

Staining in Gastrointestinal Endoscopy: Clinical Application and Limitations

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Introduction

The fine detail of the structure of the gastrointestinal mucosa is not delineated during conventional endoscopy. Yamakawa et al. [1] reported in 1966 that spraying a blue dye solution over gastric mucosa was useful in revealing such structures as the *areae gastricae*. However, the results with this method were not always satisfactory, because gastric mucosa was also colored by the dye. In 1972 Ida et al. [2] reported a new procedure for eliminating gastric mucus. Using these techniques, the results of dye-spraying are consistently effective.

Chromoscopy is a recently coined term which denotes the use of colored materials to enhance the accuracy of endoscopic examination. Many different stains have been described for use, singly or in combination, prior to or during endoscopy. In gastrointestinal endoscopy dyes or stains have primarily been utilized to enhance the detection of diseased areas, to guide endoscopic biopsies, and to obtain better visual diagnosis or characterization of small abnormalities. Tattooing is a means of permanently labeling a site in the gastrointestinal tract for future identification by intramural injection of a pigment.

In recent years there has been a natural progression to the combined use of chromoscopy and high resolution video endoscopy and magnification endoscopy [3]. A variety of dyes, stains, and pigments have been utilized in gastrointestinal endoscopy. The classification of stains used for endoscopic purposes differs from that used in histochemistry. Contrast staining, sometimes termed chromoscopy, highlights tissue topography by entering mucosal depressions and crevices. Absorptive, or vital stains identify specific epithelial types or cellular constituents by preferential staining. Reactive stains identify cellular products, for example by the color change of a pH indicator (Table 1).

Clinical Applications of Chromoscopy

Esophageal Lesions

The absorptive stains have the greatest utility for evaluating dysplastic and neoplastic diseases in the esophagus.

Lugol's solution is an iodine-based absorptive stain with an affinity for the glycogen in nonkeratinized squamous epithelium [4]. The normal esophagus turns a deep green-brown color within moments of application, gradually fading over minutes to hours. Dilutions of 1–5% are usually used and delivered *via* catheter. Dysplastic or neoplastic tissue, inflamed squamous epithelium as seen in erosive esophagitis, and nonsquamous epithelium such as the columnar-lined epithelium will not stain because glycogen is not present. Glycogen acanthosis stains more intensively than surrounding normal mucosa.

Staining with Lugol's solution may facilitate the diagnosis of early esophageal cancer and improves preoperative assessment of its extent [5–8]. This agent has enhanced the identification of early stage occult cancer in screening programs for high-risk patients such as heavy smokers, alcoholics, and those with head and neck cancer [5, 7–9]. In a recent project in China 225 patients were studied [10]. Before staining, the sensitivity for identifying high-grade dysplasia or invasive cancer by means of visible mucosal lesions was 62%, and the specificity was 79%. After staining, unstained areas identified these same lesions with a sensitivity of 96% and a specificity of 63%, and 23% of patients with severe dysplasia and 55% of those with moderate dysplasia were identified only after staining.

Lugol's staining has been used to identify the squamocolumnar junction for evaluation of Barrett's esophagus (Figure 1) and to identify areas of residual Barrett's mucosa following endoscopic mucosal ablation with, for example, photodynamic therapy or multipolar electrocoagulation [11].

Table 1 Classification of stains

Stain	Chemical name	Use
<i>Contrast stains</i>		
Indigo carmine	Sodium tindsulfonate	Contrast topography
India ink	Carbon suspension	Tattooing
<i>Vital stains</i>		
Lugol's iodine	Potassium iodide	Stains glycogen in normal squamous mucosa
Methylene blue	Methythionine chloride	Stains actively absorbing epithelium and metaplastic tissue
Toluidine blue	Diphenylamino toluphenazo thionium chloride	Stains nuclear material of malignant epithelial lesions
Cresyl violet		Stains margins of pits on colonic mucosal surface
<i>Reactive stains</i>		
Congo red	Biphenylene naphthadene sulfonic acid	Defines acid-secreting areas

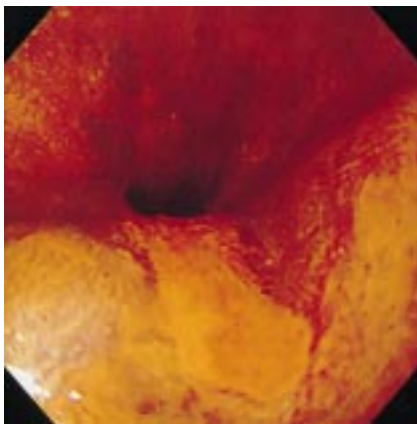


Figure 1 Lugol's staining in Barrett's esophagus. The normal esophageal mucosa is stained brown or dark brown, but Barrett's esophagus is not stained by Lugol's solution

Methylene blue is another vital stain which is used in the esophagus. This stain enters the cytoplasm of absorptive tissues such as those in the normal intestine or in intestinal metaplasia [12]. Canto et al. [13] prospectively evaluated the effectiveness of methylene blue in detecting specialized columnar epithelium and dysplasias in patients with Barrett's esophagus. The stain showed specialized columnar epithelium in 18 out of 26 patients. The methylene blue staining pattern was focal in 72% of cases and diffuse in 28%. In those patients who have a focal staining pattern biopsies should be predominantly directed to areas with positive staining to diagnose specialized columnar epithelium. There is increasing evidence that methylene blue staining can improve the rate of diagnosis of short-segment Barrett's esophagus or intestinal metaplasia at the gastroesophageal junction and cardia. In addition, methylene blue staining improves the detection rate of dysplasia and cancer with decreasing overall surveillance costs.

Methylene blue staining is reproducible and has the potential to be used for monitoring the progression of Barrett's esophagus over time and for studying the natural course of gastroesophageal reflux disease and Barrett's esophagus [13].

Toluidine blue is a vital dye which stains the nuclear material of malignant epithelial cells. It has been used for decades, in 1–2% aqueous solution, to stain oropharyngeal and esophageal lesions [14–17]. Neoplastic areas tend to stain a dark blue-violet (Figure 2). It is used to selectively stain Barrett's esophagus at endoscopy and has a sensitivity of 98% and specificity of 80% for this condition [15].

Acetic acid produces reversible intracellular cytoplasmic protein denaturation [18]. It has been used as an aid in the detection of small lesions in the uterine cervical mucosa during colposcopy [19]. This mucosa resembles that of the gastroesophageal mucosal junction. Guelrud et al. [20] hypothesized that acetic acid can help to visualize small lesions in the distal esophagus. They report the results of a prospective evaluation of the use of acetic acid to improve the detection of columnar epithelium in patients with Barrett's esophagus, who were being treated with multipolar electrocoagulation. Guelrud et al. [20] suggest that acetic acid instillation enhances the ability to detect small or indistinguishable remnant islands of columnar epithelium after endoscopic treatment of Barrett's esophagus, and that this method is safe, rapid, and inexpensive.

Gastric and Duodenal Lesions

Using a contrast dye, such as indigo carmine, several gastroduodenal diseases can be detected more easily. Indigo carmine, a widely available contrast agent, does not actually stain the gastrointestinal mucosa. At standard concentrations of 0.1–0.4%, indigo carmine is a watery solution with a deep blue color which contrasts sharply with the red gastrointestinal mucosa. Because of gravity, the solution pools in areas of depression and ulceration and fills the crevices and valleys between mucosal projections. The result is a marked highlighting of anatomical topography. Indigo carmine has been used to enhance endoscopic visualization throughout the gastrointestinal tract. It is usually sprayed on the mucosa *via* a catheter; however, it has also been given orally *via* capsule.

Using the contrast method, normal, that is acid-secreting fundic mucosa of the proximal stomach is thick and reddish, whereas pyloric (antral) mucosa and non-acid-secreting fundic mucosa affected by fundal gastritis are thin and yellowish [21]. The appearance of both non-secreting mucosae, when observed in the stomach, corresponds to atrophic gastritis. The distal border of the normal fundic type of mucosa in the stomach can be easily recognized because of the differences in mucosal characteristics and the pattern of the area gastrica. The contrast method is useful in differentiating benign from malignant ulcers and may aid in determining whether or not an ulcer is recurrent. A

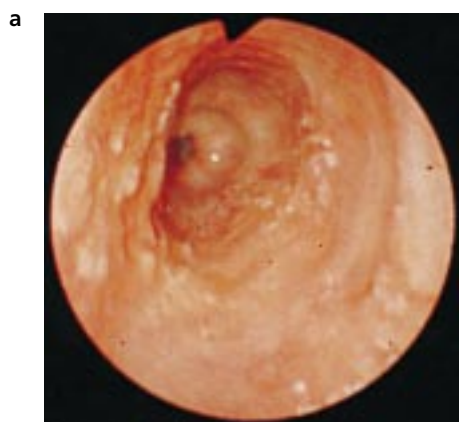


Figure 2 Superficial esophageal cancer. **a** Conventional endoscopic finding. **b** Toluidine blue staining. The mucosal cancer of this lesion is stained faint blue, and the part invaded to the submucosal layer is stained deep blue. **c** Double staining with toluidine blue and iodine solution. (Courtesy of H. Machuuchi, M.D., Tokai University, Kanagawa, Japan)

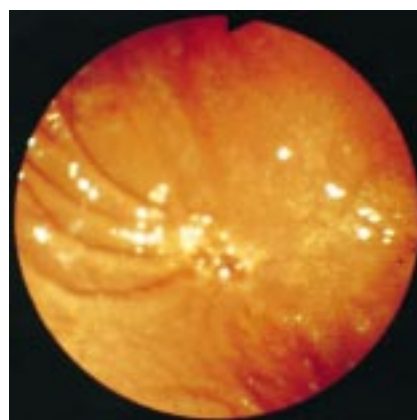
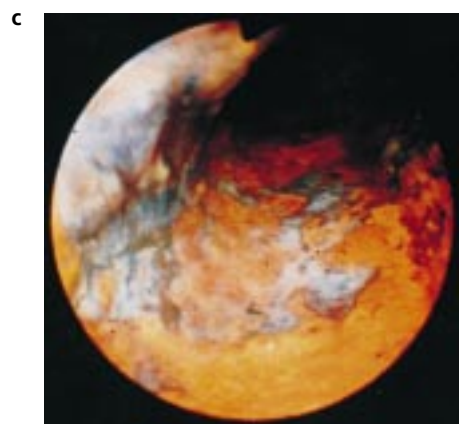
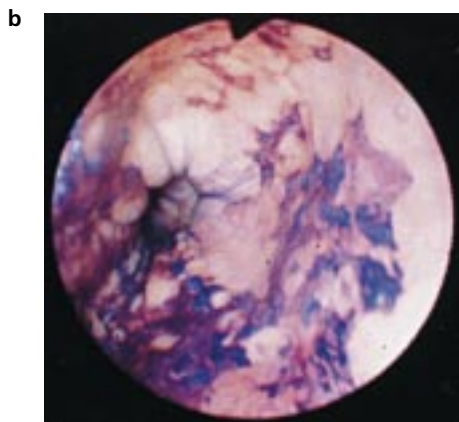
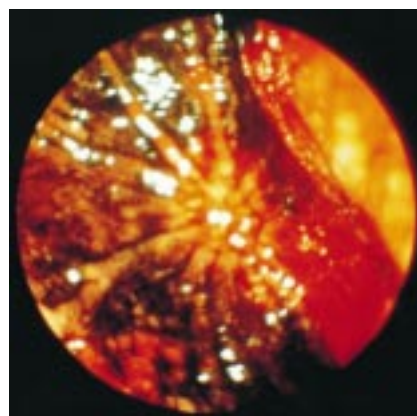
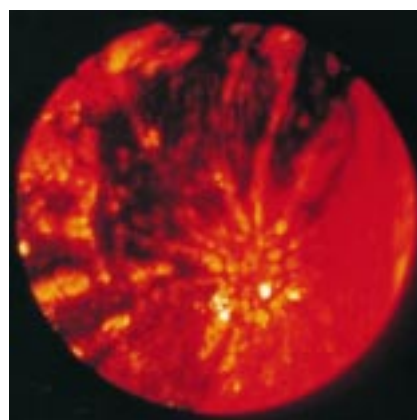


Figure 3 Chromoscopy in peptic ulcer. **a** Conventional endoscopy. **b, c** Congo red staining. (Courtesy of H. Machuuchi, M.D., Tokai University, Kanagawa, Japan)

a



b



c

small erosion which produces a very shallow depression without elevation of the surrounding mucosa is very difficult to detect endoscopically. However, using the contrast method, it is not difficult to recognize such lesions and to define their size, shape, number, and distribution. The contrast method is very effective in the detection of early gastric cancer, especially of minute and type IIB carcinoma (superficial flat type, in the Japanese classification of early gastric cancer), and in recognizing the extent of cancer infiltration [22].

The endoscopic Congo red test was developed by Okuda et al. [23] for observation of the acid-secreting area. In this test histologically normal to oxyntic gland areas are seen

as acid-secreting areas where Congo red turns from red to blue-black (Figure 3) after administration of gastrin. With this test the extent of ulcerated or histologically undifferentiated adenocarcinomas can easily be determined, because they are usually located in non-acid-secreting areas adjacent to or sometimes surrounded by acid-secreting areas [24].

The combination of methylene blue and Congo red has been described as being useful for the detection of minute or synchronous gastric cancer. Tatsuta et al. [25] reported the endoscopic diagnosis of early gastric cancer by means of an endoscopic Congo red-methylene blue test. With routine examination a correct diagnosis of minute and flat

cancers in the upper part of the stomach was made in only 27.3% and 25.0% of cases, respectively. However, using the Congo red-methylene blue test, the rates of correct diagnosis were raised significantly to 75.0% and 83.3%, respectively. In 1988 Iishi et al. [26] used the endoscopic Congo red-methylene blue test in the diagnosis of simultaneous multiple gastric cancers. A correct diagnosis of co-existing early gastric cancers was made in only 28.3% of cases by routine endoscopic examination, but using the Congo red-methylene blue test, the diagnostic rate was raised significantly to 88.9%. In this test Congo red and methylene blue are sprayed onto the surface of the stomach and are bleached within 2–5 minutes on the surface of a tumor but not on the surface of unaffected mucosa.

Endoscopic intraoperative Congo red staining is useful for guiding the extent of highly selective vagotomy [27–29]. Two outcome studies have confirmed that ulcer recurrence rates were lower when surgery was guided by this method [28,30].

The gastric application of methylene blue has a reported sensitivity of 80–98% and specificity of 89–99% in the identification of intestinal metaplasia [31,32] (Figure 4).

The villous pattern of the duodenum and individual villi are clearly defined by the contrast method. This method is helpful in determining whether a duodenal ulcer has healed completely and also in demonstrating rare duodenal malignancy more clearly.

Colonic Lesions

The colonic mucosal surface, on close observation, is granular in appearance and demarcated into small areas by what are called nonspecific grooves. When a blue dye such as indigo carmine or methylene blue is sprayed onto the surface, the groove pattern is observable without difficulty.

For observation of colorectal lesions the contrast method is commonly used. With this method accumulation of dye in concave areas highlights unevenness. Even lesions with an apparently flat surface are often minimally depressed and/or elevated. Usually, 0.1–1.0% indigo carmine solution is employed. The sprayed dye is retained in the depressed portion, making the unevenness of the lesion more conspicuous.

Recently, endoscopists have used two techniques, high magnification and/or higher resolution endoscopes along with chromoscopy, to investigate two issues [33]. The first is whether hyperplastic polyps can be distinguished from adenomatous polyps by surface characteristics, and the second issue relates to recent reports of the detection of flat neoplasms (flat adenomas and flat carcinomas).

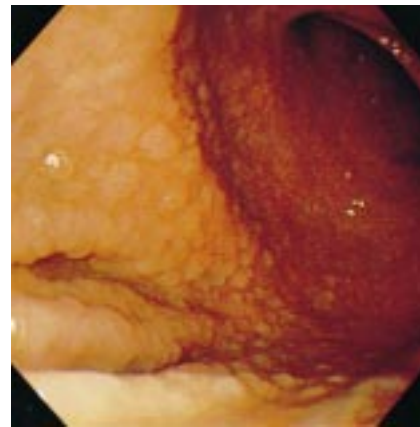
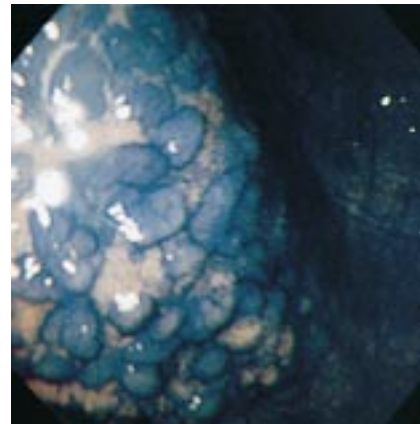


Figure 4 Intestinal metaplasia.
a Conventional endoscopy.
b After spraying with methylene blue, the intestinal metaplasia is stained deep blue

a



b

Colonic Lesions: Diminutive Polyps

Recent advances in endoscopy have facilitated the detection of small neoplastic lesions of the colon. However, diminutive lesions may not be detected during a standard colonoscopy. Contrast chromoscopy, using indigo carmine dye, can demonstrate otherwise indiscernible minute lesions, as the blue dye forms shallow pools in mucosal grooves.

Mitooka et al. [34] carried out contrast chromoscopy of the colon, using indigo carmine capsules, in 287 patients. The overall prevalence of polyps (neoplastic and non-neoplastic) was 85.7% and that of neoplastic polyps was 57.1%. Moreover, the prevalence of neoplastic polyps in 121 asymptomatic patients was 50.4%. The incidence of carcinoma was 0.5% amongst the diminutive polyps and 10.1% amongst the large polyps. Approximately 70% of all of the neoplastic polyps were located proximal to the sigmoid colon. These findings suggest that there are many more polyps present throughout the colon than previously estimated. This suggests that total colonoscopy should be done more thoroughly than hitherto, for instance, by using chromoscopy.

In 1994 George et al. [35] used 0.2% indigo carmine spray to evaluate 89 diminutive polyps in 41 patients undergoing screening sigmoidoscopy. Four polyps were adenomatous

(5%), 60 (67%) were hyperplastic, 21 (24%) were normal, and 4 (5%) were either lymphoid aggregates or inflammatory bowel disease. The endoscopists were able to correctly predict the histological characteristics in only 47% of the polyps after indigo carmine spraying (but in 68% of the hyperplastic polyps).

Axelrad et al. [36] in 1995 used 0.2% indigo carmine spray along with a Fujinon 400 series colonoscope (high-resolution, $1.5 \times$ magnification) to predict the histological nature of the polyps in 36 patients (12 retrospective, 24 prospective) during colonoscopy. To identify the endoscopic features that allow differentiation of hyperplastic from adenomatous polyps, in the first 12 patients the surface morphology of the lesions, as demonstrated by chromoscopy, was compared with the histological findings. In this phase of the study after indigo carmine spraying, the following visual criteria were defined. The hyperplastic polyp is characterized by a pit pattern with an orderly arrangement of circular dots, similar to the surrounding normal mucosa. These pits were observed well with magnification. The surface of an adenomatous polyp showed grooves that in some cases became sulci. These details were seen only with magnification. The overall diagnostic accuracy was 83%. In the prospective part of the trial the diagnostic accuracy for adenomatous polyps was 93%, and for hyperplastic polyps it was 97%. The sensitivity and specificity for distinguishing adenomatous from nonadenomatous polyps were 93% and 95%, respectively (positive predictive value 87%, negative predictive value 98%). There did not appear to be any difference in the accuracy of this trial when prediction using a magnified high-resolution method was compared with prediction using a non-magnified high-resolution method.

Colonic Lesions: Flat Adenomas and Carcinomas

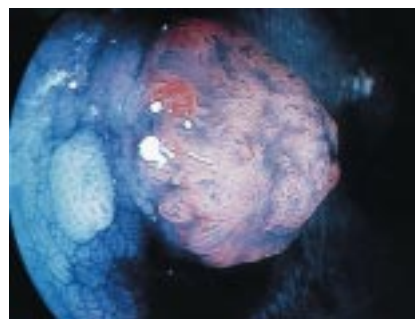
Over the last few years data have accumulated suggesting not only that small flat neoplastic lesions do exist in the colon, but also that they are much more likely than polypoid lesions to contain high-grade dysplasia, even when small. It is still difficult to identify such lesions endoscopically, but several reports have pointed out that chromoscopy is useful in their detection.

Several Japanese investigators, including Muto and Mitooka, have used methylene blue and, more recently, indigo carmine to detect and highlight these flat adenomas and adenocarcinomas [37–42] (Figure 5). In addition, many investigators have emphasized the difficulty of detecting small, flat adenomas and carcinomas at colonoscopy, and as a result they recommend using chromoscopy to enhance detection of these flat lesions.

Adachi et al. [37] indicated that the most characteristic feature of the small flat adenoma is a plaque-like reddish spot and that careful examination to detect this reddish spot is one way of locating a flat adenoma. Kuramoto et al. [38] suggested a similar way of searching for depressed adeno-



a



b

Figure 5a This is a sessile lesion, 6×7 mm in size, detected in the sigmoid colon. The surface of the lesion was so smooth that it looked like a common polyp and no other detailed findings were available. **b** The distorted structure of the surface was disclosed by direct application of indigo carmine dye and magnifying observation identified the pattern of small pits and a large one, densely and irregularly arranged. These findings gave rise to a high suspicion of this lesion's malignancy and invasiveness. (Courtesy of H. Mitooka, M.D., Kobe Kaisei Hospital, Kobe, Japan)

mas. Both groups pointed out in agreement that such lesions are difficult to detect and are easily overlooked. In this regard, they also suggested the usefulness of dye spraying for detecting such lesions, as did Matsumoto et al. [43] The detection rate of depressed adenomas by Kuramoto et al. [38] was 0.7%. Chromoscopy has not been at all popular in Western countries, but Riddell [44] and Mion et al. [45] expected chromoscopy to enhance the detection of flat depressed neoplastic lesions.

Despite the attention that has been drawn to the flat lesions by Japanese clinicians, investigators in the West have not encountered the same lesions as frequently in their patient population. Bond [46] believes that the Western population is different from that of Japan in that there is very little malignant potential in diminutive polyps. Several large US studies have not revealed high-grade dysplasia or severe atypia to the extent shown in Japanese reports. The series of Tedesco et al. [47] in 1982 of 329 diminutive polyps showed one villous adenoma. The series of Waye and colleagues [48] in 1988 of 1048 small polyps which were less than 6 mm revealed only one carcinoma (0.1%) and three villous adenomas (0.3%).

In 1995 Mitooka et al. [49] reported the detection of minute flat depressed neoplastic lesions of the colon by contrast chromoscopy using an indigo carmine capsule. In 2.8% of patients a total of 37 diminutive flat depressed neoplastic lesions of the colon, smaller than 5 mm, were detected (Figure 6) and subsequently removed by endoscopic mucosal resection. From the results of this study it is concluded that minute flat depressed neoplastic lesions are a relatively common finding when the colon is meticulously examined using chromoscopy and the magnifying colonoscope. Chromoscopy and magnification would be useful techniques in prospective surveillance for flat adenomas in the Western world.

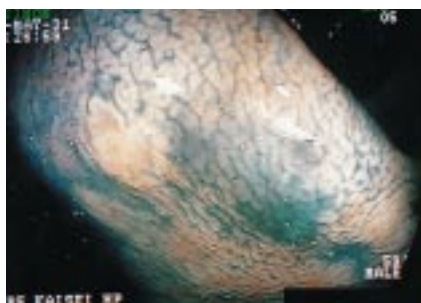
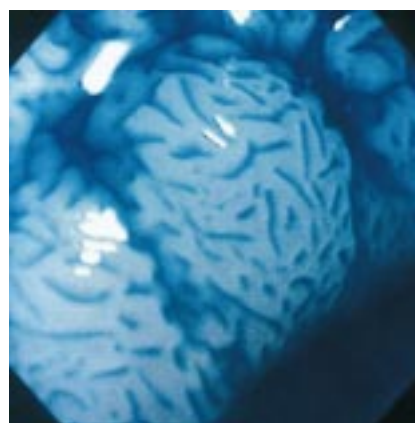


Figure 6 A depressed adenoma, 4 mm in size, detected in the ascending colon by contrast chromoscopy using indigo carmine capsules. (Courtesy of H. Mitooka, M.D., Kobe Kaisei Hospital, Kobe, Japan)

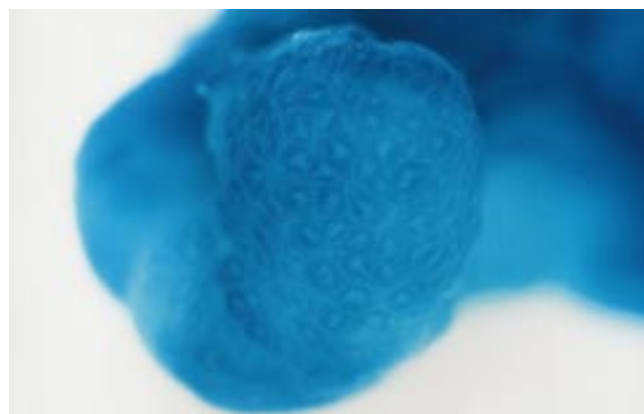
Cresyl violet in 0.2% solution has been used primarily for more specific assessment of the pit pattern of colonic lesions. Unlike the contrast dye indigo carmine, which is retained in the pits, cresyl violet is absorbed by the mucosa around the pit openings, providing a very clear definition of patterns that have histologic correlates. The staining requires very careful removal of surface debris and mucus, which is usually accomplished by washing with ample amounts of water administered through the colonoscope.

A Japanese study [50] investigated colorectal tumorous lesions using magnifying endoscopy combined with indigo carmine and cresyl violet dye, stereomicroscopy, and histologic examination. The diagnosis of small tumors by chromoscopy was based on a mucosal pit pattern classification into five types of surface: type I, round pits; type II, stellar or papillar pits; type III, large tubular or round pits; type IIIs, small tubular or round pits; type IV, branch-like or gyrus-like pits; type V, nonstructural pits. The images of these pit patterns were fundamentally similar to those obtained by stereomicroscopy; the diagnosis by chromoscopy agreed in 81.5% of cases with that of stereomicroscopy. There was also a strong correlation between the endoscopic and histologic diagnoses. Types I and II pit patterns were nontumorous epithelial tissue, while the other surface types were tumorous lesions. These results confirmed that chromoscopy is capable of discriminating tumorous from nontumorous lesions.

In 1998 Kim et al. [51] reported the usefulness of the pit patterns of colorectal tumors and magnifying colonoscopy in a study of 75 colorectal lesions (Figure 7). Comparing



a



b

Figure 7 Magnifying colonoscopy and stereomicroscopy. **a** Magnifying chromoscopy shows a large tubular pit pattern (type III). This finding suggests a neoplastic polyp. **b** Stereomicroscopy shows a stellar pit pattern (type II). This finding suggests a non-neoplastic polyp, and this polyp was confirmed as being hyperplastic

the histologic findings with assessment according to pit pattern, the diagnostic sensitivity was 83% (5/6) in type I patterns (inflammatory change and submucosal tumor); 81% (21/26) in type II (hyperplastic polyp), 45% (5/11) in type II + III (serrated adenoma), 95% (21/22) in type III (tubular adenoma), 75% (3/4) in type III + IV (tubular or tubulovillous adenoma), 75% (3/4) in type IV (tubulovillous or villous adenoma), and 100% (1/1) in type IV + III (villous adenoma with focal carcinomatous change). The overall diagnostic predictive value was 79% (59/75), and the diagnostic accuracy in differential diagnosis between neoplastic and non-neoplastic lesions was 89% (67/75). There was a good correlation between pit pattern and the histologic findings for colorectal tumors and thus, observation of the pit pattern of colorectal lesions using the magnifying endoscope with dye-contrast techniques has clinical value in assessment of the histologic character of colorectal neoplasms. In addition, we can make a differential diagnosis between neoplastic and non-neoplastic lesions and decide on treatment by observing pit patterns using magnifying colonoscopy *in vivo*.

Magnifying endoscopy allows the evaluation of the depth of invasion of malignant lesions [50]. Of those with non-structural pits and a sharply demarcated surface at endoscopic examination, 50% had a histological diagnosis of invasive cancer with involvement of the submucosal layer. If this type of lesion involves a broad area of mucosa, there is a strong suspicion of extensive malignant invasion. Thus, chromoscopy can offer useful information about the course of treatment to be taken.

This technique can also demonstrate aberrant colorectal foci (ACF). In a recent study ACF were shown to be more deeply stained than surrounding mucosa when methylene blue was used; this makes detection by magnifying endoscopy easier, and this can be useful in identifying the individual at high risk of colorectal cancer [52]. Furthermore, chromoscopy permits calculation of the ACF density on surgical specimens, with results similar to those obtained with stereomicroscopy. The ACF density has been found to be higher in patients with cancer compared with that in patients with benign lesions, both by chromoscopy and stereomicroscopy.

Colonic Lesions: Other Benign Diseases

Chromoscopy is also capable of morphologically characterizing some benign lesions of the colon. In patients with a diagnosis of Cronkhite-Canada syndrome the surface of the polyps presents more prominent crypt openings compared with juvenile polyposis; in the Peutz-Jeghers syndrome the surface of the polyps shows a much more complicated appearance, probably because of proliferation of the muscularis layer; the polyps in Cowden's disease, however, have surfaces with round cryptal openings, similar to those usually found in metaplastic polyps [53].

In a recent study chromoscopy has also been used to identify polyps in patients who have had extensive ulcerative colitis for at least 10 years [39,54,55]. The flat lesions were found more frequently. Before being sprayed with dye, they appeared as ill-defined and slightly elevated whitish areas that sometimes presented a granular surface. After staining with indigo carmine, the flat lesions were well circumscribed and without the central depression which is an endoscopic feature associated with a high grade of dysplasia and so with a high risk for cancer [55]. In 1996 Jaramillo et al. [39] reported on small, flat colorectal neoplasias in long-standing ulcerative colitis, which were detected by high-resolution electronic video endoscopy. Flat lesions in 15 out of 77 patients were diagnosed as neoplastic at histological examination. Only 7% of patients had a high-grade dysplasia without a central depression, the malignant potential being therefore considered to be low. Moreover, chromoscopy is useful in the detection of colorectal polyps in ulcerative colitis, especially those of flat appearance, which are the most difficult to identify by conventional endoscopy.

Matsumoto et al. [54] suggest that observation under magnifying chromoscopy can provide another clue to determining the severity of disease in patients with ulcerative colitis.

Endoscopic Tattooing

Endoscopic tattooing facilitates identification of sites viewed from the serosal or luminal surfaces. Since the first attempts by Ono et al. [56] to carry out intramural injections into the wall of the stomach under gastroscopic vision, this method has been developed with a variety of different aims. A major concern in this development was the desire to facilitate, both for the surgeon and the pathologist, the localization of a histologically confirmed but otherwise readily overlooked carcinomatous or precancerous lesion during endoscopic resection, during surgery, or in the resected specimen. To date, a number of dyes have been employed for this purpose, most frequently Evans blue, patent blue, and India ink. Ono et al. [56] were the first to carry out a comparative study on the use of the three above mentioned dyes and came to the conclusion that the submucosal injection of 0.5–1.0 ml of a prepared and sterilized suspension of India ink, using a sterilized injection needle, into the previously disinfected gastric mucosa sufficed to also represent a malignant lesion on the serosal side. Waldmann and Oehlert [57], and Rosch [58], all considered India ink marking of the stomach to be a useful and advisable method for the "labeling" of small carcinomas and for marking resection lines in gastric resection procedures.

Injections proximal and distal to the lesion or in all four quadrants of the lumen may improve subsequent intraoperative identification. India ink tattoos have remained visible for up to 15 years and are presumably permanent. There is limited clinical experience with indocyanine green for tattooing [59].

Several reports suggest the usefulness of this aid for subsequent operative or endoscopic identification of polypectomy sites [60–64].

Clinical Limitations of Chromoscopy

Although it is some years since Tanaka et al. [65] pointed out some problems concerning the diagnosis of minute lesions by chromoscopy, there has been little progress so far in solving them.

Problems Concerning the Endoscopic Image

Lack of objective definition or description of mucosal patterns. Even if we could obtain a clear image of the fine structure of the mucosal surface using a magnifying endoscope, this image cannot become universally useful if the terms used in decision guidelines are merely "regular" or "irregular". This means that progress in pattern analysis using an image-analyzing system is needed. Moreover, to

be practicable, the results of pattern analysis need to be obtained in real time.

Limitations in "picking up" minute lesions. As mentioned before, "picking up" by conventional endoscopic examination is a necessary precondition for the diagnosis of minute lesions by chromoscopy, but lesions cannot be "picked up" for eventual diagnoses when the color tone and irregularities on their surfaces cannot be distinguished from those of the surroundings. Improving the quality of "picking up" by conventional endoscopy also improves the accuracy of diagnosis of minute lesions.

Limitations in diagnosing minute cancers of stomach and colon. Even when endoscopy has by chance detected early-stage cancer, limitations to diagnosis still exist, because the latter is dependent on color tone and irregularities of the mucosal surface, like the "picking up" mentioned above, and earlier stage cancers show little color change or irregularity.

Change of surface pattern caused by biopsy. When we investigate minute lesions of the order of a millimeter in size, most of the surface structures may be destroyed by a single biopsy. It should be made clear whether or not the minute lesions have ever been biopsied before considering the endoscopic image. At present most reports present opinions about endoscopic imaging without considering this type of issue.

Difficulty of histologically verifying minute changes. The minute structural changes of mucosal surface which are sampled using magnifying observation might be of the order of a millimeter in size or even smaller. It is not easy to cut the sample into thin slices for tissue specimens using the tissue image. Usually the size of the tissue cut by the biopsy forceps is 6–7 mm or less, and the part viewed *via* magnifying observation might be only a part of the whole biopsy specimen even if the biopsy is taken at the exact site of the lesion. This means that one cannot be sure that the slices of tissue specimens made from the cut tissue correspond to the part viewed by magnifying observation. For example, a study on duodenal erosion, where the lesion diameter is just a few millimeters, demonstrated that the possibility that a duodenal erosion could be made into a tissue specimen is less than 32%. To solve this problem, the proposed biopsy sample should first be viewed by stereomicroscopy and separated into two parts using a micro-knife; magnifying observation is used to obtain one of the parts but is not used when the other is obtained.

Technical Problems

Longer time needed for chromoscopy. The techniques of chromoscopy are complex and confusing for endoscopic novices. The time needed for focusing when using the magnifying scope or for changing instrument variables in order to achieve good or higher quality pictures, and so on, increases the examination time.

Limitations of the examination area. The tip of the scope must be placed as close as possible to the mucosal surface in order to obtain higher quality images of minute lesions, but it is sometimes hard to find areas in which it is nearly impossible to set up good observation conditions.

Unclear endoscopic images. This technique might not detect minute lesions which were buried by the dye scattered on the mucosal surface, and in many cases it is hard to keep the focus clear, to prevent blurring, and to maintain appropriate exposure conditions.

Adverse reactions to chromoscopy. All of the endoscopic dyes are considered to be nontoxic although the maximal safe doses are not known. Occasionally indigo carmine has given rise to hypertension and bradycardia because of stimulation of alpha receptors, and has caused significant hypotensive reactions, probably related to either anaphylaxis or to an idiosyncratic drug reaction [66,67]. Concentrated Lugol's solution (50%) has caused heartburn and bronchospasm in patients with iodine sensitivity [11]. Tattooing of the stomach and colon has produced serosal or mesenteric inflammatory masses, fat necrosis, sterile abscess, focal peritonitis with microperforation, and phlegmonous gastritis [68–71]. Methylene blue has caused serious systemic reactions during parenteral administration [72].

Conclusions

Chromoscopy has been available for many years. The technical difficulty of the method is minimal. There is a learning curve with regard to endoscopic techniques, but proficiency in chromoscopy is usually attained after carrying out relatively few procedures. The cost and inconvenience are negligible. Most chromoscopy methods increase the length of an endoscopic procedure by only several minutes, and most dyes are inexpensive.

Although some staining methods remain primarily research tools with, so far, limited clinical application, chromoscopy improves the quality of gastrointestinal endoscopy, resulting in a range of diagnosis and treatment that has hitherto not been possible with conventional endoscopy. Not all patients undergoing endoscopy require chromoscopy. The indications for chromoscopy are dependent upon the knowledge of the individual endoscopist regarding clinical effectiveness. In situations where chromoscopy can be shown to be useful, the endoscopist should be able to use it as necessary. I propose that all endoscopists should learn chromoscopy as a possible endoscopic technique and should be able to use it whenever necessary in actual clinical settings.

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