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Intermediate effect markers for colorectal cancer

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Recurrence or regression of adenomatous polyps is considered to be both a biomarker of risk and an intermediate (surrogate) end-point. The observational epidemiology of adenomas resembles closely that of invasive cancer, and the findings in chemoprevention trials that have been completed closely mirror that common epidemiology. Although it is possible that both the clinical trials and the epidemiology may be wrong, these common findings suggest that adenomas in general are valid end-points.

Aberrant crypt foci show promise as biomarkers, both as markers of risk and as intermediate end-points for chemoprevention trials.

If issues of cost can be overcome, assessment of *ras* mutations in stool appears to be a promising technique for screening for large bowel neoplasms. The lack of specificity of the technique limits its utility as a sole end-point in prevention studies, however.

Mucosal proliferation has been used both as a biomarker of risk and as an intermediate end-point. The utility of these measures is not clear, however, since there has been discordance between the epidemiological findings regarding proliferation and established risk factors for colorectal neoplasia. The inherent variability of the measures and the technical problems associated with their use are further impediments. However, rectal mucosal proliferation may be suitable for studies in single institutions, or in consortia with very aggressive quality control.

Adenomatous polyps

Colorectal adenomas are well demarcated tumours of the large bowel mucosa, and are composed of crypts with cells showing features of epithelial dysplasia. This histological definition implies nothing about the gross shape of the lesion, although commonly it is polypoid, protruding into the lumen of the bowel, sometimes on a stalk. Since such adenomatous polyps are raised lesions, they are easily visible by endoscopy and (unless very large) can be removed endoscopically. Adenomatous polyps have been widely applied in large bowel chemoprevention studies both as an entry criterion and as an intermediate outcome end-point (Baron, 1996).

However adenomatous polyps are used, there is measurement error associated with their identification by endoscopy. To distinguish these polyps from non-adenomatous large bowel polyps in research studies, it is important that each polyp be biopsied for histological examination. Since the microscopic identification of adenomatous tissue is generally not difficult, the net specificity of endoscopic surveillance of the bowel can be assumed to be close to 100% if pathological examination is conducted. However, the sensitivity is likely to be somewhat lower than this. For large adenomas (1 cm or greater in estimated diameter), the miss rate is small (less than 5%; Hixson *et al.*, 1991), but for smaller polyps it has been found to be 15–25% (Hixson *et al.*, 1990; Hoff & Vatn, 1985; Rex *et al.*, 1997).

The existence of 'flat adenomas'—those that are not substantially raised above the mucosal surface—somewhat complicates this assessment. Such adenomas have been noted most often in Asia, but have also been seen in North America and Europe (Jaramillo *et al.*, 1995; Lanspa *et al.*, 1992; Owen, 1996). The presence of such adenomas may decrease the sensitivity of adenoma detection by endoscopy, but will not greatly affect the predictive value of the detected lesions, at least for the raised cancers and adenomas traditionally studied in western countries. Increased awareness of these lesions and newer endoscopic techniques such as magnifying endoscopy may resolve some of the

geographical differences in the apparent incidence and prevalence of these lesions.

Adenomas as biomarkers

Adenomatous polyps are clearly established markers of risk of colorectal neoplasia. Individuals with adenomas in the distal bowel (for example, within the reach of a sigmoidoscope) have an increased risk of proximal adenomas or cancer (Levin *et al.*, 1999; Lieberman & Smith, 1991). Advanced adenomas—those with large size (typically >1 cm), villous histology or advanced dysplasia—are more likely to harbour invasive cancer than simple adenomas (without those features) (Gatteschi *et al.*, 1991; Muto *et al.*, 1975; Shinya & Wolff, 1979). Advanced features in distal adenomas also are predictive of proximal adenomas (Levin *et al.*, 1999; Schoen *et al.*, 1998; Wallace *et al.*, 1998) as well as proximal advanced adenomas (Levin *et al.*, 1999; Schoen *et al.*, 1998; Wallace *et al.*, 1998; Zarchy & Ershoff, 1994). In some multivariate analyses, all of these factors have emerged as independent predictors (Gatteschi *et al.*, 1991), although others have found that while histology and number of polyps are independent predictors of risk, size is not (Atkin *et al.*, 1992; Levin *et al.*, 1999).

Patients with adenomas anywhere in the bowel also have an increased risk of later (metachronous) adenomas or cancer (Atkin *et al.*, 1992; Lotfi *et al.*, 1986; Simons *et al.*, 1992; Stryker *et al.*, 1987). Characteristics of the presenting adenomas that are predictive of subsequent neoplastic development include large size, villous histology, multiple adenomas, and higher degrees of dysplasia (Atkin *et al.*, 1992; Grossman *et al.*, 1989; Simons *et al.*, 1992; van Stolk *et al.*, 1998; Winawer *et al.*, 1993; Yang *et al.*, 1998). The same characteristics are also predictive of later adenomas with advanced pathological features (Atkin *et al.*, 1992; Yang *et al.*, 1998). In multivariate analyses, histology appears to explain the association of size with risk of metachronous tumours (Atkin *et al.*, 1992; Simons *et al.*, 1992). Unfortunately, in virtually all of the studies of later cancer risks in patients with adenomas, comparisons have been made with the general population, rather than with individuals screened intensively for colorectal cancer like the subjects with adenomas. Consequently, the excess cancer risks are almost certainly underestimated.

These data show that the association between adenomatous polyps and synchronous or metachronous neoplasia depends on the characteristics of the presenting polyp (Atkin *et al.*, 1992; Aubert *et al.*, 1982). In essence, it appears that while advanced lesions are predictive of both simple and advanced lesions, the simple lesions are associated only with an increased risk of synchronous or metachronous simple lesions. Since low-risk adenomas do not lead to high-risk adenomas (not to speak of cancer), it is possible that advanced and simple adenomas reflect different biological pathways.

Adenomas as surrogate end-points: the adenoma–carcinoma sequence

It is thought that the overwhelming majority of colorectal cancers arise from adenomatous tissue—this is the well known adenoma–carcinoma sequence. Evidence for the adenoma–carcinoma sequence is largely indirect. A substantial proportion of colorectal cancers have contiguous adenomatous tissue, suggesting that the former grew out of the latter (Eide, 1983; Peipins & Sandler, 1994). Also, in a high proportion of large adenomas (particularly large villous adenomas), there are foci of carcinoma (Muto *et al.*, 1975). The distribution of adenomas within the bowel differs from that of colorectal cancer (Peipins & Sandler, 1994), but large adenomas (carrying greater risk of developing malignancy) have a site distribution similar to that of cancer (Konishi & Morson, 1982; Matek *et al.*, 1986). Polyps (unknown histology) have been observed to grow in a location in which cancer was later found (Stryker *et al.*, 1987). Also, adenomas and carcinomas share a number of metabolic characteristics, including abnormalities in DNA ploidy, mucins, metabolic enzymes and cytoskeletal proteins (Tierney *et al.*, 1990). In each case, these changes follow what appears to be a progression of changes from normal mucosa through adenoma to carcinoma. It is important to note that this progression has been described largely in histopathological terms; it is actually a matter of dysplasia progressing to carcinoma. The term 'polyp', a gross pathology descriptor, has no particular histopathological meaning. The existence of the adenoma–carcinoma sequence does not necessarily imply that the polyp–carcinoma sequence holds all the time (Morson, 1984).

Perhaps the strongest evidence for the preneoplastic nature of adenomatous polyps is the somatic genetic changes seen in them. These somatic changes fit clearly into a progression, sitting between normal mucosa and invasive carcinoma (Kinzler & Vogelstein, 1996; Potter, 1999). There is a similarly high prevalence of mutations of the *APC* gene in colorectal cancer and in adenomas (even small adenomas) (Boland *et al.*, 1995; Kinzler & Vogelstein, 1996; Powell *et al.*, 1992); mutations in this gene seem to be important in turning individual cells onto a dysplastic path. The prevalence of Kirstin-*ras* (K-*ras*) and *p53* mutations increases as one considers progressively small adenomas (< 1 cm), large adenomas (≥ 1 cm) and invasive carcinoma (Boland *et al.*, 1995; Kinzler & Vogelstein, 1996). Various genetic changes have been seen at the transition from normal mucosa to small adenoma (*APC*), and from adenoma to severe dysplasia or carcinoma (*p53*, TGF-β in microsatellite unstable tumours) (Ahuja *et al.*, 1998; Boland *et al.*, 1995; Grady *et al.*, 1998).

Some mutations appear to be more common in early adenomas than in later tumours. β-Catenin mutations, for example, have been seen more commonly in small adenomas than in larger ones from the same patient population. It is possible that these mutations mark tumours that are unlikely to progress (at least in size) (Samowitz *et al.*, 1999), or this may have been a chance finding.

Assuming that colorectal cancers grow out of large adenomas and that large polyps were first small polyps, we can qualify small adenomas as precancerous lesions. Nonetheless, it is clear that only a small minority of these adenomas will progress to carcinoma within a human lifetime (Eide, 1986; Knoernschild, 1963; Peipins & Sandler, 1994) and some polyps (unknown histology) regress or disappear (Cole *et al.*, 1959; Hoff *et al.*, 1986; Hofstad *et al.*, 1994; Knoernschild, 1963; Nicholls *et al.*, 1988; Welin *et al.*, 1963). It is not known if large advanced adenomas grow from small advanced adenomas, or whether the advanced features are acquired with growth. It is also not clear that all large bowel adenocarcinomas have progressed through a stage of adenoma that could be distinctly recognized as such (and potentially removed before malignant conversion) (Bedenne *et al.*, 1992). Thus it is possible that an intervention that prevents only small, simple adenomatous polyps may not confer a benefit in terms of reduction of cancer incidence, if the adenomas prevented are those that would not progress. However, removing adenomas within the reach of a sigmoidoscope reduces the subsequent risk of cancer in the same region of the bowel, a finding that is difficult to explain on the basis of bias (Muller & Sonnenberg, 1995; Murakami *et al.*, 1990; Newcomb *et al.*, 1992; Selby *et al.*, 1992). Assuming that physical removal of adenomatous polyps does lower the risk of subsequent carcinoma, this is evidence that at least a substantial proportion of cancers would have grown from the small adenomas detected and removed. This also suggests that flat adenomas (presumably undetected by endoscopy) are not the principal precursors of carcinomas in the populations from which the reports derived.

Epidemiology of large bowel adenomas versus the epidemiology of colorectal cancer

In general, the epidemiology of large bowel adenomas appears to be similar to that of colorectal cancer itself (Potter, 1996; World Cancer Research Fund & American Institute for Cancer Research, 1997). Vegetable intake (and, less consistently, intake of fruits) has been inversely associated with risk, and red meat directly associated with both types of neoplasm. For both, cereal fibre has generally been unassociated with risk, while findings regarding dietary fat and other types of fibre have been variable. Protective effects of folate have been reported, particularly in association with high alcohol intake. In observational studies, calcium intake has been variably related to risk of both adenomas and cancer (Bergsma-Kadijk *et al.*, 1996; Martinez & Willett, 1998) and coffee intake may be inversely related to the risk of both (Giovannucci, 1998). Exercise seems inversely related to both colon cancer and colorectal adenomas, but findings regarding obesity have been variable for both types of tumour (Peipins & Sandler, 1994; Potter, 1996; World Cancer Research Fund & American Institute for Cancer Research, 1997). Associations with cholecystectomy also appear to be similar (Peipins & Sandler, 1994). Studies that included both colorectal cancers and colorectal adenomas have provided particularly interesting data in this regard, with broadly similar patterns of risk factors for invasive cancer and

adenomas (Benito *et al.*, 1991, 1993; Boutron *et al.*, 1996; Faivre *et al.*, 1997; Fuchs *et al.*, 1999; Giovannucci *et al.*, 1993, 1994, 1995a, b, 1998; Kampman *et al.*, 1994; Kearney *et al.*, 1996; Kune *et al.*, 1987, 1991; Martinez *et al.*, 1996; Platz *et al.*, 1997), although one such study suggested a difference in the effect of folate (Boutron-Ruault *et al.*, 1996).

Adenomas in chemoprevention trials

In general, the findings from intervention trials that used adenomas as end-points have confirmed those from observational epidemiology. Recurrence of sporadic adenomatous polyps has been used as an end-point for several clinical trials of calcium supplementation. The largest of these, the Calcium Polyp Prevention Study, randomized 930 subjects to treatment with placebo or 3000 mg of calcium carbonate (1200 mg of elemental calcium). There was a modest protective effect of calcium. A subsequent trial, using 2 g of elemental calcium per day reported a similar relative risk, which failed to reach statistical significance with a smaller sample size (Faivre *et al.*, 1999). In several trials, β-carotene has had no effect on adenoma occurrence (Greenberg *et al.*, 1994; Kikendall *et al.*, 1990; MacLennan *et al.*, 1995), nor has ascorbate plus α-tocopherol (Greenberg *et al.*, 1994; McKeown-Eyssen *et al.*, 1988).

In agreement with the observational studies, cereal fibre supplements did not affect sporadic adenoma recurrence in two trials, either alone (Alberts *et al.*, 2000; MacLennan *et al.*, 1995) or with a low-fat diet (MacLennan *et al.*, 1995). Two other trials that investigated a low-fat/high-fibre diet also failed to find an effect (McKeown-Eyssen *et al.*, 1994; Schatzkin *et al.*, 2000). The earlier of these studies (McKeown-Eyssen *et al.*, 1994) relied heavily on a cereal fibre supplement, and so did not focus on the fibre moieties most strongly associated with reduced risk of colorectal neoplasia. In the more recent trial (Schatzkin *et al.*, 2000), subjects in the intervention arm were advised to increase dietary fibre by increasing intake of fruits and vegetables. No data were presented regarding changes in vegetable intake during the study (separately from changes in fruit). If vegetable intake increased substantially, these negative findings may conflict with the observational data.

In familial adenomatous polyposis (FAP), vitamin C supplementation yielded weak indications of a reduction in polyp burden (Bussey *et al.*, 1982), although in a larger study, ascorbate plus α-tocopherol had no effect (DeCosse *et al.*, 1989). There were weak suggestions that cereal fibre was similarly beneficial in conjunction with ascorbate plus α-tocopherol (DeCosse *et al.*, 1989). Several studies of sulindac have shown clear evidence that treatment with this nonsteroidal anti-inflammatory drug (NSAID) can lead to a reduction in polyps (Giardiello *et al.*, 1993; Labayle *et al.*, 1991; Nugent *et al.*, 1993).

Aberrant crypt foci

Aberrant crypt foci are groups of crypts that are morphologically altered: delineated from the surrounding glands, larger than normal, often with dilated lumina (Fenoglio-Preiser & Noffsinger, 1999). Vital stains such as methylene blue accentuate the aberrant crypt foci. The lesions are histologically heterogeneous, variably including hyperplastic and dysplastic crypts. The multiplicity of aberrant crypt foci (i.e., the number of crypts involved) can vary from one to as many as several hundred.

Aberrant crypt foci were first noted in rats treated with carcinogens, and several aspects of their occurrence suggest that they may be preneoplastic (Bird, 1995; McLellan *et al.*, 1991; Pretlow, 1995). Their numbers correlate with the dose of carcinogen used, and aberrant crypt foci have not often been seen in untreated animals, in those treated with carcinogens that target other organs or in those treated with toxic non-carcinogenic compounds such as cholic acid (Bird, 1995; Pretlow *et al.*, 1992). With increasing time after carcinogen administration, the number, size and degree of dysplasia of the aberrant crypt foci increase. Known promoters or inhibitors of colorectal carcinogenesis seem to have the same effect on the number and multiplicity of aberrant crypt foci (Bird, 1995). Clonal expansion and dysplasia may be separate processes (Bird, 1995; Jen *et al.*, 1994). In these experimental situations, aberrant crypt foci have been found to have some of the features of carcinogenesis, such as hyperproliferation and mutated *p53* (Fenoglio-Preiser & Noffsinger, 1999; Olivo & Wargovich, 1998; Pretlow, 1995; Roncucci, 1992). A natural history

study found that after administration of carcinogen, areas of aberrant crypt foci were likely to later contain adenomas or carcinoma, although some areas of aberrant crypt foci clearly regressed (Shpitz *et al.*, 1996). Aberrant crypt foci have been widely used in animal studies of chemopreventive agents. In one large-scale investigation of numerous agents, the response of aberrant crypt foci to the interventions was thought to correlate reasonably well with earlier efficacy studies (Olivo & Wargovich, 1998; Wargovich *et al.*, 1996).

Aberrant crypt foci have also been observed in the human colorectal mucosa. Relatively high numbers have been found in the bowel mucosa of patients with FAP or with sporadic colorectal cancer (Fenoglio-Preiser & Noffsinger, 1999; Nascimbeni *et al.*, 1999; Roncucci *et al.*, 1991), but are also seen in patients with non-neoplastic bowel disorders (Fenoglio-Preiser & Noffsinger, 1999). The numbers of aberrant crypt foci are progressively higher in the more distal regions of the bowel (Bouzourene *et al.*, 1999; Roncucci *et al.*, 1991; Shpitz *et al.*, 1998; Yamashita *et al.*, 1995), although dysplastic aberrant crypt foci may be preferentially found more proximally (Nascimbeni *et al.*, 1999; Roncucci *et al.*, 1998).

Aberrant crypt foci may be observed *in vivo* using magnifying endoscopy and methylene blue staining (Takayama *et al.*, 1998). There appears to be an association between the appearance of the lumina of the crypts and dysplastic aberrant crypt foci (Fenoglio-Preiser & Noffsinger, 1999; Roncucci *et al.*, 1991). Although one study reported excellent concordance between endoscopic appearance and histological characteristics (Takayama *et al.*, 1998), it is likely that there is considerable measurement error in the assessment of aberrant crypt foci with magnifying endoscopy.

Some aberrant crypt foci seem to be monoclonal, and hence neoplastic (Fenoglio-Preiser & Noffsinger, 1999; Siu *et al.*, 1999). In humans, aberrant crypt foci have been found to display some of the characteristics of colorectal carcinogenesis: hyperproliferation, overexpression of carcinoembryonic antigen, MSI phenotype, and somatic *APC* and K-*ras* mutations (Augenlicht *et al.*, 1996; Fenoglio-Preiser & Noffsinger, 1999; Heinen *et al.*, 1996; Polyak *et al.*, 1996; Shpitz *et al.*, 1997; 1998). K-*ras* mutations are very common (in various studies ranging up to 100%; Fenoglio-Preiser & Noffsinger, 1999), but *APC* and *p53* mutations are relatively uncommon (Fenoglio-Preiser & Noffsinger, 1999; Jen *et al.*, 1994; Losi *et al.*, 1996; Otori *et al.*, 1998; Pretlow *et al.*, 1993; Smith *et al.*, 1994; Yamashita *et al.*, 1995). *APC* mutations in aberrant crypt foci may correlate with dysplastic histology (Otori *et al.*, 1998). Human aberrant crypt foci with carcinoma *in situ* have been observed (Konstantakos *et al.*, 1996; Siu *et al.*, 1997). In one study, a correlation of number of aberrant crypt foci with numbers of adenomas was observed (Takayama *et al.*, 1998). The histology of aberrant crypt foci is not uniform among the crypts: determination of the hyperplastic/dysplastic nature of the aberrant crypt foci may require serial sectioning, and mixed foci are common (Nascimbeni *et al.*, 1999; Pretlow, 1995; Siu *et al.*, 1997). It is possible that hyperplastic aberrant crypt foci may evolve into dysplastic aberrant crypt foci (Otori *et al.*, 1995). On the other hand, it has been suggested that aberrant crypt foci are precursors of both adenomas and hyperplastic polyps (Fenoglio-Preiser & Noffsinger, 1999). Aberrant crypt foci have been used in only one human chemoprevention study—a non-randomized parallel arm study that found sulindac to be very effective in inducing regression of aberrant crypt foci (Takayama *et al.*, 1998).

Stool K-*ras*

The mucosa of the large bowel is a rapidly proliferating tissue, turning over completely in four days (Smith-Ravin *et al.*, 1995). Despite the enzymes and debris present in the stool environment, sufficient DNA from cells shed into the lumen is passed in stool to permit amplification with the polymerase chain reaction (PCR) (Sidransky *et al.*, 1992). The amount of DNA obtained per 100 mg stool has varied from less than 1 µg to 9 µg. In patients with large bowel carcinoma, it is possible that the proportion of the DNA in stool derived from the tumour itself could be sufficient to permit amplification and detection (Sidransky *et al.*, 1992). The K-*ras* gene is particularly attractive for use in stool diagnostics because, when present, the mutations typically cluster in a few codons and are therefore relatively easy to detect.

Assay for mutated K-*ras* reveals a high proportion of carcinomas that themselves contain mutated K-*ras* (Hasegawa *et al.*, 1995; Nollau *et al.*, 1996; Ratto

et al., 1996; Sidransky *et al.*, 1992). Adenomas with mutated *ras* can also be detected in this way (Hasegawa *et al.*, 1995; Sidransky *et al.*, 1992). The sensitivity of the approach clearly depends on the methods used (Hasegawa *et al.*, 1995); sensitivity may be lower for lesions in the right bowel (Hasegawa *et al.*, 1995). Colonic effluent from bowel preparations has also been used as a source material (Tobi *et al.*, 1994), as have washings obtained during endoscopy (Smith-Ravin *et al.*, 1995). This technique revealed mutated *ras* in some patients with a history of colorectal neoplasia or with a family history, although colonoscopy disclosed no neoplasm (Tobi *et al.*, 1994; Villa *et al.*, 1996). Mutated *ras* has also been detected in the stool of patients with pancreatic cancer ductal hyperplasia (Caldas *et al.*, 1994).

Other molecular targets have also been described. A deletion in the *APC* gene has been detected in stool (Nollau *et al.*, 1996), but this issue has not been otherwise studied. The large size of the *APC* gene makes effective screening of this gene difficult in tumours, not to speak of stool. Similar considerations apply to *p53*. The amount of DNA on the surface of stool has also been investigated in one study: in individuals with colorectal cancer, a greater amount of DNA was detected (Loktionov *et al.*, 1998). The stool content of mRNA for CD44, a cell surface glycoprotein, has also been associated with large bowel adenocarcinoma (Yamao *et al.*, 1998).

Assessment of K-*ras* in stool has limited sensitivity for detection of large bowel neoplasia—the proportion of colorectal cancers that contain mutated *ras* is typically less than 50% (Andreyev *et al.*, 1998), although adenomas and aberrant crypt foci may have a higher prevalence of *ras* mutation (Jen *et al.*, 1994; Martinez *et al.*, 1999; McLellan *et al.*, 1993).

Mucosal proliferation

Mucosal proliferation is thought to play a prominent role in carcinogenesis, and consequently, has appeal as a biomarker. Mucosal proliferation in epithelial tissues was first measured using tissue explant culture of biopsy specimens, labelling dividing cells with tritiated thymidine, which is incorporated into DNA during S phase (Risio, 1994; Rozen, 1992). This technique suffers from the disadvantages of being time-consuming and requiring radioactive materials; and other, simpler labelling agents have superseded tritiated thymidine. One of these, bromodeoxyuridine, is also incorporated into DNA during S phase in tissue explant culture, but it can be recognized by immunohistochemistry, and so avoids the use of radioactive materials. Immunohistochemical detection of endogenous nuclear proteins, proliferating cell nuclear antigen and Ki-67 has also been used to assess proliferation, although these antigens are expressed over a wider range of the cell cycle than thymidine and bromodeoxyuridine (Biasco *et al.*, 1994; Risio, 1994). These techniques are relatively easy to use, since uptake of the label during explant culture is not required (Biasco *et al.*, 1994; Einspahr *et al.*, 1997; Risio, 1994; Rozen, 1992). The whole crypt mitotic count is another measure of proliferation for the bowel (Goodlad *et al.*, 1991; Murray *et al.*, 1995; Tosteson *et al.*, 1996). In limited studies, it did not show strong correlation with the labelling index (the proportion of cells in a crypt that are labelled) computed using proliferating cell nuclear antigen (Keku *et al.*, 1998; Murray *et al.*, 1995). Whole crypt production rate (Allan & Jewell, 1983) and flow cytometry (Nakamura *et al.*, 1995) have also been used in various studies.

There are clear impediments to the use of mucosal proliferation as a biomarker of risk or as an intermediate-effect biomarker. Application in epidemiological studies has been difficult, in part because of the demands on personnel to properly handle the biopsy specimens (Baron *et al.*, 1995a). Whatever the label, these techniques require manipulation of biopsy specimens in such a way that crypt architecture is maintained and then fixation until labelling and scoring. Scoring involves computation of the labelling index. Although measurement of proliferation indices can be reproducible (Bostick *et al.*, 1997a; Einspahr *et al.*, 1997; Lyles *et al.*, 1994), careful assessments of the variability of the measurements (Anti *et al.*, 1994a; Bostick *et al.*, 1997a; Lyles *et al.*, 1994; Macrae *et al.*, 1994; McShane *et al.*, 1998) have clearly identified the potential for measurement error and intra-subject variability.

Proliferation in the upper (luminal) parts of the crypt is a more sensitive marker of increased proliferation, and consequently the labelling index is also commonly computed in crypt compart-

ments (typically quintiles of cells by position from bottom to top of crypt (Rozen, 1992). There is only a weak correlation between the overall labelling index and the proliferation in the upper crypts (Bostick *et al.*, 1997a; Risio *et al.*, 1991). Although there are differences in the labelling indices generated by the various labels, in general they are highly correlated (Bostick *et al.*, 1997a; Diebold *et al.*, 1992; Earnest *et al.*, 1993; Kubben *et al.*, 1994; Lacy *et al.*, 1991; Richter *et al.*, 1992; Weisgerber *et al.*, 1993). Nonetheless, some investigators have found proliferating cell nuclear antigen to be a less effective label than others (Bromley *et al.*, 1996; Risio *et al.*, 1993). No consistent differences have emerged in proliferation measurements in various segments of the large bowel (Cats *et al.*, 1991; Terpstra *et al.*, 1987).

Many studies have demonstrated that colorectal carcinomas and adenomas exhibit a higher labelling index than normal-appearing mucosa (Kanemitsu *et al.*, 1985; Risio *et al.*, 1988, 1993; Shpitz *et al.*, 1997). Moreover, the normal mucosa of individuals with colorectal cancer or colorectal adenomas seems to have higher indices than the mucosa of unaffected individuals (Bleiberg *et al.*, 1985; Lipkin *et al.*, 1987; Paganelli *et al.*, 1991; Risio *et al.*, 1991; Roncucci *et al.*, 1991; Stadler *et al.*, 1988; Terpstra *et al.*, 1987). The proliferative zone also has been observed to shift towards the lumen (Ponz de Leon *et al.*, 1988; Risio *et al.*, 1991; Terpstra *et al.*, 1987; Wilson *et al.*, 1990). A few studies, however, found no association of proliferation with the presence of adenomas or cancer (Keku *et al.*, 1998; Jass *et al.*, 1997; Kashtan *et al.*, 1993; Nakamura *et al.*, 1995; Wong *et al.*, 1995). There has been only one published investigation of the relationship between proliferative indices and subsequent neoplasia: high indices were predictive of adenoma recurrence (Anti *et al.*, 1993).

Although data are not consistent, FAP patients may have increased proliferation indices (Deschner & Lipkin, 1975; Lipkin *et al.*, 1984; Mills *et al.*, 1995; Nakamura *et al.*, 1993), and there have been indications of increased proliferation in normal-appearing mucosa of affected and unaffected subjects in other types of high-risk families (Cats *et al.*, 1991; Gerdes *et al.*, 1993; Lipkin *et al.*, 1984, 1985; Lynch *et al.*, 1985; Patchett *et al.*, 1997). One small study found no evidence of hyperproliferative epithelium in patients with hereditary nonpolyposis colon cancer (HNPCC) (Jass *et al.*, 1997). Increased proliferation has also been observed in normal mucosa of patients with hyperplastic polyps (Risio *et al.*, 1995).

In some studies, proliferation has been seen to increase with age (Paganelli *et al.*, 1990; Roncucci *et al.*, 1998), but in several large investigations, no age association was seen (Bostick *et al.*, 1997b; Caderni *et al.*, 1999; Keku *et al.*, 1998). In observational studies, mucosal proliferation has not been consistently associated with intake of any nutrient (Bostick *et al.*, 1997 b; Caderni *et al.*, 1999; Keku *et al.*, 1998). However, there have been suggestions that high intake of red meat may be associated with increased proliferation (Bostick *et al.*, 1997 b; Caderni *et al.*, 1999).

There have been numerous clinical trials of dietary supplementation that studied proliferation as an end-point. In one trial, supplementation with wheat bran had no effect on proliferation (overall, or in the upper crypt) (Alberts *et al.*, 1997), although in a smaller study (that used crypt cell production rate), decreased proliferation was reported (Rooney *et al.*, 1994). In another crossover study, a low-fat diet in association with oat bran or wheat bran had no effect on overall labelling index or proliferation in the upper crypt (Macrae *et al.*, 1997). In a five-day study, a high-fat diet (with much of the fat given as a bolus) was associated with a higher overall labelling index than a lower-fat diet or a high-fat diet without the bolus (Stadler *et al.*, 1988). Fish oil (eicosapentaenoic and docosahexaenoic acids) decreased proliferation both overall and in the upper crypt (Anti *et al.*, 1992, 1994b), and subjects given a fish oil supplement had lower proliferation than subjects given corn oil (Bartram *et al.*, 1993). In another study, only subjects with initially high proliferation had a response to fish oil (Anti *et al.*, 1994b), suggesting a component of regression to the mean.

In one investigation, a combination of vitamin A, α-tocopherol and ascorbic acid reduced proliferation in the upper crypts (Paganelli *et al.*, 1992). In another study, vitamin C lowered proliferation in all crypt compartments, while β-carotene (9 mg per day for a month) significantly reduced it only in the lower parts of the crypt, and α-tocopherol had no effect (Cahill *et al.*, 1993). Another study found that supplementation with 30 mg β-carotene did not affect proliferation in any crypt

compartment (Frommel *et al.*, 1995), although an analysis, published only in an abstract, indicated a beneficial effect (lower proliferation) in the upper crypt (Macrae *et al.*, 1991).

Mucosal proliferation has been most intensively studied with regard to calcium supplementation. Here, the findings are quite mixed, as in the epidemiology of carcinoma and adenoma. Some studies have shown a decrease in proliferation with calcium supplementation (Barsoum *et al.*, 1992; Bostick *et al.*, 1995; Cats *et al.*, 1995; Lipkin & Newmark, 1985; Wargovich *et al.*, 1992), while others have shown no effect (Baron *et al.*, 1995b; Bostick *et al.*, 1993; Stern *et al.*, 1990; Weisgerber *et al.*, 1996) or even an increase (Gregoire *et al.*, 1989; Kleibeuker *et al.*, 1993). Two studies of high versus low consumption of dairy food also came to differing conclusions (Holt *et al.*, 1998; Karagas *et al.*, 1998).

Treatment with NSAIDs, consistently found to be inversely associated with risk of colorectal cancer and colorectal adenoma, appears to be unrelated to mucosal proliferation (Aoki *et al.*, 1996; Earnest *et al.*, 1993; Labayle *et al.*, 1991; Pasricha *et al.*, 1995; Spagnesi *et al.*, 1994). However, in one study of FAP, sulindac lowered the labelling index (Nugent *et al.*, 1993).

The observational epidemiology of large bowel mucosal proliferation is not well developed, but some published findings (NSAIDs, age) have diverged from clinical trial and epidemiological findings. However, in clinical trials, the mixed findings regarding calcium supplementation could be taken to reflect the weak findings in observational studies and the mixed epidemiological results.

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