed with the colposcope (Figure 7.1). These two vascular appearances may occur together and may be found in low-grade (CIN 1) lesions. The patterns do not necessarily appear throughout the whole lesion.

Coarse punctation (Figure 7.3) and *coarse mosaics* (Figures 7.1 and 7.2) are formed by vessels having larger calibre and larger intercapillary distances, in contrast to the corresponding fine changes. Coarse punctation and mosaicism tend to occur in more severe neoplastic lesions such as CIN 2, CIN 3 lesions and early preclinical invasive cancer. Sometimes, the two patterns are superimposed in an area so that the capillary loops occur in the centre of each mosaic 'tile'. This appearance is called umbilication (Figure 7.1).

Leukoplakia (hyperkeratosis)

Leukoplakia or hyperkeratosis (Figure 7.4) is a white, well-demarcated area on the cervix that may be apparent to the unaided eye, before the application of acetic acid. The white colour is due to the presence of keratin and is an important observation. Usually leukoplakia is idiopathic, but it may also be caused by chronic foreign body irritation, HPV infection or squamous neoplasia. No matter where the area of leukoplakia is located on the cervix, it should be biopsied to rule out high-grade CIN or malignancy. It is not usually possible to colposcopically evaluate the vasculature beneath such an area.

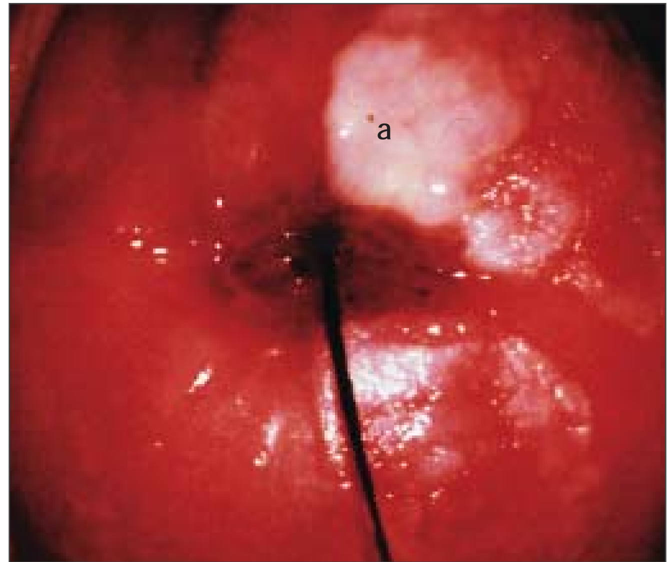


FIGURE 7.4: Hyperkeratosis (leukoplakia) (a)

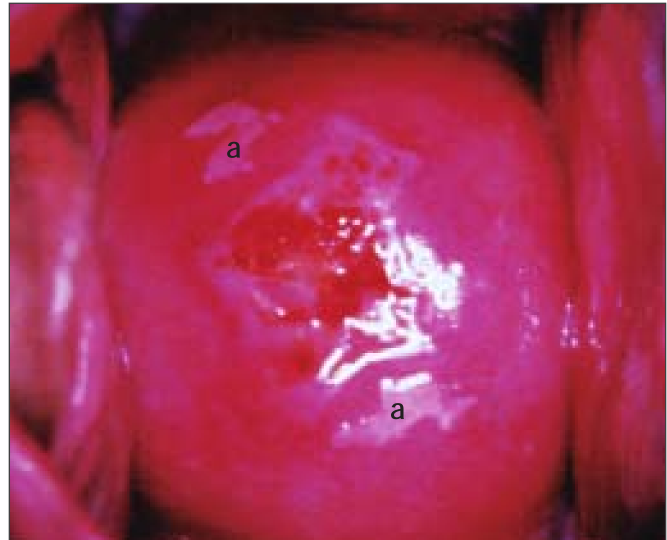
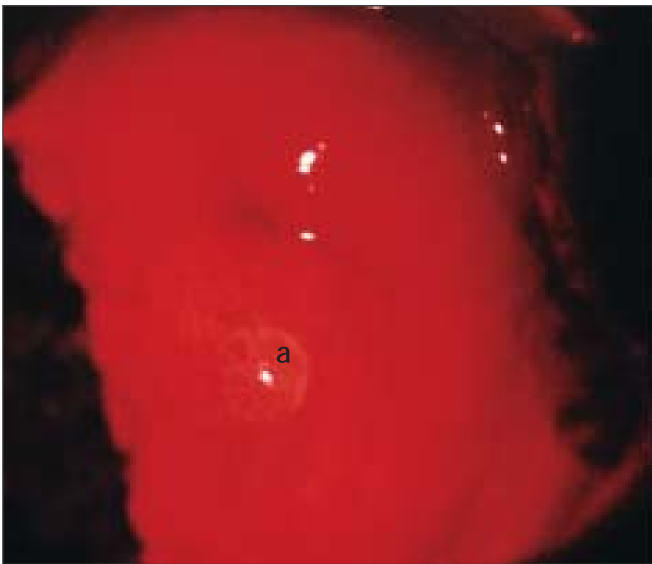


FIGURE 7.5: The geographic satellite lesions (a) far away from the squamocolumnar junction suggestive of condyloma

Condylomata

An exophytic lesion on the cervix usually represents and exhibits the characteristic features of a condyloma (Figures 7.5- 7.8). Condylomata are multiple, exophytic lesions, that are infrequently found on the cervix, but more commonly in the vagina or on the vulva. Depending on their size, they may be obvious to the naked eye. They present as soft pink or white vascular growths with multiple, fine, finger-like projections on the surface, before the application of acetic acid. Under the colposcope, condylomata have a typical appearance, with a vascular papilliferous or frond-like surface, each element of which contains a central



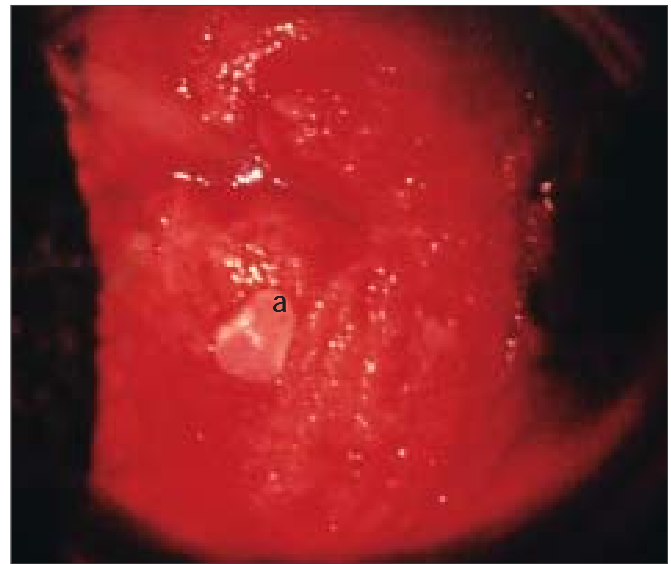


FIGURE 7.6: Exophytic condyloma in the posterior lip of the cervix (a) before and after 5% acetic acid application

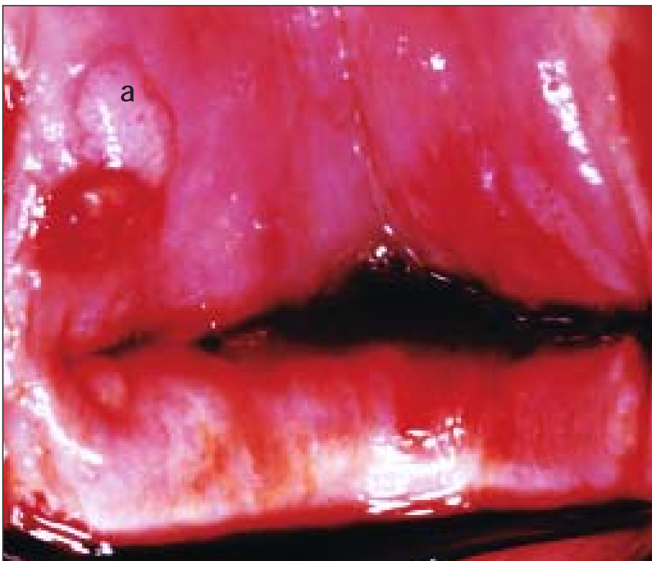


FIGURE 7.7: Exophytic condyloma in the cervix (a) after application of acetic acid

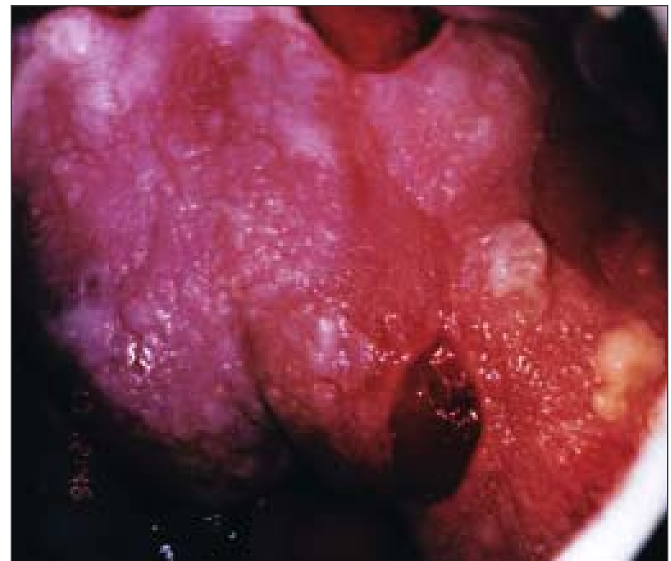


FIGURE 7.8: Condyloma with an encephaloid (cerebriform) pattern

capillary. Occasionally, the surface of a condyloma may have a whorled, heaped-up appearance with a brain-like texture, known as an encephaloid pattern (Figure 7.8). Often, the surface of the lesion may be densely hyperplastic. These lesions may be located within, but are more often found outside the transformation zone. After application of acetic acid, there is blanching of the surface with acetowhite change persisting for some time. A condyloma at the squamocolumnar junction can sometimes be confused with a prominent area of columnar epithelial villi. Both tend to be acetowhite, but condyloma is whiter. It is always prudent to obtain a biopsy to confirm the diagnosis of any exophytic lesion and to rule out malignancy. Condylomatous lesions may not take up iodine stain or may stain only partially brown.

After the application of 5% acetic acid solution

The observation of a well demarcated, dense, opaque, acetowhite area closer to or abutting the squamocolumnar junction in the transformation zone after application of 5% acetic acid is critical. In fact, it is the most important of all colposcopic signs, and is the hallmark of colposcopic diagnosis of cervical neoplasia. The degree to which the epithelium takes up the acetic

acid stain is correlated with the colour tone or intensity, the surface shine, and the duration of the effect, and, in turn, with the degree of neoplastic change in the lesion. Higher-grade lesions are more likely to turn dense white rapidly. Abnormal vascular features such as punctation, mosaicism and atypical vessels are significant only if these are seen in acetowhite areas.

The acetic acid dehydrates cells and reversibly coagulates the nuclear proteins. Thus, areas of increased nuclear activity and DNA content exhibit the most dramatic colour change. The most pronounced effects are observed in high-grade lesions and invasive cancer. A direct correlation exists between the intensity of the dull, white colour and the severity of the lesion. Less differentiated areas are associated with an intensely opaque, dull-white appearance of lesions in the transformation zone.

Flat condyloma and low-grade CIN may uncommonly present as thin, satellite acetowhite lesions detached (far away) from the squamocolumnar junction with geographical patterns (resembling geographical regions) and with irregular, angular or digitating or feathery margins (Figures 7.9- 7.13). Many low-grade CIN lesions reveal less dense, less extensive and less complex acetowhite areas close to or abutting the squamocolumnar junction with well demarcated, but irregular, feathery or digitating margins (Figures 7.10-7.16) compared with high-grade CIN lesions (Figures 7.17-7.27). High-grade lesions show well demarcated, regular margins, which may sometimes have raised and rolled out edges (Figures 7.25 and 7.26). High-grade lesions like CIN 2 or CIN 3 have a thick or dense, dull, chalk-white or greyish-white appearance (Figures 7.17-7.27). They may be more extensive and complex lesions

Table 7.1: Surface extent of acetowhite areas associated with cervical neoplasia

Cervical neoplasia	Cases	One lip of cervix (%)	Both lips (%)
CIN 1	27	21 (78)	6 (22)
CIN 2	30	17 (57)	13 (43)
CIN 3	87	36 (41)	51 (59)
Early invasive cancer	66	10 (15)	56 (85)

Adapted from Burghart *et al.*, 1998

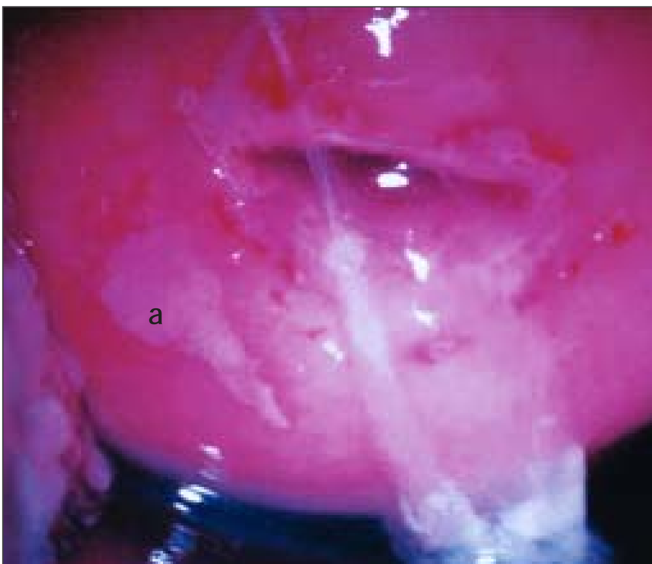


FIGURE 7.9: Geographic satellite lesion after application of 5% acetic acid (a) far away from the squamocolumnar junction, suggestive of low-grade lesion

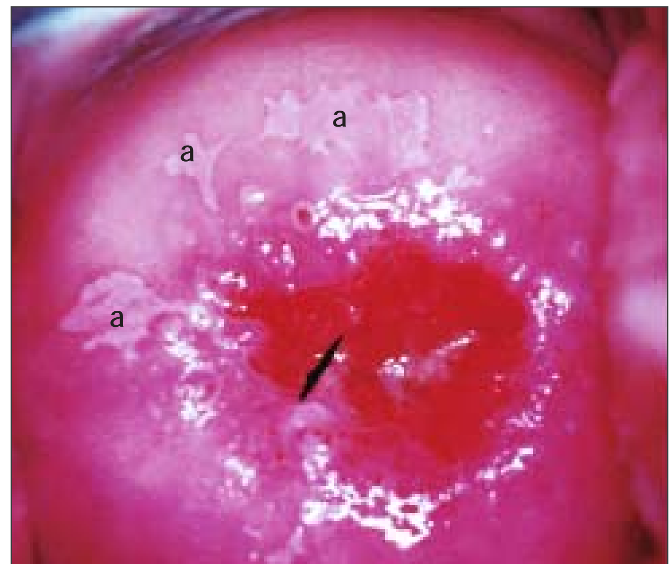


FIGURE 7.10: Geographic satellite lesions after application of 5% acetic acid (a) far away from the squamocolumnar junction, suggestive of low-grade lesions

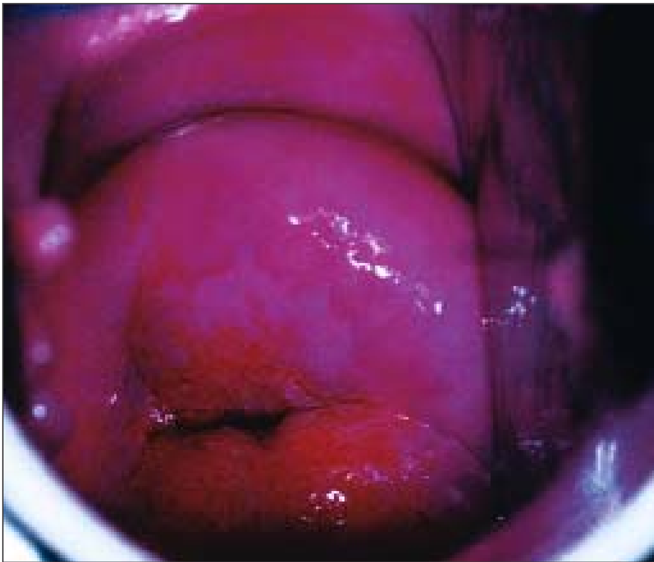


FIGURE 7.11: Thin acetowhite lesion with geographic margins in the upper lip. Histology indicated CIN 1

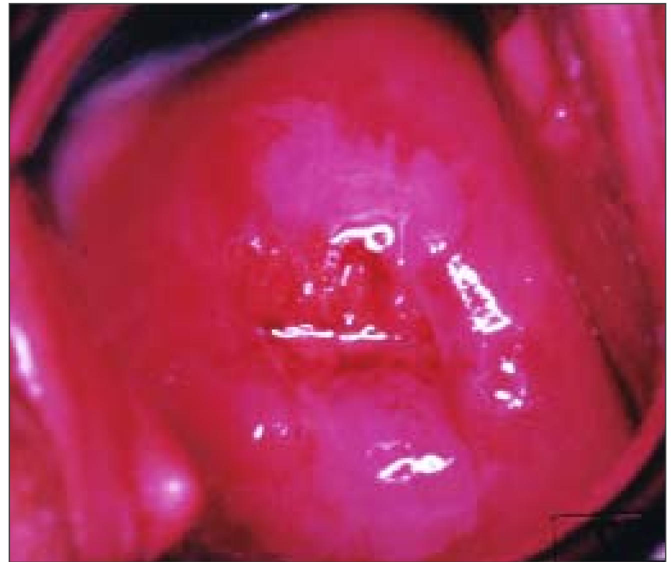


FIGURE 7.13: Mildly dense acetowhite lesions arising from the squamocolumnar junction in 12 and 6 o'clock position with irregular geographical margins, which on histology proved to be CIN 1 lesion

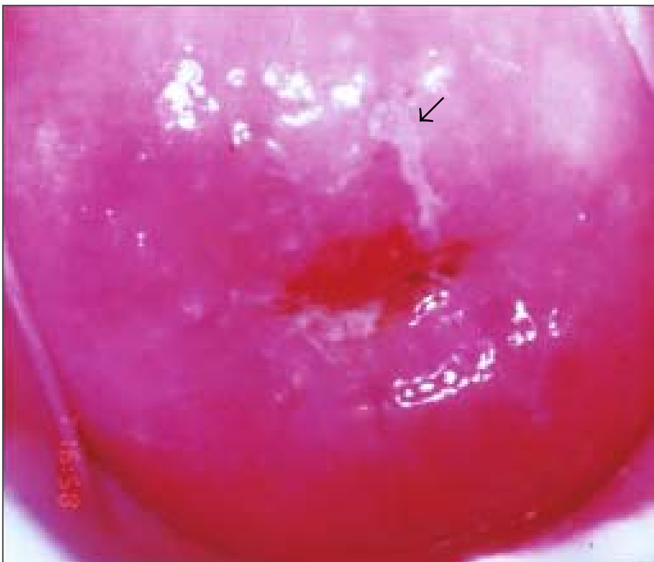


FIGURE 7.12: Mildly dense, thin, elongated acetowhite lesion with regular margins abutting the squamocolumnar junction. Note the fine mosaic at the distal end of the lesion. Histology indicated CIN 1

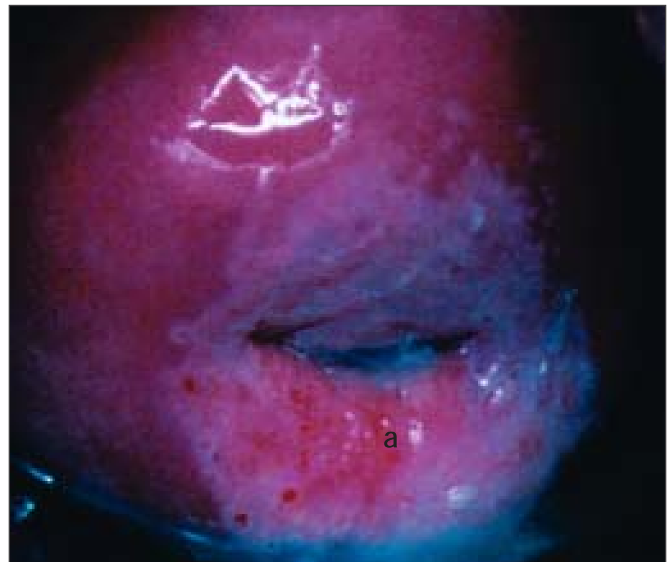


FIGURE 7.14: Note the circumorificial acetowhite CIN 1 lesion with irregular margin and fine mosaics (a)

extending into the endocervical canal (Figures 7.22-7.27) compared with low-grade lesions. High-grade lesions often tend to involve both the lips (Burghardt *et al.*, 1998) (Table 7.1). Severe or early malignant lesions may obliterate the external os (Figures 7.22 and 7.25).

As lesions become more severe, their surfaces tend to be less smooth and less reflective of light, as in normal squamous epithelium. The surfaces can become irregular, elevated and nodular relative to the surrounding epithelium (Figures 7.20 and 7.23-7.27).

The line of demarcation between normal and abnormal areas in the transformation zone is sharp and well delineated. High-grade lesions tend to have regular, sharper borders (Figures 7.17, 7.18, 7.19, 7.21,

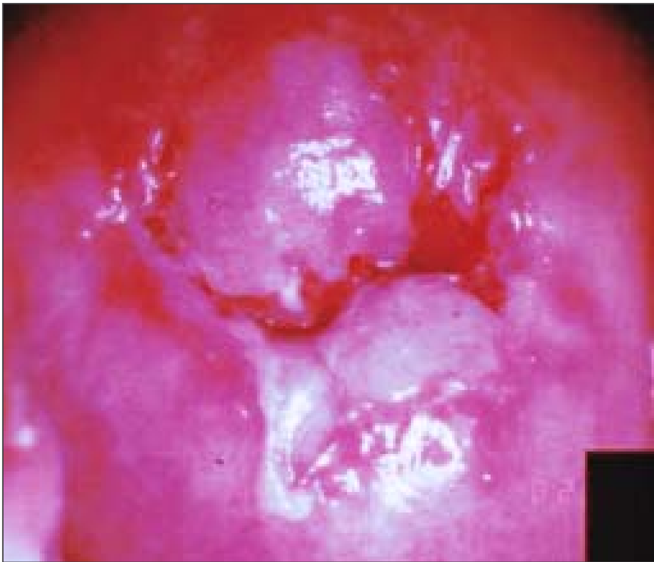


FIGURE 7.15: Moderately dense acetowhite lesions with irregular margins in the anterior and posterior lips (CIN 1)

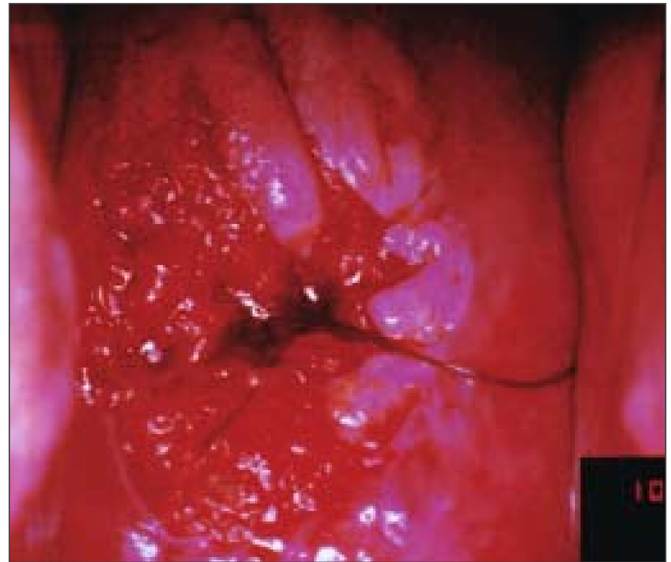


FIGURE 7.17: Moderately dense acetowhite lesions with well defined margins and coarse punctations in the anterior lip and in 3 o'clock position (CIN 2 lesion)

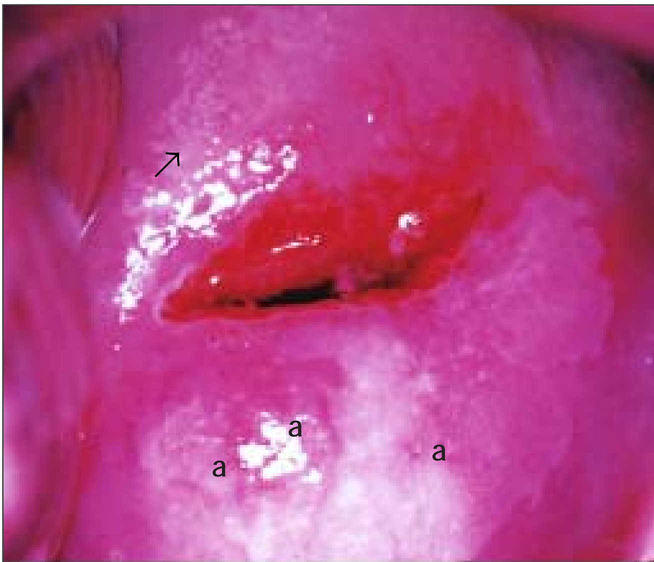


FIGURE 7.16: Circumorificial, mild to dense acetowhite lesion with fine mosaic (arrow). Histology indicated CIN 1. Note the internal borders within the lesion (a)

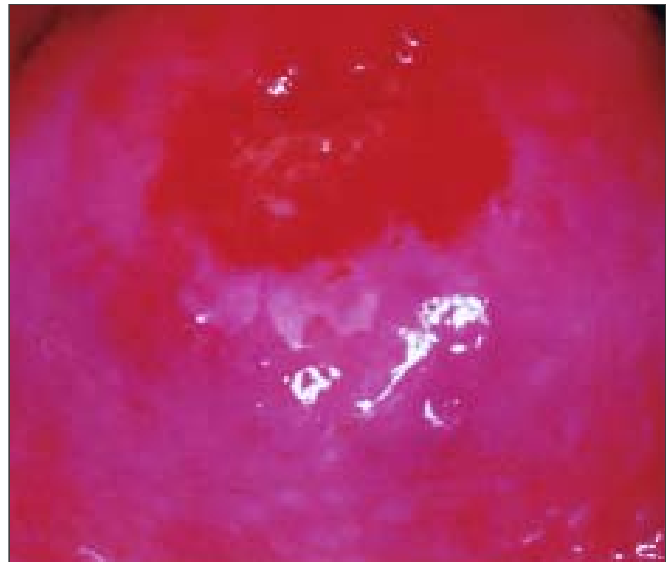


FIGURE 7.18: Dense, well defined acetowhite area with regular margins and coarse mosaic (CIN 2 lesion)

7.23, 7.25 and 7.26) than low-grade lesions (Figures 7.13-7.16). Visualization of one or more borders within an acetowhite lesion ('lesion within lesion') (Figure 7.21) or a lesion with differing colour intensity (Figure 7.16) is an important observation indicating neoplastic lesions, particularly high-grade lesions. The crypt openings that are involved in high-grade precursor lesions may have thick, dense and wide acetowhite rims called cuffed crypt openings (Figure 7.26). These are whiter and wider than the mild, line-like acetowhite rings that are sometimes seen around normal crypt openings.

The cardinal features that should differentiate between the CIN lesions and immature metaplasia are the less dense and translucent nature of the acetowhitening associated with metaplasia, and the

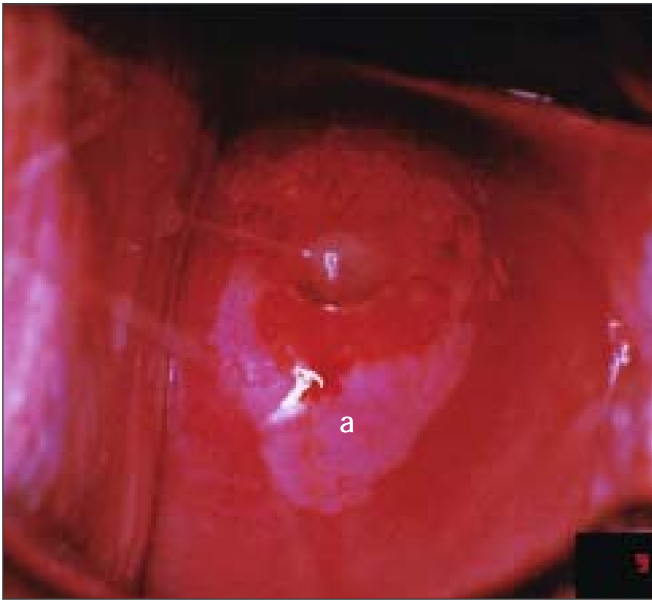


FIGURE 7.19: A dense acetowhite lesion with varying colour intensity and coarse mosaics (a) in a CIN 2 lesion

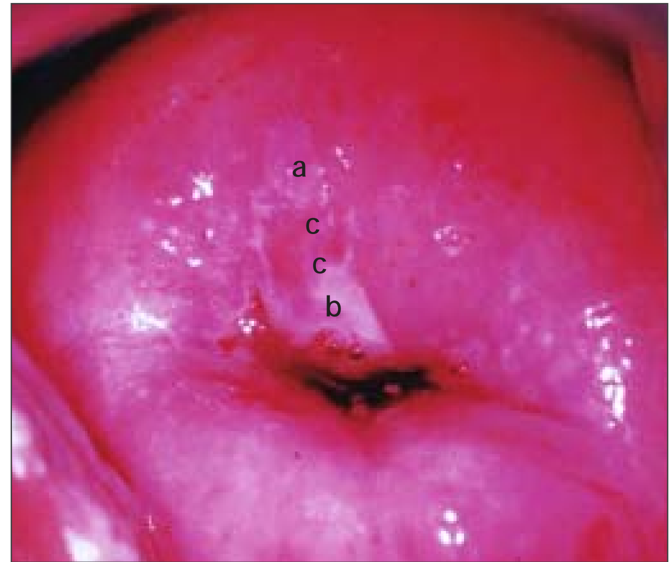


FIGURE 7.21: An acetowhite lesion arising at 12 o'clock position, abutting the squamocolumnar junction. Note the two colour intensities in the same lesion (a and b) with an internal border within the same lesion (c). This is an example of a lesion within a lesion

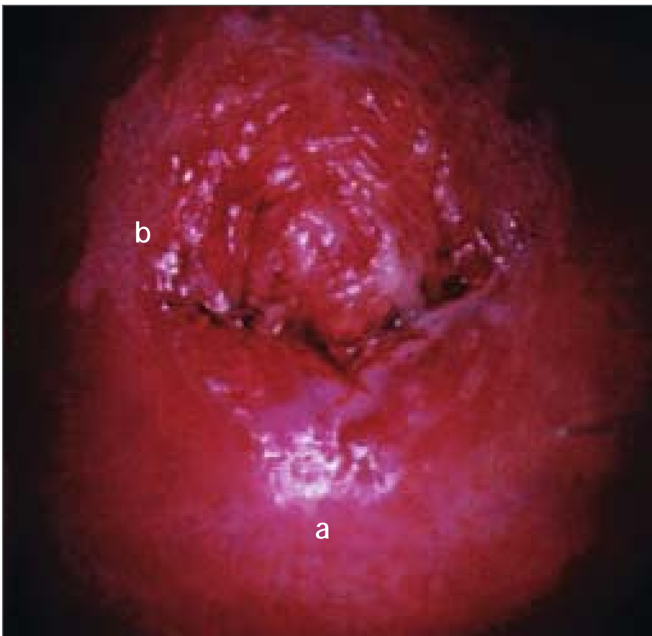


FIGURE 7.20: Acetowhite lesions with coarse punctation (a) and mosaics (b) in a CIN 2 lesion

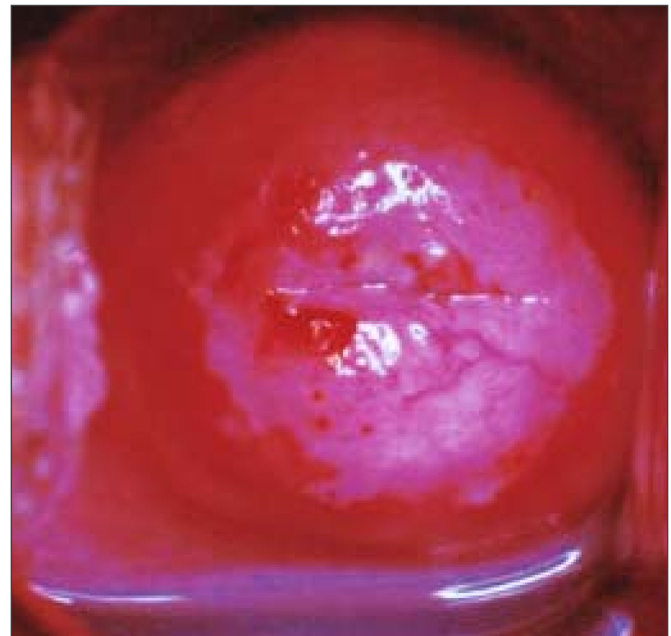


FIGURE 7.22: A circumorificial dense opaque acetowhite area with coarse mosaics (CIN 3 lesion)

lack of a distinct margin between the acetowhite areas of immature metaplasia and the normal epithelium. The line of demarcation between normal epithelium and acetowhite areas of metaplasia in the transformation zone is diffuse and invariably blends with the rest of the epithelium (Figures 6.8-6.13). The finger-like or tongue-like projections of the metaplastic epithelium often point towards the external os centripetally (Figures 6.11 and 6.12). The acetowhite lesions associated with CIN are invariably

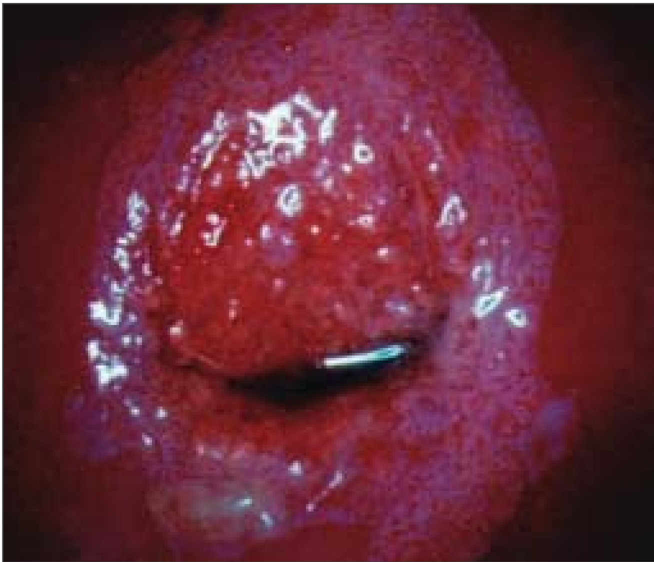


FIGURE 7.23: A dense acetowhite lesion with regular margin and coarse, irregular punctation in a CIN 3 lesion

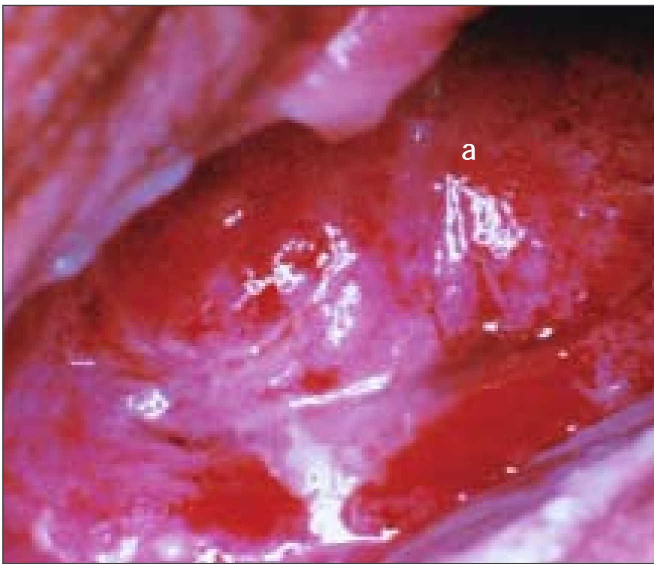


FIGURE 7.24: Coarse mosaics (a) in a CIN 3 lesion

located in the transformation zone closer to or abutting, and appearing to arise from, the squamocolumnar junction (Figures 7.11-7.21). They spread centrifugally, pointing away from the external os. The line of demarcation between normal squamous epithelium, inflammatory lesions, and regenerating epithelium is also diffuse (Figures 9.2 and 9.5).

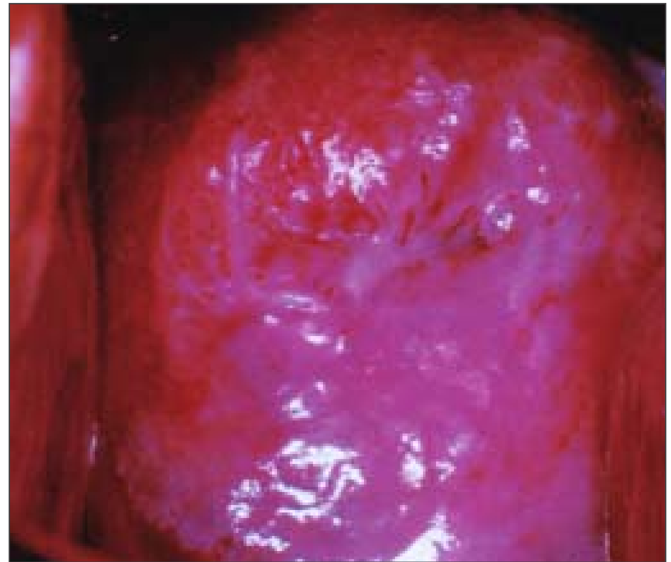


FIGURE 7.25: Note the intensely dense, complex, acetowhite lesion (CIN 3 lesion) with raised and rolled out margins, obliterating the external os

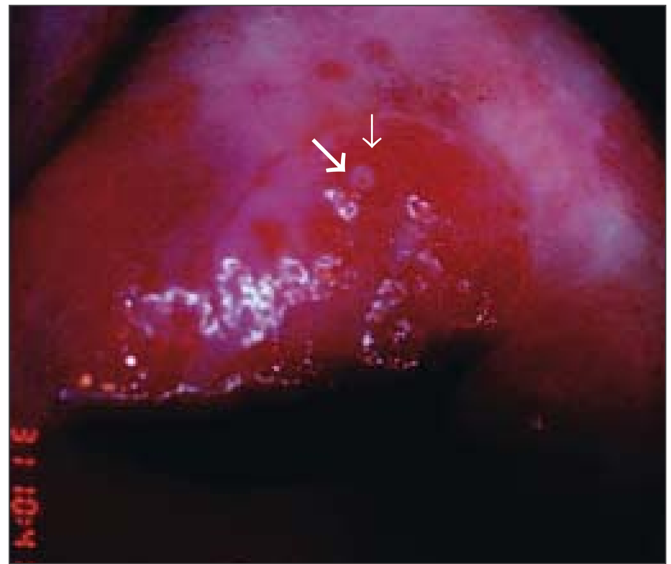


FIGURE 7.26: A dense acetowhite lesion with raised and rolled out margins with a cuffed crypt opening (dense arrow) and coarse mosaics with umblication, suggestive of a CIN 3 lesion

To summarize, acetowhite staining is not specific for CIN and may also occur, to some extent, in areas of immature squamous metaplasia, the congenital transformation zone, inflammation and healing and regenerative epithelium. However, acetowhite changes associated with CIN are found localized in the transformation zone, abutting the squamocolumnar

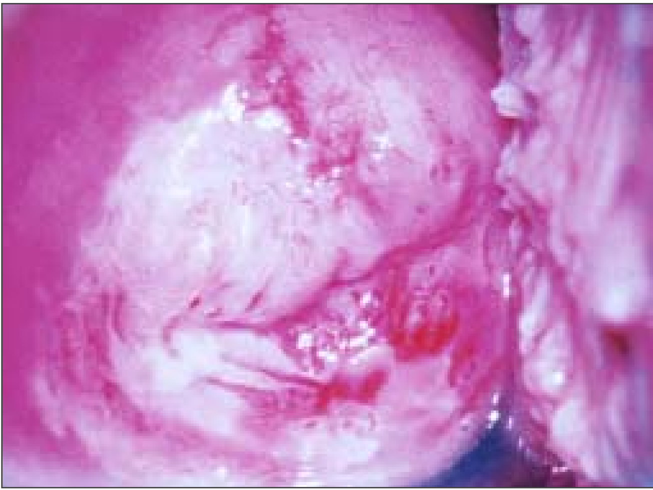


FIGURE 7.27: A dense acetowhite, opaque, complex, circumorificial CIN 3 lesion

junction and well demarcated from the surrounding epithelium. Low-grade lesions tend to be thin, less dense, less extensive, with irregular, feathery, geographic or angular margins and with fine punctation and/or mosaic; sometimes, low-grade lesions may be detached from the squamocolumnar junction; and atypical vessels are seldom observed in low-grade lesions. On the other hand, high-grade lesions are associated with dense, opaque, grey white, acetowhite areas with coarse punctation and/or mosaic and with regular and well demarcated borders; these lesions often involve both lips and may occasionally harbour atypical vessels; CIN 3 lesions tend to be complex, involving the os.

After application of Lugol's iodine solution

Lugol's iodine solution is abundantly applied with a cotton swab to the whole of the cervix and visible parts of the vagina. The periphery of the cervix, fornices and vaginal walls must be observed until the epithelium is strongly stained dark brown or almost black by iodine. Normal vaginal and cervical squamous epithelium and mature metaplastic epithelium contain glycogen-rich cells, and thus take up the iodine stain and turn black or brown. Dysplastic epithelium contains little or no glycogen, and thus does not stain with iodine and remains mustard or saffron yellow (Figures 7.28-7.32). This colour difference is helpful in distinguishing normal from abnormal areas in the transformation zone that have shown faint acetowhitening. Columnar epithelium does not stain with iodine and immature metaplasia only partially stains, if at all. Atrophic epithelium also stains partially with iodine and this makes interpretation difficult in post menopausal women. Condylomatous lesions also do not, or only partially, stain with iodine (Figure 7.33).

Atypical epithelium of CIN may be less firmly attached to the underlying stroma, from which it may easily detach or peel off, after repeated application with different solutions, resulting in a true erosion (epithelial defect) exposing the stroma. Such true

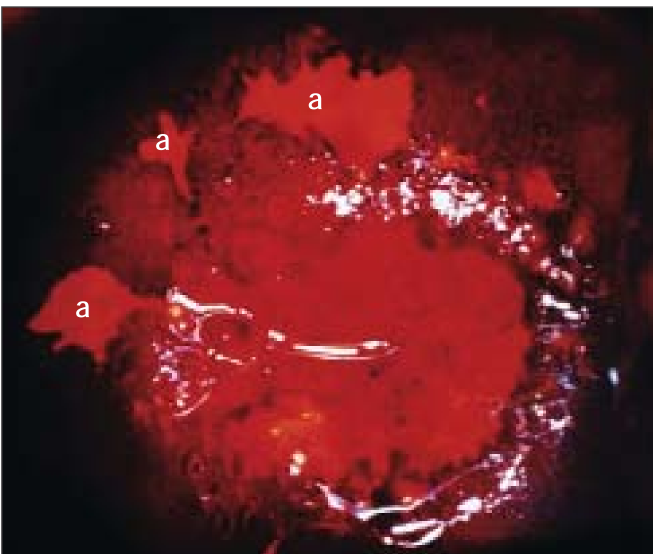


FIGURE 7.28: Satellite lesions (a) do not stain with iodine after the application of Lugol's iodine solution and remain as thin yellow areas (see the appearance after acetic acid application in Figure 7.10)

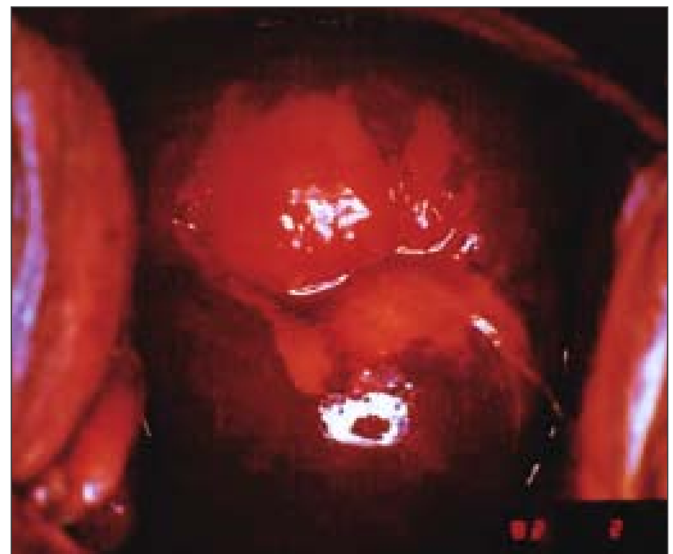


FIGURE 7.29: A CIN 1 lesion with a mustard yellow iodine-negative area with irregular margins (see the appearance after acetic acid application in Figure 7.15)

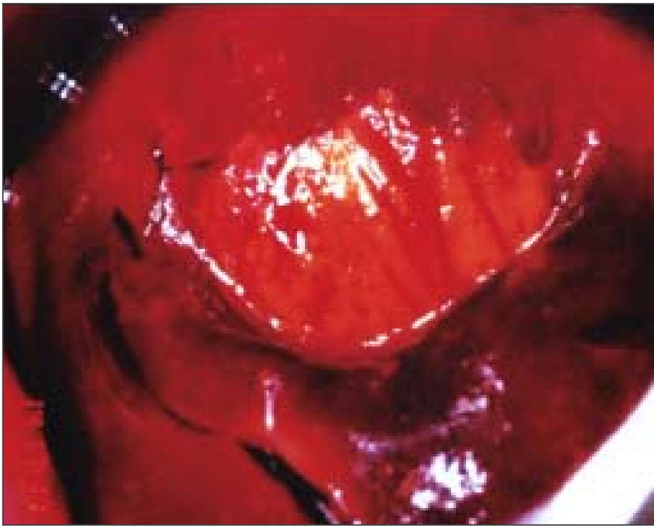


FIGURE 7.30: Mustard yellow iodine-negative area in the anterior lip (CIN 2 lesion) after the application of Lugol's iodine solution

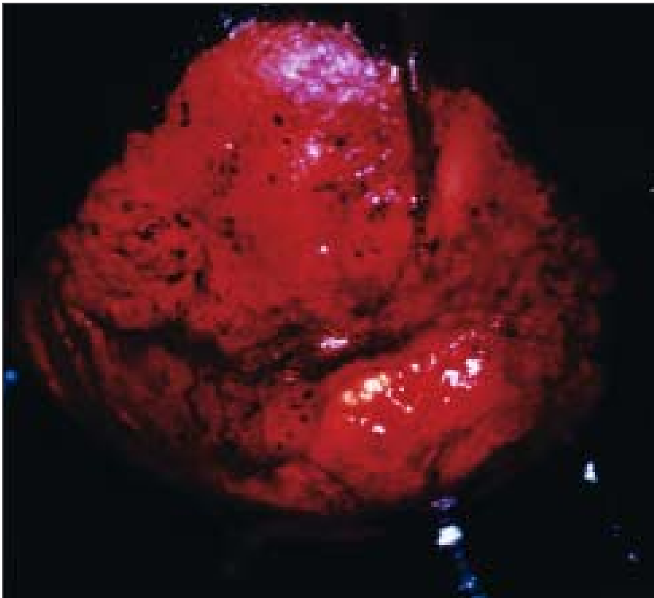


FIGURE 7.31: Dense saffron yellow iodine-negative area of a CIN 3 lesion after the application of Lugol's iodine solution. Note the surface irregularity.

erosions may easily be observed after iodine application, as the stroma does not stain with iodine.

Determining the nature of the lesion

The colposcopic detection of CIN essentially involves recognizing the following characteristics: the colour tone, margin and surface contour of the acetowhite

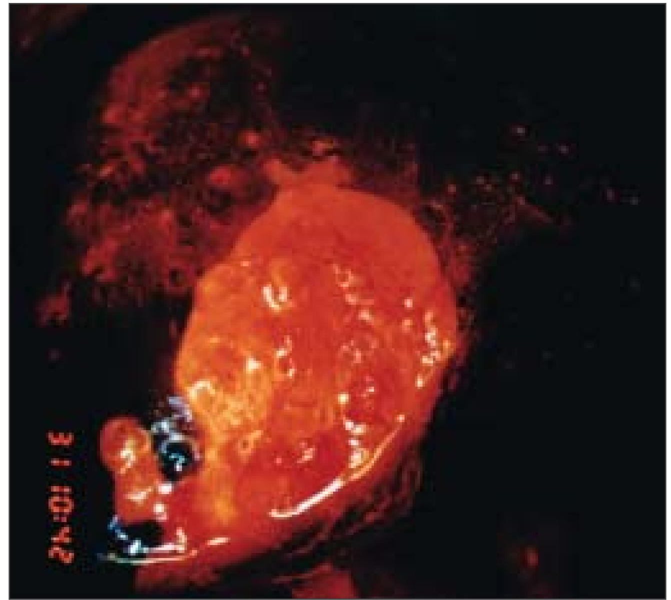


FIGURE 7.32: A dense mustard yellow iodine-negative area in the upper lip suggestive of CIN 3 lesion (see the appearance after acetic acid application in Figure 7.26)

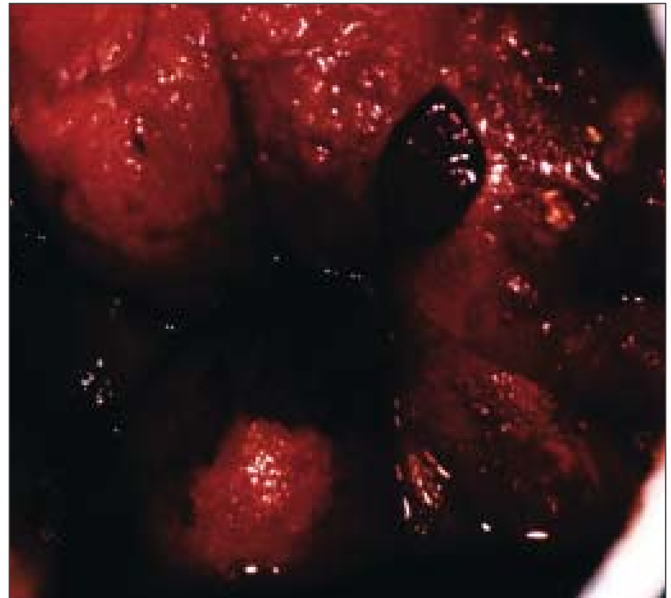


FIGURE 7.33: A condylomatous lesion does not stain with iodine (see the appearance after acetic acid application in Figure 7.8)

epithelium in the transformation zone, as well as the arrangement of the terminal vascular bed and iodine staining. Variations in quality and quantity of the above atypical appearances help in differentiating CIN from physiological, benign, infective, inflammatory and reactive changes in the cervix. Grading schemes, based on these variations may guide the colposcopic diagnosis.

Table 7.2: Modified Reid colposcopic index

Feature	0 points	1 point	2 points
Colour of acetowhite (AW) area	Low-intensity acetowhitening; snow-white, shiny AW; indistinct AW; transparent AW; AW beyond the transformation zone	Grey-white AW with shiny surface	Dull, oyster-white; Grey
AW lesion margin and surface configuration	Feathered margins; angular, jagged lesions; flat lesions with indistinct margins; microcondylomatous or micropapillary surface	Regular lesions with smooth, straight outlines	Rolled, peeling edges; internal demarcations (a central area of high-grade change and peripheral area of low-grade change)
Vessels	Fine/uniform vessels; poorly formed patterns of fine punctuations and/or fine mosaic; vessels beyond the margin of transformation zone; fine vessels within microcondylomatous or micropapillary lesions	Absent vessels	Well defined coarse punctation or coarse mosaic
Iodine staining	Positive iodine uptake giving mahogany brown colour; negative uptake of lesions scoring 3 points or less on above three categories	Partial iodine up-take by a lesion scoring 4 or more points on above three categories – variegated, speckled appearance	Negative iodine uptake by a lesion scoring 4 or more points on the above three criteria

Scoring: A score of 0 to 2 points = Likely to be CIN 1; 3-4 points = Overlapping lesion: likely to be CIN 1 - 2; 5 to 8 points = Likely to be CIN 2 - 3 lesions.

Table 7.3: Grading abnormal colposcopic findings using two categories

Grade	Findings
1. Insignificant	The acetowhite epithelium is usually shiny or semitransparent. The borders are not sharp, with or without fine-calibre vessels (fine punctation and/or fine mosaic), which have ill-defined patterns and short intercapillary distances. There is an absence of atypical vessels.
2. Significant	Dense acetowhite or grey opaque epithelium is sharply bordered. There are dilated calibre, irregular shaped or coiled vessels (coarse punctation and/or mosaic). Atypical vessels and sometimes irregular surface contour indicate either imminent or invasive cancer.

Adapted from Coppleson *et al.*, 1993 b

We recommend that the student should become familiar with the current colposcopic terminology given in Appendix 4 and use this to record the colposcopic findings (Stafl & Wilbanks, 1991).

The colposcopist is also encouraged to make a colposcopic prediction (or 'diagnosis') at the end of the colposcopic session in terms of normal (or negative), low-grade CIN, high-grade CIN, invasive cancer, other (e.g., inflammation etc.) and unsatisfactory colposcopy. Use of a scoring or grading system may guide colposcopic interpretation and diagnosis in a less subjective manner and helps developing a systematic approach to colposcopy. The modified Reid colposcopic score (Table 7.2 and Appendix 5) based on the colposcopic index proposed by Reid & Scalzi (1985) is quite useful for this purpose. We recommend that beginners routinely use this scoring system to decide whether or not a lesion is CIN and to select biopsy sites. An alternative may be a two-class grading system developed by Coppleson et al (1993) (Table 7.3). We also recommend the student to use the above systems only when an acetowhite area is observed.