In Vivo Vital Staining as an Aid to Identification of Esophagogastric Mucosal Junction in Man

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A method is described for accurate, rapid and safe differential staining of squamous and columnar cells in the esophagus. In all 10 patients studied during elective esophagoscopy, instillation of Lugol's solution into the distal esophagus stained the squamous epithelium a gray-black. Columnar epithelium and areas of inflammation remained unstained. A sharp circumferential line at the squamocolumnar junction was obtained in patients with a normal esophagus; this line was irregular in patients with esophagitis. The procedure does not interfere with further histologic staining for permanent slides. Lugol's solution reacts with glycogen in squamous cells, as demonstrated by the PAS and PAS-diastase methods. Under these conditions the effect of Lugol's solution on thyroid function studies other than protein-bound iodine is minimal and of short duration. This staining procedure has significant clinical and research potential because it allows the endoscopist to locate, rapidly and accurately, the squamocolumnar junction, its relationship to other anatomic or pathologic entities and the extent as well as the proximal borders of esophagitis.

Localizing the esophageal squamocolumnar junction and its position, relative to the physiologic lower esophageal sphincter, hiatus hernia or Schatzki ring, have been subjects of controversy (1–6). It is sometimes difficult to accurately identify the squamocolumnar junction endoscopically during life. The procedure described herein provides a simple, safe, rapid and accurate method of identifying this important landmark without interfering with subsequent histopathologic studies of biopsy material.

MATERIALS AND METHODS

Patients

Ten patients with normal mucosa or mild hyperemia at elective esophagoscopy* were studied. Patients with mass lesions, severe or erosive esophagitis, esophageal varices or other significant pathology were excluded, as were patients with known iodide sensitivity.

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Procedure

1. The flexible esophagoscope was passed routinely with the patient in the left lateral position. If esophagoscopy excluded significant pathology, photographs of the distal esophagus, including the suspected squamocolumnar junction, were taken. A small polyethylene catheter was passed through the esophagoscope and 3 to 5 ml of 5% Lugol's solution (5% elemental iodine and 10% KI in aqueous solution) was flushed into the distal esophagus, approximately 2 cm above the suspected esophagogastric junction. After 1 minute, excess solution was washed away with distilled H_2O. Additional photographs were obtained and the stained and the unstained areas were biopsied. Biopsy specimens were submitted in 10% formaldehyde for histologic examination.

2. To obtain a larger sampling of this area, 20 to 30 ml of Lugol's solution was introduced blindly into the esophagus of 4 anesthetized fasting dogs through a nasogastric tube.

* Olympus Fiberoptic Esophagoscope

The animals were immediately sacrificed and the distal esophagus and proximal stomach removed for study.

3. The effect of Lugol's solution on thyroid function studies was determined in 2 normal volunteers who drank 5 ml of the solution. Protein-bound iodine, T_4 by column, free T_4, T_4 by Murphy–Patee, and T_3 were determined prior to ingestion and again 1 hour, 1 day, 2 days, 1 week and 2 weeks after ingestion. In addition, radioactive iodine uptake was studied in 1 volunteer prior to ingestion of Lugol's solution, and 1 day and 1 week afterward.

RESULTS

Application of Lugol's solution resulted in dark, gray-black discoloration of esophageal squamous epithelium in all patients. Columnar epithelium of the stomach either appeared unchanged or was a light brown, clearly distinguishable from the squamous esophageal mucosa. Biopsies obtained just proximal and distal to the line of demarcation confirmed the presence of squamous and columnar mucosa, respectively. In 4 patients with a normal esophagus, a sharp circumferential line was obtained (Figure 1). In 6 patients with esophagitis, areas of inflammation did not take vital staining, resulting in either patchy areas of staining or an irregular, interrupted circumferential margin. Biopsies obtained on either side of the line of demarcation in patients with esophagitis showed normal squamous epithelium from the stained area and mucosal inflammation from the unstained area. Over a 15- to 20-minute period the vital stain disappeared. Dogs that had received Lugol's solution also exhibited a sharp demarcation between squamous and columnar epithelium at the gastroesophageal junction (Figure 2).

To preserve the iodine staining of squamous epithelium in histologic sections, cryostat preparations of unfixed material were used without mounting media. Sections prepared in this manner revealed staining in superficial squamous cells but not in columnar cells (Figure 3). PAS and PAS-diastase methods (7) confirmed the presence of glycogen within squamous epithelial cell cytoplasm, in humans as well as in dogs. Similar staining of areas of inflammation revealed PAS-positive material that was not glycogen, as demonstrated by the PAS-diastase technic. Iodine staining of epithelium was not preserved in routinely processed biopsy specimens, since alcohol, formalin fixation or mounting media removed the stain.

The effect of a single large dose of Lugol's solution on thyroid function studies was minimal and of short duration. There was no change in T_4 by the Murphy–Pattee technic or T_3; within 24 to 48 hours T_4 by column and free T_4 were normal. In 1 subject the protein-bound iodine returned to normal within 1 week, but in a second patient it was still elevated at 2 weeks. In 1 subject radioactive iodine uptake remained markedly suppressed 24 hours after the ingestion of Lugol's solution; however, it returned to preingestion levels after 1 week.

DISCUSSION

In 1933 Schiller (8) first described the use of Lugol's solution to differentiate normal from

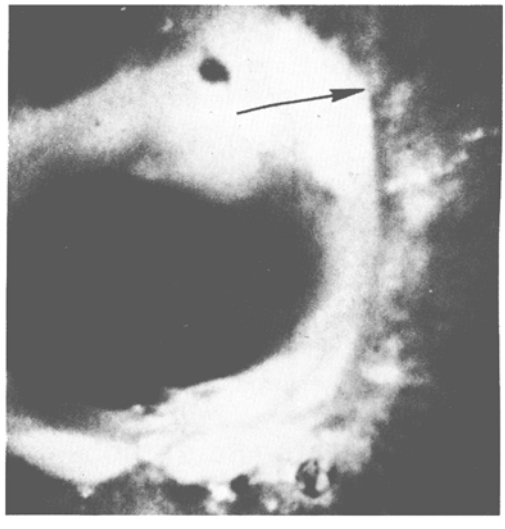

Fig 1. Endoscopic appearance of stained squamocolumnar junction. The lumen is seen distally as a dark circle to the left. **Arrow** identifies circular squamocolumnar junction, in which stained squamous cells appear as the dark peripheral border and unstained columnar cells appear as the white area.

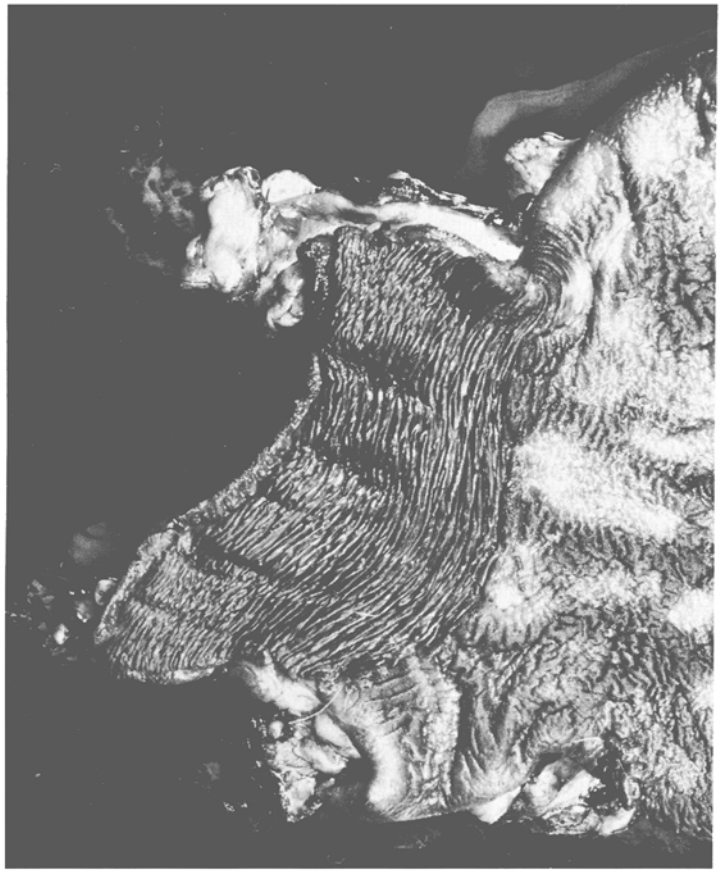

Fig 2. Dog esophagogastric junction is stained with Lugol's iodine. The squamocolumnar junction is outlined by selective staining of squamous epithelium, to the left of the photograph. Columnar epithelium on the right stains poorly or not at all.

abnormal cervical squamous epithelium. Adapting this procedure to delineate mucosal characteristics of the distal esophagus provides the endoscopist with a simple, rapid, safe method for accurately identifying the squamocolumnar junction.

Lugol's solution produces a gray-black vital stain by the reaction of iodide with glycogen (7, 9). Mature nonkeratinized squamous cells contain large amounts of glycogen (as demonstrated by PAS and PAS-diastase methods) (7) and consequently tend to stain selectively with Lugol's solution. By staining the esophagus, the extent of normal squamous epithelium is delineated. Histologically proven areas of inflammation fail to stain with Lugol's solution, presumably because glycogen is absent. This pattern of response is similar to that obtained with the Schiller test, in which the cervix is swabbed or sprayed with Lugol's or Gram's solution and rinsed off after 30 seconds. Glycogen-containing cells of the cervix and vagina take up the iodine, producing a deep mahogany color; however, there is usually no uptake in the presence of cancer, trauma or inflammation (10, 11).

Since iodine is removed by fixation with alcohol or formaldehyde, and by routine staining and mounting media, unstained, unmounted frozen sections are needed to demonstrate the location of stained cells (Figure 3). Conversely, this feature ensures that prior staining will have no deleterious effect on biopsies obtained for routine histologic examination.

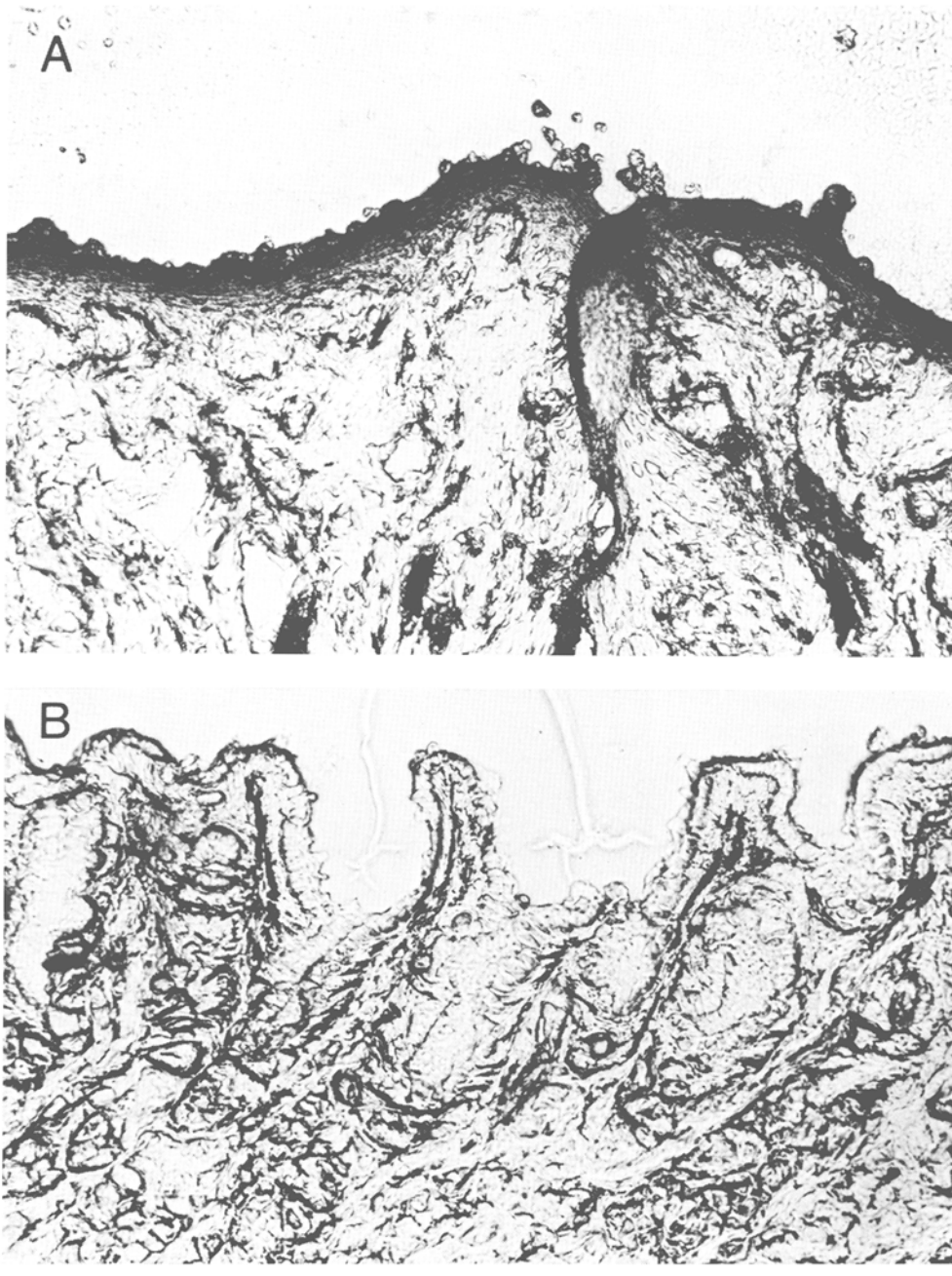

Fig 3. Frozen section of dog esophagogastric junction stained with Lugol's iodine. **A.** Intense iodine staining of squamous epithelium of esophagus. **B.** Lack of staining of columnar epithelium just distal to A.

The gradual diminution in staining observed in living subjects over a 15- to 20-minute period may represent either leeching out of iodine or desquamation of stained superficial squamous epithelium. Human cadavers were usually unacceptable for study because anaerobic glycogenolysis causes glycogen to disappear rapidly. However, in a report of a newly described entity, glycogenic acanthosis, glycogen in discrete mucosal esophageal plaques was stained by Lugol's solution in fixed autopsy specimens (12).

Simultaneous studies by Brodmerkel (13) suggest that half-strength Lugol's solution (2.5%) may provide a better differential stain because there is less superficial staining of columnar cells. When the distal esophagus contains excessive mucus, prior installation of dilute acetic acid to dispose of mucus may improve the results.

The single dose of iodide had only a transient effect on serum thyroid function tests. Assuming that the elemental iodine in Lugol's solution would be completely absorbed from the gastrointestinal tract as iodide, 500 to 800 mg of iodide was administered. This is approximately equivalent to 10 to 18 drops of a saturated solution of potassium iodide (SSKI), a dose comparable to that used for chronic obstructive lung disease but greater than that used in routine treatment of hyperthyroidism (2 to 6 mg) (14). Radioactive iodine uptake returns to normal within 2 weeks after inorganic iodide is administered (15); it did so within 1 week in our patient. Several weeks are required for serum protein-bound iodine to return to normal after chronic administration of iodide (in doses similar to the single dose used in our studies) (16). T_4 (by column chromatography) may also be transiently affected by a large dose of inorganic iodide (17). Total serum T_4 may be measured accurately by competitive binding technics immediately after Lugol's solution has been administered.

Staining the esophageal mucosa with Lugol's solution during esophagoscopy is a potentially valuable clinical and research tool for the endoscopist. This method should make it much easier to locate the squamocolumnar junction relative to hiatus hernia or Schatzki ring. Since inflamed areas fail to stain, the extent and proximal borders of esophagitis can be quickly assessed. A report in the German literature on the use of 1 to 2% Lugol's solution confirms the value of this technic in delineating the border between gastric and esophageal mucosa and revealing areas of esophagitis (18). Submucosal tumors covered by intact normal mucosa will usually take the vital stain, while tumors with mucosal involvement will not (13). Although suitable patients were not available for study, one would expect this method to identify areas of heterotopic columnar epithelium in the esophagus, as seen with Barrett's esophagus.

Endoscopic examination of esophageal mucosa stained with Lugol's solution provides the endoscopist with a safe, accurate method of identifying the squamocolumnar junction.

ACKNOWLEDGMENTS

The authors wish to thank Drs. Raphael Garcia-Bunnel and Paul Davis for their helpful suggestions.

Acknowledgment is made to the Gerontology Research Center, NICHD, for use of facilities provided under its guest scientist program.

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