imply that germline hemi-allelic methylation of *MLH1* could be transmitted vertically.

A paper from Ward's group arrived at a different conclusion with respect to germline hemi-allelic methylation or epimutation of *MLH1*.[3] They documented two additional subjects carrying an *MLH1* epimutation, who also met clinical criteria indicative of a diagnosis of "HNPCC". Additionally, the epimutation was present in spermatozoa of one of the affected subjects. The second finding not only fitted with a germline defect but also provided evidence for vertical transmission of the defect. The authors therefore advanced the concept of *MLH1* epimutation as a new cause of HNPCC.[3] Nevertheless, it may be questioned if epimutations can in fact be inherited. Although germline hemi-allelic methylation was indeed shown in single members of two families that met certain clinical criteria for HNPCC, this is hardly surprising as the search for the epimutation was conducted exclusively in members of families registered in cancer family clinics. This ascertainment bias aside, it is now clear (as stated above) that when a family happens to meet a particular clinical definition of "HNPCC" this does not automatically prove the existence of an underlying altered DNA mismatch repair gene (the basis for Lynch syndrome).[4] Although one of the affected subjects indeed showed methylation of *MLH1* in spermatozoa, this was in <1% of spermatozoa.[3] Should such an affected sperm succeed in fertilising an ovum, subsequent clearance of methylation during early embryogenesis would negate the effects of vertical transmission of the affected allele.

Ward *et al* subsequently showed the de novo origin of germline hemi-allelic methylation of *MLH1* in a male subject who was shown to have inherited the methylated allele from his mother in whom the same allele was not methylated.[15] These authors nevertheless continued to claim that *MLH1* epimutation was "weakly" heritable, although they also contradicted themselves in the same paper by asserting that there was no evidence that *MLH1* epimutation could be inherited. Wong *et al* now cite the four preceding reports on this topic as providing evidence for the heritability of germline epigenetic change.

In summary, the balance of evidence suggests that genetic mechanisms will be found to at least partially explain the evolution of CIMP-positive CRCs and will account for a subset of families that may mimic Lynch syndrome. On the other hand, there is no evidence to support the inheritance of *MLH1* epimutation.

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References

1 **Frazier ML**, Xi L, Zong J, *et al.* Association of the CpG island methylator phenotype with family history of cancer in patients with colorectal cancer. *Cancer Res* 2003;**63**:4805–8.
2 **Ward RL**, Williams R, Law M, *et al.* The CpG island methylator phenotype is not associated with a personal or family history of cancer. *Cancer Res* 2004;**64**:7618–21.
3 **Suter CM**, Martin DIK, Ward RL. Germline epimutation of MLH1 in individuals with multiple cancers. *Nat Genet* 2004;**36**:497–501.
4 **Lindor NM**, Rabe K, Petersen GM, *et al.* Lower cancer incidence in Amsterdam-1 criteria families without mismatch repair deficiency. Familial colorectal cancer type X. *JAMA* 2005;**293**:1979–85.
5 **Samowitz WS**, Albertsen H, Herrick J, *et al.* Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. *Gastroenterology* 2005;**129**:837–45.
6 **Weisenberger DJ**, Siegmund KD, Campan M, *et al.* A distinct CpG island methylator phenotype in human colorectal cancer is the underlying cause of sporadic mismatch repair deficiency and is tightly associated with BRAF mutation. *Nat Genet* 2006;**38**:787–93.
7 **Samowitz WS**, Sweeney C, Herrick J, *et al.* Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. *Cancer Res* 2005;**65**:6063–9.
8 **Young J**, Barker MA, Simms LA, *et al.* BRAF mutation and variable levels of microsatellite instability characterize a syndrome of familial colorectal cancer. *Clin Gastroenterol Hepatol* 2005;**3**:254–63.
9 **Jass JR**, Cottier DS, Pokos V, *et al.* Mixed epithelial polyps in association with hereditary non-polyposis colorectal cancer providing an alternative pathway of cancer histogenesis. *Pathology* 1997;**29**:28–33.
10 **Cui H**, Cruz-Correa M, Giardiello FM, *et al.* Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. *Science* 2003;**299**:1753–5.
11 **Sasaki J-I**, Konishi F, Kawamura YJ, *et al.* Clinicopathological characteristics of colorectal cancers with loss of imprinting of insulin-like growth factor 2. *Int J Cancer* 2006;**119**:80–3.
12 **Nakagawa H**, Chadwick RB, Peltomaki P, *et al.* Loss of imprinting of the insulin-like growth factor II gene occurs by bi-allelic methylation in a core region of H19-associated CTCF-binding sites in colorectal cancer. *Proc Natl Acad Sci USA* 2001;**98**:591–6.
13 **Gazzoli I**, Loda M, Garber J, *et al.* A hereditary nonpolyposis colorectal carcinoma case associated with hypermethylation of the hMLH1 gene in normal tissue and loss of heterozygosity of the unmethylated allele in the resulting microsatellite instability-high tumor. *Cancer Res* 2002;**62**:3925–8.
14 **Miyakura Y**, Sugano K, Akasu T, *et al.* Extensive but hemiallelic methylation of the hMLH1 promoter region in early-onset sporadic colon cancers with microsatellite instability. *Clin Gastroenterol Hepatol* 2004;**2**:147–56.
15 **Hitchins M**, Williams R, Cheong K, *et al.* MLH1 germline epimutations as a factor in hereditary nonpolyposis colorectal cancer. *Gastroenterology* 2005;**129**:1392–9.

Methylene blue but not indigo carmine causes DNA damage to colonocytes in vitro and in vivo at concentrations used in clinical chromoendoscopy

Identification of mucosal abnormalities is aided by the use of dyes during colonoscopy (chromoendoscopy).[1] Two dyes that have found particular favour are methylene blue and indigo carmine.[2 3]

Methylene blue, which, unlike indigo carmine, is taken up by cells, induces cellular DNA damage in vitro via the generation of singlet oxygen when photoexcited by white light.[4] In contrast, indigo carmine appears to be photostable and to possess little potential to damage genetic material in vitro.[5 6] A recent clinical study has shown that the extent of DNA damage (particularly oxidative DNA damage) in human oesophageal cells is increased after methylene blue chromoendoscopy.[7] Additional iatrogenic oxidative DNA damage to epithelial cells is of particular concern in such precancerous tissue because of the association between oxidative DNA damage, mutagenesis and the development of malignancy.[8] We hypothesised that indigo carmine would induce less DNA damage than methylene blue both in vitro in cultured colon cells during simulated chromoendoscopy conditions and in vivo in colonic biopsy samples collected at chromoendoscopy.

We used the alkaline comet assay to determine DNA damage in the cells treated with methylene blue/indigo carmine and white light. This is a sensitive technique for analysing and measuring such damage in mammalian cells, with the percentage of DNA in the comet tail being linearly related to DNA damage.[9] The inclusion of the DNA repair enzyme, Fapy-DNA-glycosylase (FPG), in the comet assay results in the excision of oxidised guanines to yield additional DNA strand breaks that are detectable in the comet assay.[10] This allows an estimation of specific oxidative DNA damage to cells. In all the experiments described below, the cell viability exceeded 70%.

To simulate chromoendoscopy in vitro, 50 µl of either 0.1% methylene blue or 0.1% indigo carmine dye was added to a monolayer of cultured $CaCo_2$ adenocarcinoma cells for 2 min in the presence and absence of cold white light. Only low levels of DNA damage are found in cells in both the alkaline and the FPG-modified comet assay when the exposure is to white light alone (fig 1). Treatment with indigo carmine either in the light or in the dark, or with methylene blue in the dark did not result in any major change in the extent of DNA damage compared with controls. In contrast, cells treated with methylene blue in the light showed a salient increase in DNA damage compared with controls in both the alkaline and the FPG-modified comet assay ($p<0.001$).

For in vivo experiments, ethical approval and patient consent were obtained to take biopsy

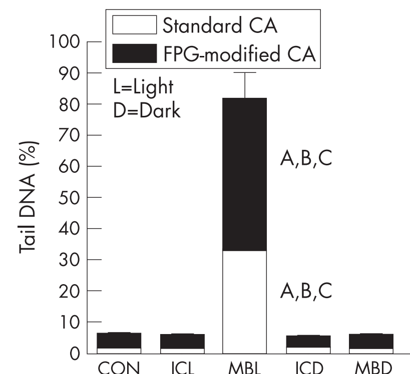

Figure 1 $CaCo_2$ cells exposed to 0.1% methylene blue (MB) or 0.1% indigo carmine (IC) either in white light or in the dark for 2 min. Control cells (CON) were treated with white light only (no dye). Data are presented as mean (SE) tail DNA (%) for three experiments. Both the DNA damage from the alkaline comet assay (CA; no fill) and the additional damage with Fapy-DNA-glycosylase (FPG; fill) are shown. (A) Significance at $p<0.001$ relative to control cells in the alkaline or FPG-modified comet assay. (B) Significance at $p<0.001$ in methylene blue versus indigo carmine cells under the same experimental conditions (either in the light or in the dark) in the alkaline or the FPG-modified comet assay. (C) Significance at $p<0.001$ in methylene blue-treated cells in the light versus in the dark in the alkaline or FPG-modified comet assay.

Table 1 Median DNA damage measured by the standard comet assay and the Fapy-DNA-glycosylase- modified comet assay before and after methylene blue and indigo carmine dye spraying in the two groups of patients

Patient group	n	Median DNA damage (IQR) before spraying (%)	Median DNA damage (IQR) after spraying (%)	p Value (Wilcoxon's test)
IC spraying (standard comet assay)	10	7.2 (5.1–15.8)	5.3 (4.1–11.7)	0.084
IC spraying (FPG-modified comet assay)		10.5 (6.0–32.1)	12.3 (5.9–17.3)	0.492
MB spraying (standard comet assay)	10	5.9 (4.9–8.1)	12.0 (5.2–19.8)	0.014
MB spraying (FPG-modified comet assay)		9.8 (7.5–14.0)	34.3 (23.9–58.8)	0.002

FPG, Fapy-DNA-glycosylase; IC, indigo carmine; IQR, interquartile range; MB, methylene blue.
Biopsy samples were digested with 100 µl of 0.5% pronase and 100 µl of 0.3% collagenase in 800 µl DMEM at 37˚C for 1 h, to create a single-cell suspension for comet assay analysis.

samples from colonic mucosa during routine endoscopic examination. Mucosal biopsy samples were taken from the same area of the colon before and after the application of 2 ml of 0.1% methylene blue or indigo carmine dye onto the colonic mucosa.

Patients in the methylene blue chromoendoscopy group, but not those in the indigo carmine group, had significantly greater DNA damage in biopsy samples after dye spraying than before the application of dye in both the alkaline ($p = 0.014$) and the FPG-modified comet assay ($p = 0.002$; table 1).

Of the 10 patients receiving methylene blue chromoendoscopy, eight had higher levels of DNA damage, post-endoscopy, measured by alkaline comet assay, and all had higher levels of DNA damage, measured by the FPG-modified comet assay.

Our study highlights the potential for the induction of DNA damage by methylene blue when used as a dye during colonoscopy. It is reasonable to suggest that any iatrogenic DNA damage induced in colonocytes by dye spraying should be avoided where possible, particularly in high-risk groups such as patients with ulcerative colitis. The efficacy of methylene blue versus indigo carmine during chromoendoscopy has not been formally compared, but if assumed to be equal, indigo carmine rather than methylene blue should be considered for use.

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Ethical approval was given by the Harrogate Local Research Ethics Committee to collect biopsy samples from 20 patients undergoing elective sigmoidoscopic and colonoscopic examinations for a variety of clinical indications at the Endoscopy Department at The General Infirmary at Leeds (Ethics reference number 04/Q1107/16).

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References

1 **Bruno MJ**. Magnification endoscopy, high resolution endoscopy, and chromoscopy; towards a better optical diagnosis. *Gut* 2003;**52**:iv7–11.
2 **Kiesslich R**, Fritsch J, Holtmann M, *et al*. Methylene blue aided chromoendoscopy for the detection of intraepithelial neoplasia and colon cancer in ulcerative colitis. *Gastroenterology* 2003;**124**:880–8.
3 **Rutter MD**, Saunders BP, Schofield G, *et al*. Pancolonic indigo carmine dye spraying for the detection of dysplasia in ulcerative colitis. *Gut* 2004;**53**:256–60.
4 **Boiteux S**, Gajewski E, Laval J, *et al*. Substrate specificity of the *Escherichia coli* FPG protein (formamidopyrimidine-DNA glycosylase): excision of purine lesions in DNA produced by ionising radiation or photosensitisation. *Biochemistry* 1992;**31**:106–10.
5 **Abbruzzetti S**, Viappiani C, Murgida DH, *et al*. Non-toxic water-soluble photocalorimetric reference compounds for UV and visible excitation. *Chem Phys Lett* 1999;**304**:167–72.
6 **Rhee Y**, Termini J, Valentine M. Oxidative base damage in DNA detected by reverse transciptase. *Nucl Acids Res* 1995;**23**:3275–82.
7 **Olliver JR**, Wild CP, Sahay P, *et al*. Chromoendoscopy with methylene blue and associated DNA damage in Barrett's oesophagus. *Lancet* 2003;**362**:373–74.
8 **Volkovova K**, Dunsinska M, Collins AR. From oxidative DNA damage to molecular epidemiology. *J Appl Biomed* 2005;**3**:1–5.
9 **Wong VWC**, Szeto YT, Collins AR, *et al*. The comet assay: a biomonitoring tool for nutraceutical research. *Curr Top Nutraceutical Res* 2005;**3**:1–14.
10 **Collins AR**. Measurement of oxidative DNA damage using the Comet Assay. In: Lunce J, Griffiths HR, eds. *Measuring in vivo oxidative damage*. Chichester: Wiley, 2000:81–94.

Ménétrier's disease

The Editor's quiz in *Gut* (1993;**52**:1572) featured a most interesting presentation of Ménétrier's disease (hypertrophic protein losing gastropathy). There is a recognised association between Ménétrier's disease and infection with *Helicobacter pylori*. In two retrospective studies, between 30% and 90% of patients with Ménétrier's disease were associated with *H pylori*.[1–4] Further, Bayerdörffer *et al* reported marked improvement of the condition after eradication of *H pylori*. Is it possible that the reported case in the Editor's quiz was colonised with *H pylori* and if so, would the patient benefit from eradication treatment?

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References

1 **Wolfsen HC**, Herschel A, Carpenter A, *et al*. Ménétrier's disease: a form of hypertrophic gastropathy of gastritis. *Gastroenterology* 1993;**104**:1310–19.
2 **Bayerdörffer E**, Ritter MM, Hatz R, *et al*. Ménétrier's disease and Helicobacter pylori. *N Engl J Med* 1993:60.
3 **Herz R**, Lombardi E, Wipping F, *et al*. Helicobacter pylori-associated hypertrophic gastritis. Imitation of Ménétrier's disease. *Fortschr-Med* 1992;**110**:37–40.
4 **Lepore MJ**, Smith FB, Bonanno CA. Campylobacter-like organisms in patients with Ménétrier's disease. *Lancet* 1988:466.