TECHNIQUE

Chromoendoscopy - a Complementary Diagnostic Method in Upper Digestive Endoscopy

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Abstract

Dye-staining is a complementary technique of digestive endoscopy contributing to an increased diagnostic accuracy of this method (sensitivity, specificity) and especially of endobiopsy.

The present study analyses four types of dye-staining methods - contrast, staining, reaction and mixed method, presenting the principles, indication and interpretation of results for each technique.

Due to the fact that these methods are cheap and easy to perform and therefore accessible to any endoscopist, we recommend their use as routine investigation methods in certain diseases of the upper gastrointestinal tract. Thus, we would recommend the use of the Lugol test in oesophageal diseases and Congo red and/or methylene blue test for the stomach.

Key words

Chromoendoscopy-indigo carmine test-methylene blue test-toluidine blue test-Lugol test-Congo red test

Rezumat

Coloraţiile intravitale sunt tehnici complementare endoscopiei digestive, contribuind la ameliorarea performanţelor diagnostice ale acesteia (sensibilitate, specificitate) şi mai ales ale endobiopsiei.

Lucrarea noastră analizează 4 categorii de metode, prezentând principiul fiecărei tehnici, indicaţiile şi interpretarea rezultatelor.

Fiind ieftine şi simplu de efectuat, deci accesibile oricărui endoscopist, recomandăm aplicarea de rutină a acestor metode diagnostice, mai ales a testului cu Lugol pentru esofag şi a testului cu roşu de Congo şi/sau albastru de metilen pentru stomac.

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Introduction

Intravital dye staining has been widely used in clinical and experimental medicine.After the introduction of flexible digestive endoscopy in 1958, this method was adopted in gastroenterology as well. Intravital dye staining was developed in the eighties with the introduction of the Lugol test, borrowed from gynaecology, in defining the oesogastric boundary (Voegali), diagnosing oesophageal cancer (1) and oesophagitis (2).

Although these methods have an unquestionable beneficial diagnostic role (3, 4), low cost (5) and are technically accessible to any endoscopist, they are still not being used as routine investigation.

Several terms have been used in literature for these methods: "chromoendoscopy" (6), "chromoscopy" (7), "in vivo dye staining" (8), "in vivo dye scattering" (9), "vital staining" (9) etc.

There are several methods of chromoendoscopy (10) (Table I). The most commonly used staining solutions are Lugol's solution (for the oesophagus), methylene blue and Congo red (for the stomach).

Table I. Classification of chromoendoscopy

Methods	Solutions
Contrast methods	Indigo carmine
	Evans blue
	Methylene blue
Staining methods	Toluidine blue
	Methylene blue
Reaction methods	Lugol's solution
	Congo red
Mixed method	Congo red+Methylene blue

Techniques

The method is based on the principle of assessing different colours occurring in various areas of an organ, differences induced by certain functional and/or structural alterations that are otherwise invisible to the eye.

Intavital staining of the gastro-intestinal mucosa is possible either by epithelial absorption (diffusion), or by permeation of degenerated (necrosing) cells by the substances that are used.

The dye may be sprayed either before endoscopy, through an oesophageal or gastric tube, after which the patient changes his position several times so that the substance may come into contact with the entire tubular segment or it may be sprayed during endoscopy using a thin catheter introduced through the opening for the biopsy pinch. The second method is preferable as it requires smaller quantities of solution, thus proving less costly and decreasing the risk of adverse reactions and also it is possible to spray strictly the desired area. In addition, this method allows a previous investigation of the gastro-intestinal tract by classical endoscopy and the assessment of areas with minimal mucosal alteration (colour, granularity, relief), which would be better revealed by dyeing.

If the examined area is covered with a lot of mucus, this should be washed away with water, pronasis, dimethyl–polysiloxan or sodium bicarbonate before dyeing.

The results will be assessed 2-5 to 15 minutes after dyeing. Biopsy samples will then be taken from the areas with altered colour.

Contrast methods

These methods use non-absorbing dyes or dyes which do not react with the oeso-gastro-intestinal epithelium (indigo carmine, Evans blue and in certain areas, methylene blue) (9, 11-12).

The methods bring out the architecture of the mucosa by storing the dye in its grooves and depressions and by enhancing any differences of relief (Fig. 1).

The most commonly used dye in this category is indigo carmine 0.5-1%, which permits the assessment of early gastric cancer (swellings or indentations). The method is also useful in diseases with malignant degeneration risk (13), showing any change of relief (gastric mucosal atrophy) (14), intestinal metaplasia (14-15), gastric ulcer (16), gastric polyps (17).

The method can be used with the same indications in the examination of other segments of the gastro-intestinal tract: oesophagus (18), duodenum (19), colon (20-23).

Toluidine blue test

Toluidine blue is a dye with great affinity for the nuclear DNA as compared to the cytoplasmatic RNA, so that multinucleic epithelial cells or cells with reverse nucleocytoplasmatic ratio (dysplasia or epithelial atypia) are coloured in a darker blue (warm image) as compared to the areas with normal mucosa (hypochromia) (9, 24-26).

The test with toluidine blue uses a 1-2% solution and is indicated in the diagnosis of Barrett oesophagus (27), oesophageal cancer (9, 25-27) (squamous neoplasia but not glandular neoplasia) (18) or gastric cancer (9). The differences of colour between the normal and the pathological mucosa occur within 10-15 minutes, therefore the patients should be anaesthetized. It requires preliminary washing with acetic acid to remove mucus.

As it is a rather laborious method, this test is less often used, although it has certain unquestionable advantages: diagnosis of cancer (in association with endobiopsy), definition of the tumoral extension establishing the line for resection (25) and assessment of the efficacy of therapeutic methods (surgery, radiotherapy, chemotherapy).

Methylene blue test

Methylene blue 0.05-0.7 to 2% reveals the areas with inflammation, those rich in mucus and those with metaplasia (intestinal metaplasia in the stomach and gastric metaplasia in the duodenum) (Fig. 2). In this test the pathological areas stain blue with methylene blue. The test with methylene blue brings out inflamed areas, being stored, for example, in areas where an ulcer might develop. After the healing of the ulcer, the area returns to its normal appearance and colour affinity (12, 28). On chromoendoscopic examination, complete healing was defined as a scarred area covered with granular patterns, while incomplete healing was defined as a scarred area surrounded only marginally by granular patterns (28). Thus, the staining with methylene blue is useful in monitoring gastric ulcer, in finding patients with delayed healing or patients with recurrent ulcer. The hyperchromic areas surrounding the ulcer scar should be examined histopathologically.

Lugol test

Unlike methylene blue which stains the columnar epithelium, Lugol's solution stains the squamous epithelium (9).

In vivo staining with iodine (iodinated iodine solution) has been used since 1926 when the Lham-Shiller test for the diagnosis of epithelial dysplasia and atypia of the external os was first introduced. This technique is still considered as extremely useful, mainly in gynaecology where it is used either as a separate method or as a compulsory phase in colonoscopy. The method allows for the differentiation of normal multistratified epithelium from a typical epithelium (metaplasia, dysplasia and especially neoplasia), which is poor in glycogen and pale (iodine-negative) in contrast with the normal tissue which appears dark brown.

Lugol's solution used in the exploration of the oesophagus has a concentration of 1-2 to 5%. The iodine contained in the solution has affinity for the glycogen (this phenomenon is not yet completely understood), so that when the solution is sprayed on the unkeratinized squamous epithelium of the oesophagus, the mucosa takes a brown-corrugated aspect (Fig. 3), related to the intracellular glycogen content and to the thickness of the glycogen-

containing cell layer. In the normal squamous epithelium, the upper three fourths of the epithelium contain glycogen, in the intercellular space in the middle layer and inter- and intracellular spaces in the upper layer. In the abnormal epithelium (Lugol-negative areas) the glycogen-containing cells are either absent or present only in the upper thin layer of the epithelium.

The Lugol staining pattern consists of two main components: the staining intensity of the lesion and the degree of the marginal clearness.

According to the affinity for iodine, the Lugol test reveals four types of areas (29): hyperstaining (grade I), normal greenish brown staining (grade II) (Fig. 3), less intense staining (grade III) and unstained (grade IV) (Fig. 4). Hyperstaining areas appear only in glycogenic acanthosis. Grade III is usually characteristic for oesophagitis (Fig. 5), epithelial atrophy, but it is mostly suggestive of metaplasia (columnar epithelium has a low glycogen content) (Fig. 6) or of moderate to mild dysplasia. Grade IV generally indicates a severe epithelial dysplasia, in situ carcinoma or invasive cancer.

The marginal clearness reflects the abrupt or dull transition from the lesion to the adjacent normal epithelium. Oesophagitis, atrophy or mild dysplasia have dull margins, whereas severe dysplasia and carcinoma have abrupt, sharp margins.

Oesophagochromoscopy with Lugol, alongside other in vivo staining methods (toluidine blue, indigo carmine), is successfully used today in the early diagnosis of oesophageal cancer (5, 9, 18, 26, 29-34). The widespread applicability of these methods for the early diagnosis of cancer is confirmed by the introduction of the following terms referring to the early types of oesophageal cancer in the standard terminology of the World Organization of Digestive Endoscopy (OMED) (35): white (elevated), red (erosive), mixed (erosive and elevated) and occult (visible only by in vivo staining). Owing to its acceptable sensitivity, the Lugol test meets the necessary requirements for a screening method for early oesophageal cancer in symptomatic patients and in persons with risk for oesophageal diseases: alcoholics, smokers, patients with long-term peptic or postcaustic oesophagitis, Barrett oesophagus. The method may also be useful in population screenings in regions with a high incidence of oesophageal cancer, for example, China. Lugol test is useful whenever upper digestive endoscopy, performed for various gastro-duodenal diseases shows minor alterations of the oesophageal mucosa: alterations in colour, granularity, distensibility, circumscribed surface irregularities etc. The test also makes possible the diagnosis of synchronous or metachronous oesophageal cancers in patients in whom oral and oro-pharingo-oesophageal cancers had previously been diagnosed (5, 36-39). Lugol staining may also be useful before or during surgery in order to assess the extent of the tumour (29, 40-41). Another possible application of chromoendoscopy with Lugol might be in the postoperative or postradiotherapy monitoring of the patients.

Lugol test is not a diagnostic method for oesophageal ulcer because the margin of the ulcer does not stain and is white, while the ulcer base is dark, brown, probably due either to the pooling of Lugol solution, haemorrhage, exudates or the direct exposure of the muscular layer. However, the method is useful in monitoring the scarring process (42).

Although it is quite inexpensive and easy to perform, the Lugol test has certain inconveniences, the greatest one being the adverse reactions to iodine. In order to avoid such incidents, allergic patients whould be excluded from this test. Chromoendoscopy with Lugol and other substances is a time consuming additional method, which requires careful premedication and accurate endoscopic technique. Another inconvenience reffers to the fact that Lugol's solution induces a histological artifact in epithelial cells, most pronounced in dysplastic cells and may lead to histological interpretation errors (43). The pathologist should recognize these artifacts: cellular shrinking, cytoplasmic eosinophilia and vacuolization, development of visible intercellular spaces and nuclear pyknosis with loss of chromatin detail.

Congo red test

Congo red, a nontoxic azine, is a pH-type marker, which changes its colour proportionally to H^+ ion concentration (44): pH > 5 - the area exposed to the dye ranges from pale to pink; pH ≤ 5 - the area becomes bright red; pH < 3 - the colour ranges from dark blue to black (Fig. 7). The acid-secretory areas are pointed out by the change of the indicator colour to blue-black. This change in colour may occur either spontaneously, after 2-5 minutes, or it may occur after stimulating the gastric secretion by a vagal mechanism (administration of insulin) (45), or by a hormonal mechanism (administration of histamine (44), penta- (46) or tetragastrin (47).

The test with Congo red is useful in diagnosing and defining the acid-secretory areas with oxyntic mucosa, including the oesophagus (46, 48-49), antrum (50) and duodenal bulb (45, 51-52).

As Congo red assesses gastric pH, the method is used to study the effectiveness of certain antisecretory methods: vagotomy (53-54), H_2-inhibitors (55), inhibitors of the proton pump.

The assessment of the ratio oxyntic/nonoxyntic mucosa, using the test with Congo red, is very important. Its importance lies in tracking down persons that are predisposed to duodenal ulcer, patients that already have duodenal ulcer with aggravating tendencies (late healing or lack of response to antisecretory medication) or patients at high risk of recurrence (56-58).

The test makes possible the diagnosis of atrophic gastritis of the corpus and fornix and it assesses the risk for developing gastric cancer, which is proportional to the extension of the areas of mucosal atrophy (59).

Due to the various changes in colour, the Congo red test allows the differentiation of oxyntic glandular polyps from the nonoxyntic ones (60) (Fig. 8), as well as of the benign ulcer (surrounded by a gastritis area - Fig. 9) from the malignant one (Fig. 10) (47, 58, 61).

Congo red - methylene blue test

The combined method is necessary as the simple test with Congo red only discovers and assesses the extension of ulcerated or undifferentiated adenocarcinomas, which are usually surrounded by an acid-secretory area (61). In the case of polypoid or differentiated adenocarcinomas, the test with Congo red does not make the differentiation from the nonsecretory adjacent mucosa possible. However, these types of carcinomas occur in areas with severe intestinal metaplasia that can be visualized using methylene blue.

Table II. Interpretation of the test with Congo red and methylene blue

Dye	Positive test	Pathological findings in:
Congo red	Acid-secretory areas coloured in blue-black	Ulcerated or undifferentiated adenocarcinoma
	Nonacid secretory areas coloured in red or pale	Severe superficial gastritis or atrophic gastritis in the form of nonacid-secretory islands in the fornix and gastric corpus
Methylene blue	Areas of inflammation or intestinal metaplasia coloured in more intense blue (vital staining)	Atrophic gastritis
		Intestinal metaplasia
	Differences of relief (dye accumulations in depressed areas) or disorganisation of gastric mucosa architecture (contrast-chromoendoscopy)	Gastric ulcer
		Gastric polyps
		Gastric polyposes
		Gastric cancer
Congo red + Methylene blue	Discoloured/white areas	Gastric cancer

In order to obtain maximum contact of the mucosa with the methylene blue, it is necessary to remove the adherent mucus before spraying the dye. Thus, besides standard premedication that precedes endoscopy (anticholinergic drugs + diazepam/midazolam), the patient is also given proteinase (20.000 units), sodium bicarbonate (50 ml 0.2 M) and dimethylpolysiloxan (20 mg). These drugs are administered about half an hour before endoscopy and in the interim period, the patient changes position several times, so that the entire surface of the stomach may be covered. After the introduction of the gastroscope, the aspiration of the gastric juice and the routine examination of the stomach, the actual staining is performed. The entire gastric mucosa is sprayed with methylene blue 0.05% through a polyethylene catheter that has been introduced in the orifice of the biopsy pinch. The gastric microstructure is examined, followed by the introduction of a mixture of Congo red 0.3% and sodium bicarbonate 0.2 M. The blue coloured areas become discoloured. Then, maximal doses of histamine or gastrin are administered. The excess substances are aspired and over the next 5-15 minutes the changes in the colour of the mucosa are noted. The areas affected by cancer turn to white (underlying mechanism not defined yet) in contrast with the areas of normal mucosa coloured in red or blue-red (47, 62).

The test is useful in the diagnosis of early cancers (62-64), in the preoperative assessment of the extension of an advanced gastric cancer, or in finding a synchronous gastric cancer (65). The change of the colour to white is faster and more intense in early polypoid or superficial cancer. The tinctorial change is also more intense in differentiated cancers as compared to undifferentiated ones (47).

The combined method allows the simultaneous discovery of other mucosal damage (erosive gastritis, atrophic gastritis, intestinal metaplasia), that are more or less obvious by classical endoscopy (56-69). Biopsy samples must be taken from the areas displaying pathological changes in colour.

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As a conclusion, dye staining represents a complementary method that enhances the diagnostic accuracy of upper digestive endoscopy. The advantages of chromoendoscopy, i.e. easy to perform, low costs, accessible to any endoscopist, increased sensitivity and specificity of gastro-oesophageal endoscopy and endobiopsy, are much greater than its disadvantages, i.e. a laborious technique that increases the patient's discomfort and the theoretical possibility of certain accidents and incidents, aspiration of solutions in the respiratory tract and/or allergy to substances. However, most of these disadvantages can be eliminated if chromoendoscopy is used as a routine investigation. The necessary solutions and materials should be prepared so as to be available whenever needed. Finally, the use of these techniques as a routine investigation method brings us a step closer to the early diagnosis of gastrooesophageal cancers and, implicitly, to better therapeutic results in this category of disease.

References

1. Brodmerkel GJ Jr. Schiller's test, an aid in esophagoscopic diagnosis. Gastroenterology 1971; 60: 813.
2. Nothmann JB, Wright RJ, Schuster MM. In vivo vital staining as an aid to identification of esophagogastric mucosal junction in man. Dig Dis 1972; 17: 912-924.
3. Volkov BP, Shiraliev DK, Stepanova OV, Shengelidze VV. Khromoezofagogastroduodenoskopiia. Vestn Khir 1981; 127: 27-32.
4. Racz I, Toth E. A gyomornyalkahartya chromoendoscopies vizsgalata. Orv Hetil 1989; 130: 2635-2638.
5. Hiroyasu I, Hitoshi S, Isamu O, Masanobu K. The frequency of a concomitant early esophageal cancer in male patients with oro-pharingeal cancer. Cancer 1994; 73: 2038-2041.

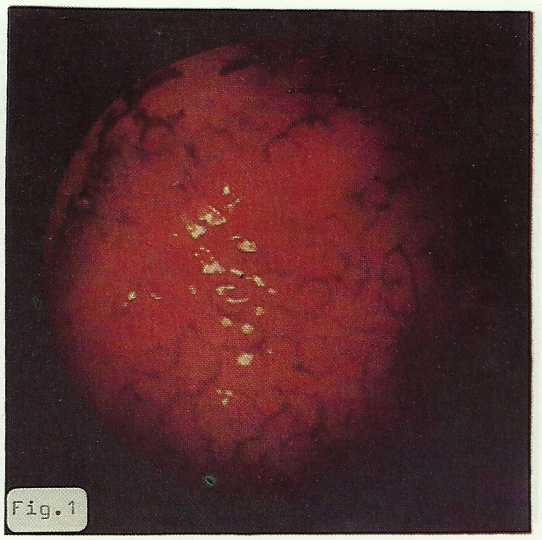


Fig. 1 Staining with indigo carmine. Normal areae gastricae pattern.

Fig. 2 Staining with methylene blue. Biliary reflux gastritis with areas of intestinal metaplasia.

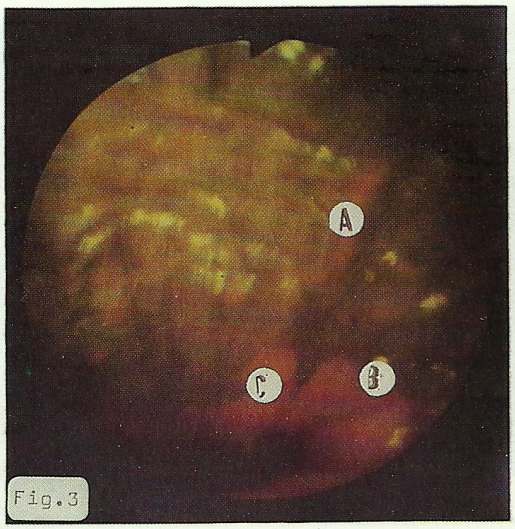


Fig. 3 Lugol test. Views of normal oesophageal squamous mucosa (A), ora serrata or Z line (B) and normal gastric columnar mucosa (C).

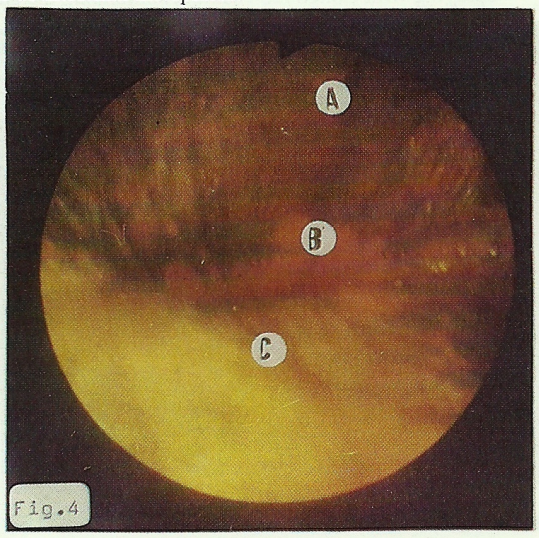


Fig. 4 Lugol test. Normal staining areas (grade II)-A; less intense staining area (grade III)-B; unstained area (grade IV)-C, indicating an invasive oesophageal carcinoma.

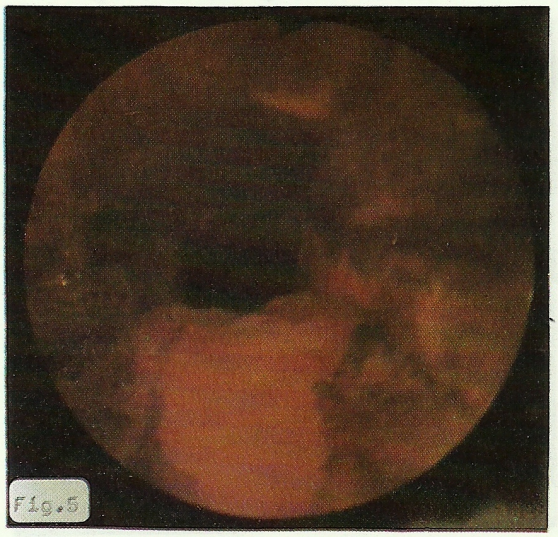


Fig. 5 Lugol test. Discoloured area=the gastro-oesophageal junction proximally prolonged on the right wall of the oesophagus. Histopathology: peptic oesophagitis-like alterations.

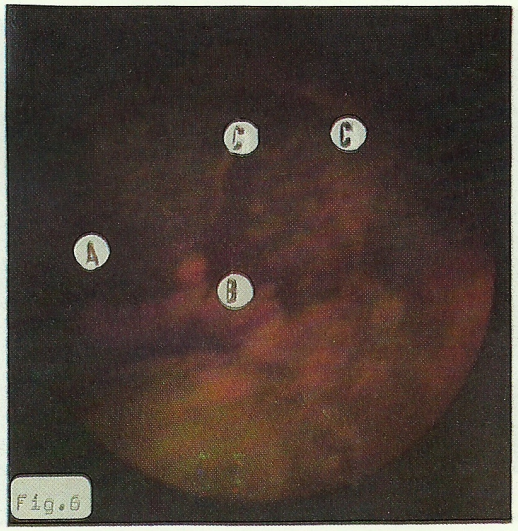
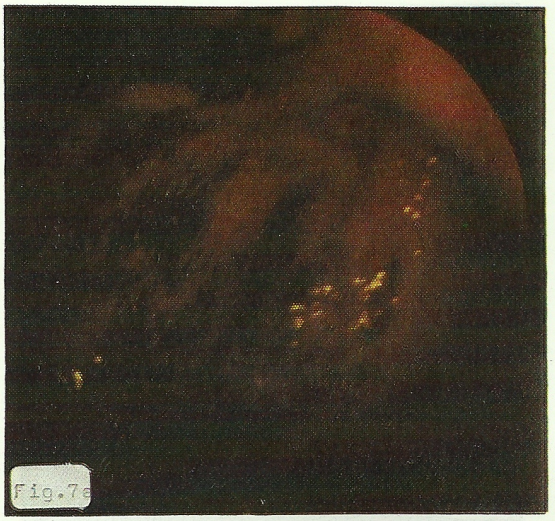


Fig. 6 Lugol test. Barrett oesophagus (cardial type at histopathological exam). View of normal squamous mucosa (A), Z line (B) and island of oesophageal metaplasia (C).



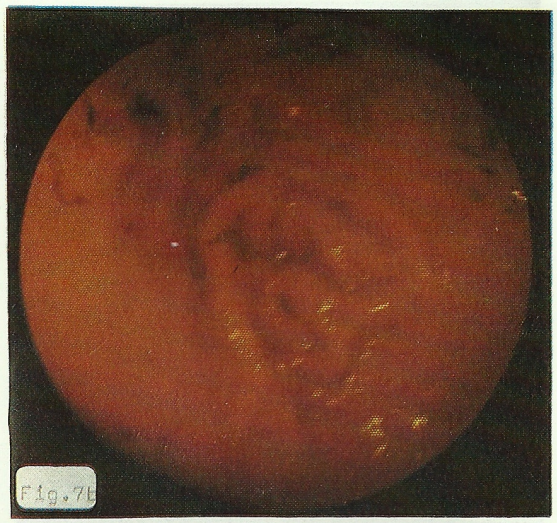


Fig. 7 Congo red indicates areas with pH ≤ 5 (red) and areas with pH < 3 (black). 7.a: Gastric corpus two minutes after spraying with Congo red (without stimulating the gastric secretion). 7.b.: Gastric antrum five minutes after staining.

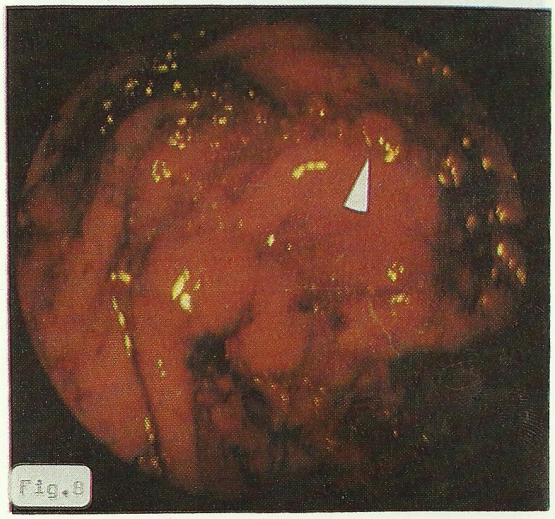


Fig. 8 Congo red test. Small polyps with nonoxyntic type suprajacent mucosa (arrow).

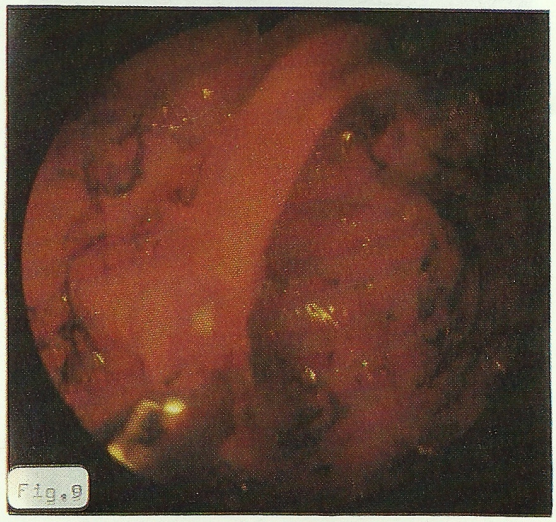
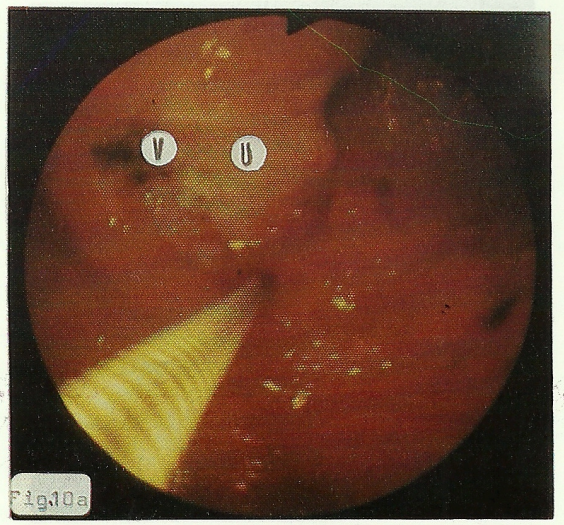


Fig. 9 Congo red test. Benign ulcer in stage H. Chromoendoscopy shows nonacid-secretory mucosa surrounding the ulcer . Biopsy of margins and adjacent mucosa: gastritis-like changes.



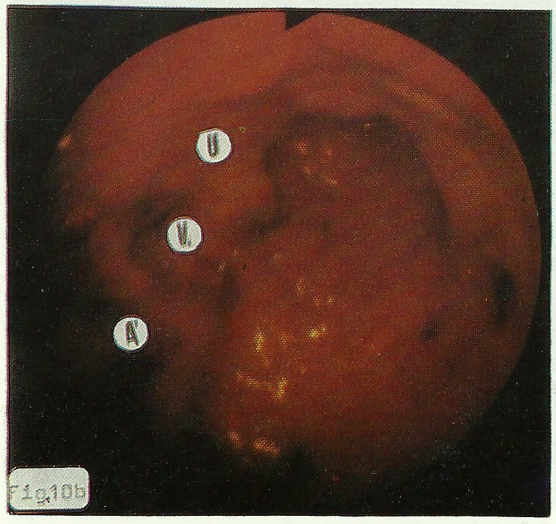


Fig. 10 Gastric ulcer (U) in stage A, with a thrombosed vessel (V) before (10.a) and after (10.b) staining with Congo red. Acid-forming areas (A) border an ulcerous crater. Histopathology: gastric adenocarcinoma.

6. Bychkov VV, Aleksandrov AN, Salenkov VA. Sravnitel'naia otsenka metodov priamoi i nepriamoi khromoendoscopii verkhnikh otdelov psichevaritel'nogo kanala.. Klin Khir 1990; 5: 22-23.
7. Time LI, Kruchinin EZ, Savvin IN, Volkov AV. Znachenie khromoscopii v otsenke funktsional'nogo sostoianiia zheludka i dvenadtsatiperstnoi kishki. Voen Med Zh 1984; 12: 53-54.
8. Ida K, Mashimoto Y, Kawai K. In vivo staining of gastric mucosa. Endoscopy 1975; 7: 18-24.
9.Tytgat GNJ. Investigation of gastric disease. In: Gusvavsson S, Kumar D, Graham DY (eds): *The Stomach*. New York: Churchill Livingstone 1992; 159.
10. Aparcero M. Cromoscopia en del diagnostico del cancer gastrico. GEN 1989; 43: 120-123.
11. Kitamura T, Mai M, Ueda H et al. Clinicopathological study of minute stomach cancer. Gan No Rinsho 1986; 32: 965-970.
12. Tatsuta M, Iishi H, Okuda S. Evaluation of chromo-endoscopy for diagnosis of histological repair of gastric ulcer. Nippon Rinsho 1992; 50: 94-97.
13. Sinev IV, Gavrilenko IV, Balalykin AS et al. Khromoendofibroskopiia a metilenovym sinim v differentsial'noi diagnostike dobrocachestvennykh i zlokakhestvennykh porazhenii zheludochno-kishechnogo trakta i bronkhov. Ter Arkh 1981; 53: 70-73.
14. Kohli Y, Kato T, Suzuki K et al. Incidence of atrophic gastritis with age in Japan and Canada. Jpn J Med 1987; 26: 158-161.
15. Barreto ZR, Garduno I, Sernal FS et al. Effect of routine endoscopy on critical assessment in diagnosis of inestinal metaplasia at Mexico General Hospital. In: Nishi M, Sugano H, Takahashi T (eds)Proceedings of The 1st International Gastric Cancer Congress 1995, March 29- April 1; Kyoto; Monduzzi Ed 1995: 442-443.
16. Oguro Y. Endoscopic diagnosis and follow-up study of peptic ulcer. Nippon Naika Gakkai Zasshi 1991; 80: 22-26.
17. Yang HJ, Wang JI, Wang TH et al. Diagnosis of gastric polypoid by magnifying endoscopy and dye endoscopy. Taiwan I Hsueh Hui Tsa Chih 1991; 90: 371-374.
18. Lambert R. Endoscopic detection and treatment of early esophageal cancer: a critical analysis. Endoscopy 1995; 27: 12-18.
19. Nagakawa H, Tsukamoto Y, Naitoe Y. Function of the minor duodenal papilla in humans as determined under duodenoscopy using indigo carmine dye and pH sensor. Endoscopy 1991; 23: 59-63.
20. Mitoka H, Fujimori T, Ohno S et al. Chromoscopy of the colon using indigo carmine dye with electrolyte lavage solution. Gastrointest Endosc 1992; 38: 373-374.
21. Tada M, Kawai K. Research with the endoscope. New techniques using magnification and chromoscopy. Clinics Gastroenterol 1986; 15: 417-437.
22. Iishi H, Kitamura S, Nakaizumi A et al. Clinicopathological features and endoscopic diagnosis of superficial early adenocarcinomas of the large intestine. Dig Dis Sci 1993; 38: 333-337.
23. Karita M, Cantero D, Okita K. Endoscopic diagnosis and resection treatment for flat adenoma with severe dysplasia. Am J Gastroenterol 1993; 88: 1421-1423.
24. Herlin P, Marnay J, Jacob JH et al. A study of the toluidine blue dye test. Endoscopy 1985; 15: 4-7.
25. Fontolliet C, Monnier P. The histological reliability of vital staining with toluidine blue. Acta Endoscopica 1991; 21: 617-621.
26. Norberto L, Cusumano A, Bonavina L et al. Endoscopic vital staining in the diagnosis of esophageal cancer. In: Siewert JR, Hölscher AH (eds). *Diseases of the esophagus*. Berlin: Springer 1993; 135-136.
27. Chobanian SJ, Cattau EL Jr, Winters C Jr et al. In vivo staining with toluidine blue as an adjunct to the endoscopic detection of Barrett's esophagus. Gastrointest Endosc 1987; 33: 99-101.
28. Tatsuta M, Iishi H, Okuda S. Histological features and recurrence of completely and incompletely healed gastric ulcers classified by methylene blue dye method. Endoscopy 1987; 19: 193-197.
29. Mori M, Adachi Y, Matsushima T et al. Lugol staining pattern and histology of esophageal lesions. Am J Gastroenterol 1993; 88: 701-705.
30. Ormeci N, Okten I, Gungor A et al. Role of dye endoscopy with Lugol in the diagnosis of esophageal carcinoma. In: Nishi M, Sugano H, Takahashi T (eds). Proceedings of the 1st International Gastric Cancer Congress 1995 March 29- April 1; Kyoto; Monduzzi Ed 1995; 1: 436-437.
31. Shimazu H, Kobori O, Shoji M et al. Superficial carcinoma of the esophagus. Gastroenterol Jpn; 18: 409-416.
32. Nishizawa M, Okada T, Hosoi T, Makino T. Detecting early esophageal cancers, with special reference to the intraepithelial stage. Endoscopy 1984; 16: 92-94.
33. Sugimachi K, Ohno S, Matsuda H et al. Lugol-combined endoscopic detection of minute malignant lesions of the thoracic esophagus. Ann Surg 1988; 208: 179-183.
34. Adachi Y, Kitamura K, Tsutsui S et al. How to detect early carcinoma of the oesophagus. Hepatogastroenterol 1993; 40: 207-211.
35. Maratka Z. Terminology, definitions and diagnostic criteria in digestive endoscopy. OMED database of digestive endoscopy. 2nd edition. Hamburg; Normed Verlag 1989.
36. Papazian A, Descombes P, Capron JP, Lorriaux A. Frequence du cancer de l'oesophage synchrone des cancers des voies aerodigestives supérieures (100 cas): interêt des colorations vitales au Lugol et au bleu de toluidine. Gastroenterol Clin Biol 1985; 9: 16-22.
37. Chisholm EM, Williams SR, Leung JW, Chung SC et al. Lugol's iodine dye-enhanced endoscopy in patients with cancer of the oesophagus and head and neck. Eur J Surg Oncol 1992; 18: 550-552.
38. Okumura T, Aruga H, Inohara H et al. Endoscopic examination of the upper gastrointestinal tract for the presence of second primary cancers in head and neck cancer patients. Acta Otolaryngol Suppl (Stockh) 1993; 501: 103-106.
39. Ina H, Shibuya H, Ohashi I, Kitagawa M. The frequency of a concomitant early esophageal cancer in male patients with oral and oropharyngeal cancer. Screening result using Lugol dye endoscopy. Cancer 1994; 73: 2038-2041.
40.Takeshita K, Habu H, Saito N et al. Endoscopic evaluation of gastric cancer infiltrating the lower esophagus. Surg Endosc 1992; 6: 62-67.

41. Endo M, Takeshita K, Kaweno T, Inous H. Minimally invasive esophageal surgery. Endoscopic resection of mucosal cancer of the esophagus. Dis Esophagus 1994; 7: 24-25.
42. Ţuculanu D, Romoşan I, Tudor C et al. Doxycycline induced oesophageal ulcer. Rom J Gastroenterol 1995; 4: 107-110.
43. Benda JA, Lamoreaux J, Johnson SR. Artifact associated with the use of strong iodite solution (Lugol's) in cone biopsies. Am J Surg Pathol 1987; 11: 367-374.
44. Holm L, Flemstrom G. Microscopy of acid transport at the gastric surface in vivo. J Intern Med Suppl 1990; 732P: 91-95.
45. Kunzle JE, Ziliotto A Jr, Brunaldi JE et al. Teste do vermelho congo com estimulo pela insulina para avaliacao da integralidade vagal apos vagotomia. AMB Rev Assoc Med Bras 1982; 28: 8-12.
46. Ottenjann R, Kunert H, Kuhner W, Seib HJ. Magenschleim Haut-inseln im zervikalen Osophagus. Ihre potentielle pathogenetische Bedeutung. Dtsch Med Wschr 1983; 108: 246-249.
47. Tatsuta M, Okuda S, Tamura H et al. Endoscopic diagnosis of early gastric cancer by the endoscopic Congo red-methylene blue test. Cancer 1982; 50: 2956-2960.
48. Ziliotto A Jr, Kunzle JE, Vianna RJ Jr. Esofago de Barrett postesofagite peptica e alcalina. Arq Gastroenterol 1984; 21: 167-171.
49. Hamilton JW, Thune RG, Morrissey JF. Symptomatic ectopic gastric epithelium of the cervical esophagus. Dig Dis Sci 1986; 31: 337-342.
50. Popiela T, Turczynowski W, Karcz D et al. Long-term results of highly selective vagotomy in the treatment of duodenal ulcer patients using the intraoperative endoscopic Congo red test to identify the parietal cell antrum-corpus borderline. Hepatogastroenterology 1993; 40: 267-271.
51. Ikeda K, Sannohe Y, Murayama H et al. Heterotopic gastric mucosa in the duodenum-reaction to Congo red under fiberscopic observation. Endoscopy 1982; 14: 168-170.
52. Carrick J, Lee A, Hazell S et al. Campylobacter pylori, duodenal ulcer and gastric metaplasia: possible role of functional heterotopic tissue in ulcerogenesis. Gut 1989; 30: 790-797.
53. Donahue PE, Yoshida J, Richter HM et al. Can the use of endoscopic Congo red test decrease the incidence of incomplete proximal gastric vagotomy? Gastrointest Endosc 1987; 33: 427-431.
54. Chisholm EM, Raimes SA, Leong HT et al. Proximal vagotomy assessed by the endoscopic Congo red test. Br J Surg 1993; 80: 737-739.
55. Tatsuta M, Iishi H, Okuda S. Effects of cimetidine on the healing and recurrence of duodenal ulcers and gastric ulcers. Gut 1986; 27: 1213-1218.
56. Tatsuta M, Okuda S, Tamura H. Healing and recurrence of duodenal ulcers in relation to the acid-secreting area. Endoscopy 1982; 14: 11-13.
57. Tatsuta M, Okuda S. Age-related changes in the acid-secreting area in patients with duodenal ulcer. Endoscopy 1983; 15: 243-245.
58. Tatsuta M, Iishi H, Okuda S. Location of peptic ulcers in relation to antral and fundal gastritis by chromoendoscopic follow-up examinations. Dig Dis Sci 1986; 31: 7-11.
59. Tatsuta M, Iishi H, Nakaizumi A et al. Fundal atropic gastritis as a risk factor for gastric cancer. Int J Cancer 1993; 53: 70-74.
60. Tatsuta M, Okuda S, Tamura H, Taniguchi H. Polyps in the acid-secreting area of the stomach. Gastrointest Endosc 1981; 27: 145-149.
61. Tatsuta M, Okuda S, Taniguchi H, Tamura H. Gross and histological types of early gastric carcinoma in relation to the acid-secreting area. Cancer 1979; 43: 317-321.
62. Tatsuta M, Okuda S, Taniguchi H. Diagnosis of minute cancers by the endoscopic Congo red-methylene blue test. Endoscopy 1983; 15: 252-256.
63. Tatsuta M, Iishi H, Okuda S, Taniguchi H. Diagnosis of early gastric cancers in the upper part of the stomach by the endoscopic Congo red-methylene blue test. Endoscopy 1984; 16: 131-134.
64. Tatsuta M, Iishi H, Okuda S et al. The association of Helicobacter pylori with differentiated-type early gastric cancer. Cancer 1993; 72: 1841-1845.
65. Iishi H, Tatsuta M, Okuda S. Diagnosis of simultaneous multiple gastric cancers by the endoscopic Congo red-methylene blue test. Endoscopy 1988; 20: 78-82.
66. Ikeda T, Senoue I, Hara M et al. Gastric pseudopolyposis: a new clinical manifestation of type A gastritis. Am J Gastroenterol 1985; 80: 82-90.
67. Prokrotniek II, Strelis EA, Pominova VB et al. Prognoz khronicheskoi iazvy zheludka po dannym gastrokhromoskopicheski morfologicheskoi otsenki slizistoi obolochki zheludka. Ter Arkh 1986; 58: 45-48.
68. Tatsuta M, Iishi H, Okuda S. Relationship of erosive gastritis to the acid secreting area and intestinal metaplasia and the healing effect of pirenzepine. Gut 1987; 28: 561-565.