REVIEW

Colorectal Cancer: Molecules and Populations

John D. Potter

The epidemiology and molecular biology of colorectal cancer are reviewed with a view to understanding their interrelationship. Risk factors for colorectal neoplasia include a positive family history, meat consumption, smoking, and alcohol consumption. Important inverse associations exist with vegetables, nonsteroidal anti-inflammatory drugs (NSAIDs), hormone replacement therapy, and physical activity. There are several molecular pathways to colorectal cancer, especially the APC (adenomatous polyposis coli)–β-catenin–Tcf (T-cell factor; a transcriptional activator) pathway and the pathway involving abnormalities of DNA mismatch repair. These are important, both in inherited syndromes (familial adenomatous polyposis [FAP] and hereditary nonpolyposis colorectal cancer [HNPCC], respectively) and in sporadic cancers. Other less well defined pathways exist. Expression of key genes in any of these pathways may be lost by inherited or acquired mutation or by hypermethylation. The roles of several of the environmental exposures in the molecular pathways either are established (e.g., inhibition of cyclooxygenase-2 by NSAIDs) or are suggested (e.g., meat and tobacco smoke as sources of specific blood-borne carcinogens; vegetables as a source of folate, antioxidants, and inducers of detoxifying enzymes). The roles of other factors (e.g., physical activity) remain obscure even when the epidemiology is quite consistent. There is also evidence that some metabolic pathways, e.g., those involving folate and heterocyclic amines, may be modified by polymorphisms in relevant genes, e.g., *MTHFR* (methylenetetrahydrofolate reductase) and *NAT1* (*N*-acetyltransferase 1) and *NAT2*. There is at least some evidence that the general host metabolic state can provide a milieu that enhances or reduces the likelihood of cancer progression. Understanding the roles of environmental exposures and host susceptibilities in molecular pathways has implications for screening, treatment, surveillance, and prevention. [J Natl Cancer Inst 1999; 91:916–32]

This review is divided into five parts; its primary purpose is to provide an overview of the epidemiology and molecular biology of colorectal cancer and to consider some of the links between the two. There is a brief recap of the descriptive epidemiology, which is followed by a more detailed consideration of major environmental risk factors. The third section outlines the inherited syndromes that carry a markedly elevated risk of colorectal cancer. A discussion of the role of high-prevalence genetic polymorphisms in metabolizing enzymes and the way in which these may interact with environmental exposures comprises the fourth section. The final section discusses some of the somatic genetic changes in relation to the environmental, genetic, and other host influences. In this review, gene names are italicized.

Descriptive Epidemiology

Colorectal cancer is the fourth most common incident cancer and the second most common cause of cancer death in the United States, with approximately 130 000 new cases and 55 000 deaths per year *(1).* Colon and rectal cancers share many environmental risk factors and are both found in individuals with specific genetic syndromes; however, there are some differences in etiology. Worldwide, an estimated 875 000 cases of colorectal cancer occurred in 1996, accounting for 8.5% of all new cases of cancer *(2).* Incidence rates vary approximately 20-fold around the world, with the highest rates seen in the developed world and the lowest in India *(3,4).* Colon cancer is the only cancer that occurs with approximately equal frequency in men and women *(5);* however, in high-incidence areas such as North America and Australia, as well as in Japan and Italy where rates are rising rapidly, rates in men now exceed those in women by as much as 20%. Rectal cancer is up to twice as common in men as in women. Five-year relative survival following diagnosis of colon cancer is around 55% in the United States *(6).* Rectal cancer may have a better overall survival where screening is more common.

The international differences, migrant data, and recent rapid changes in incidence rates in Italy, Japan, urban China, and male Polynesians in Hawaii *(3,4)* show that colon cancer particularly is highly sensitive to changes in the environment. Among immigrants and their descendants, incidence rates rapidly reach those of the host country, sometimes within the migrating generation *(7,8).* The 20-fold international difference may be explained, in large part, by dietary and other environmental differences; indeed, although incidence rates in Japan have been low even until quite recently, the highest rates in the world are now seen among Hawaiian Japanese *(3).* However, colorectal cancer has long been known to occur more frequently in certain families *(9),* and there are several rare genetic syndromes that carry a markedly elevated risk *(10–12).* Colorectal cancer is thus causally related to both genes and environment.

Individual-Level Epidemiology

Diet, Nutrients, and Foods

Most of the caveats regarding the interpretation of dietary data are well known and will not be repeated here. For extensive treatment of those issues, the reader is referred to the publication by Willett *(13)* and the World Cancer Research Fund (WCRF) report *(14).* We have recently reviewed both case–control and cohort studies of the associations between dietary factors and

Correspondence to: John D. Potter, M.D., Ph.D., Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N. MP702, P.O. Box 19024, Seattle, WA 98109-1024 (e-mail: jpotter@fhcrc.org).

See "Note" following "References."

risk of colorectal neoplasia *(14–16),* and the reader is referred to those publications for many of the relevant citations and for more detail. The major findings are provided here, along with the most recent publications. As a central underpinning to the interpretation of data, the reader is also referred to criteria for drawing causal inferences from epidemiologic studies, first articulated by Hill *(17).* Discussion of causality specifically in relation to diet and cancer is found in chapter 3 of reference *(14).*

Vegetables, fruits, fiber, and micronutrients. There are at least seven published cohort studies of colorectal neoplasia and consumption of vegetables and fruits. Four studies [*see* references *(14–16)*] examined vegetable and fruit consumption and colon cancer risk and reported modest (and not entirely consistent) findings of lower risk in association with higher consumption. One study reported on rectal cancer in Seventh-day Adventists, showing a small, statistically nonsignificant reduction in risk with higher consumption of green salad. The most recent cohort study of Seventh-day Adventists *(18)* notes a somewhat lower risk of colorectal cancer with higher intake of a variety of plant foods (statistically significant only for legumes). One study of adenomatous polyps in men *(19)* reported an approximate halving of risk with higher intake of vegetable and fruit fiber.

There are at least 21 case–control studies of colon cancer and vegetable and fruit consumption, and 17 of these studies reported some degree of reduced risk with higher consumption of at least one category of vegetable or fruit *(14,15).* Decreased risks of colon cancer have been particularly consistent for raw vegetables, green vegetables, and cruciferous vegetables. A meta-analysis of six case–control studies of vegetables and colon cancer *(20)* found a combined odds ratio (OR) of 0.48 (95% confidence interval [CI] = 0.41–0.57) for highest versus lowest quintiles of consumption. There are 13 case–control studies of rectal cancer; 10 reported on statistical significance and, of these 10 studies, eight showed a significant inverse association for at least one vegetable or fruit category. Results were most consistent for cruciferous vegetables. Each of five case–control studies of adenomas that examined vegetables as a broad category found an inverse association that was, however, not always statistically significant *(14,15).* Studies of fruit consumption and colorectal cancer risk are less abundant, and the findings are less clear than those for vegetables.

Foods high in fiber include vegetables as well as grains. A role for dietary fiber in colon carcinogenesis was first proposed by Burkitt *(21).* Data from cohort studies were weakly supportive of the fiber hypothesis, with two studies finding no association and two finding a weak inverse association. The most recent cohort study of total dietary fiber *(22)* found no association with either carcinoma or adenoma in women. Only one prospective study has provided data on rectal cancer; there was little evidence of an association. A combined analysis of 13 case–control studies found a reduction in colorectal cancer risk with increasing intake of dietary fiber *(23).* Similar findings have been reported in a meta-analysis of 16 case–control studies *(20).* Fiber from vegetables and cereals, in a prospective study *(19),* has been associated with a more than halving of risk for colorectal adenomas in men. Case–control studies *(14)* have found inverse associations with total fiber, fiber from cereals, and fiber from vegetables and fruits.

There are no clearly supportive data from intervention trials in patients with familial adenomatous polyposis (FAP) (except in a *post-hoc* analysis of adherent individuals) *(24),* and fiber had no statistically significant effect on the occurrence of metachronous sporadic adenomas *(25).*

Overall, the relationship between fiber intake and risk of colorectal cancer, although often inverse, is somewhat inconsistent, not the least because of the heterogeneous nature of fiber and differences in the way in which fiber is measured *(26);* nonetheless, the limited data on humans do not suggest a stronger effect for any source or type of fiber. The WCRF report *(14)* concluded, "evidence that diets rich in vegetables protect against cancers of the colon and rectum is convincing." It was also concluded *(14),* "diets high in fiber possibly decrease the risk of colorectal cancer" but "the data on fruit are more limited and inconsistent; no judgement is possible."

Other nutrients have been invoked to explain the reduced risk of colorectal cancer in association with consumption of vegetables *(16,27).* Many of these nutrients are not readily measurable in epidemiologic studies, but several micronutrients have been reported on (including carotenoids, ascorbate, and folate). The reader is referred to the WCRF report *(14)* for more detail.

Freudenheim et al. *(28),* who first proposed the folate–colorectal cancer hypothesis, found lower risks of both colon and rectal cancers in association with high folate intakes in their case–control study. Total folate intake and dietary folate were not associated, however, with differences in risk of colon cancer in a cohort of men *(29).* Nonetheless, an increase in risk of colon cancer was seen among men with low intakes of folate and methionine and high intakes of alcohol. Similar results were shown for adenomatous polyps in the same cohort study *(30).* In a large multicenter case–control study, Slattery et al. *(31)* found no association between micronutrients involved in methyl-group metabolism and risk of colon cancer. The role of genetic variability in folate metabolism is discussed below in the section entitled "Genetic Predisposition—High-Prevalence Polymorphisms." Overall, the data suggest that the relationship between risk of colorectal cancer and intake of vegetables is more consistent (as well as having a considerably larger literature) than any of the specific micronutrients.

Meat and fat. To mid-1997, seven cohort studies (reported in eight papers) had examined meat intake and the risk of colorectal neoplasia. *See* references *(14,15)* for original citations and greater detail. A study of Seventh-day Adventists—a predominantly vegetarian population—reported that meat intake was not associated with risk of colorectal cancer. The Nurses' Health Study reported that women who consumed red meat frequently compared with women who consumed red meat rarely had a statistically significant 2.5-fold increase in risk of colon cancer. Men who consumed five or more servings per week of beef, pork, or lamb had a moderately increased risk of colon cancer when compared with men who consumed these meats less than once per month. In contrast, the American Cancer Society cohort showed no difference in risk between uppermost and lowest quintiles of meat consumption in either sex. The Iowa Women's Health Study and The Netherlands and Finnish cohorts also showed no increase in risk with meat consumption. Two of the four cohort studies that examined intake of processed meat showed statistically significant higher risks of colorectal cancer with higher consumption, and one showed a weakly elevated risk. Only one study was null. Thus, there are only studies showing increased risk and those that are null. As the WCRF report *(14)* notes, of the 16 estimates of relative risk associated with meat consumption reported in these cohort studies, eight were

greater than 1.5 or statistically significantly greater than 1.0, eight were between 0.75 and 1.5, and none was less than 0.75 or statistically significantly less than 1.0; i.e., the weight of the evidence points to an elevated risk, even though the findings are neither strong nor consistent. A cohort study among New York women *(32)* did not find an association with meat consumption. Most recently, two other cohort studies *(18,33)* focused on meat consumption among low-consuming populations. One study *(33)* found no association with colorectal cancer mortality, although the researchers were able to detect an association between meat consumption and coronary heart disease. The other study *(18)* found an elevated risk in Seventh-day Adventists in association with higher consumption of both red meat and white meat.

In the literature to date, there are at least 26 case–control studies of colorectal neoplasia (23 of cancer and three of adenomatous polyps). As with the cohort studies, almost all estimates of risk are increased or null with higher intake of meat. Each of 16 studies conducted in Europe, South America, Australia, and the United States reported one or more statistically significant elevated ORs with higher consumption of meat; *see* references *(14,15)* for original citations and greater detail. Some of these elevated risks were specific to, or more marked in, women; some findings were more obvious for the rectum than for the colon. Two other studies in Australia and the United States showed weak increases in risk with higher intakes of meat. Seven of the 26 studies were essentially null. Again, as noted in the WCRF report *(14),* among the 86 estimates of risk associated with these studies, 47 were greater than 1.5 or statistically significantly greater than 1.0, 31 were between 0.75 and 1.5, and eight were less than 0.75 or statistically significantly less than 1.0 (of these eight, only five were statistically significantly less than 1.0 and the other three were associated with pork in studies in Australia and Belgium); these last three studies showed statistically significant increases in risk with higher consumption of beef. The other two findings of reduced risk with higher consumption came from the study in China and were seen for rectal, but not for colon, cancer. Five of the studies that reported on processed or cured meats found statistically significantly elevated risks, whereas the other four showed no association.

Gerhardsson de Verdier et al. *(34)* reported an OR for colon cancer of 2.7 (95% CI = 1.4–5.9) for the most frequent consumers of fried meat with a heavily browned surface; they reported a higher risk (OR = 6.0; 95% CI = 2.9–12.6) for rectal cancer. Schiffman and Felton *(35)* also reported a 3.5-fold increase in risk for those preferring well-done meat. Comparable findings have been seen in a case–control study of adenomatous polyps *(36).* Heterocyclic amines are produced when meat is cooked. These compounds and their genetically variable metabolism are discussed below in the section entitled "Genetic Predisposition—High-Prevalence Polymorphisms."

Whether the weaker findings in the cohort studies compared with the case–control studies are due to selection or recall bias in the latter or increasing time from baseline in the former remains to be resolved *(37).* It is not yet clear whether the risk that is observed involves animal fat (the epidemiologic data are discussed below), processing, or cooking methods.

As with the exploration of constituent nutrients in vegetables, epidemiologic studies have been undertaken to explore the association between risk of colorectal cancer and some of the important measurable nutrients found in meat (including fat, saturated/animal fat, protein, and iron).

The large majority of the case–control studies of fat reported increased risks in association with higher intakes; ORs ranged from 1.3 to 2.2. Again, *see* references *(14,15)* for original citations and greater detail. Eleven studies attempted to distinguish fat from total energy intake. Four of the five cohort studies found no association, whereas the Nurses' Health Study reported that total fat intake in the uppermost versus the lowest quintile was associated with a twofold increased risk of colon cancer. Results from five case–control studies that adjusted for energy intake were mixed; three studies found no association, and two found statistically significant increases in risk. A cohort study of colorectal cancer among Hawaiian-Japanese men reported decreased risk with higher intakes of total fat.

Howe et al. *(38)* reported on a combined analysis of 13 case–control studies of colorectal cancer, involving 5287 case patients and 10 478 control subjects from various populations with differing cancer risks and diets. There was no evidence of any increased risk with any dietary fat variable after adjustment for total energy intake. Furthermore, there were no statistically significant associations for any type of fat in subgroup analyses by sex, age, or anatomic location of the cancer.

Thus, recent cohort studies and the combined analysis of 13 case–control studies have failed to find clear evidence for the association of colorectal cancer with dietary fat intake observed in many early studies.

Five cohort studies have reported specifically on saturated or animal fat. One study reported that high intake of saturated fat was associated with a reduced risk of colon cancer and a moderately increased risk of rectal cancer. In contrast, the much larger Nurses' Health Study of women found that those with the highest intakes of animal fat were at almost twofold greater risk than those with the lowest intakes. Weaker increases in risk were seen with high consumption of saturated fat. A small increase in risk for colon cancer was reported for women but not for men in The Netherlands cohort. No substantial associations between saturated or animal fat were seen in two other cohort studies. Fourteen case–control studies have examined associations between intakes of saturated and/or animal fat and the risk of colon, rectal, or colorectal cancer; results from these studies were inconsistent.

In summary, of the 19 cohort and case–control studies, 11 showed some evidence of elevated risk associated with higher intakes of saturated/animal fat, two showed weak inverse associations, and six show no association. Thus, while the evidence is consistent with a stronger risk with saturated/animal fat than with total fat, the data are not entirely convincing. Giovannucci and Goldin *(39)* concluded that the association with red meat consumption does not appear to be mediated by its lipid content. Evidence for explanations via protein and iron appears weaker still *(14).*

Overall, the data suggest a stronger association of colorectal cancer with meat than with any of the associated nutrients and that, while both processing of meat and saturated/animal fat are possibly associated with an increased risk of colorectal cancer, neither total fat nor total protein seems to play a major role.

Calcium. The association between higher intake of calcium and colorectal neoplasia has been explored in both cohort and case–control studies [*see* references *(14,15)*]. The results are tantalizing, but not consistent, although most of the evidence

suggests a reduced risk or no association. There are intervention data to show that calcium reduces proliferation in the upper part of the colonic crypt *(40)* and observational data that calcium reduces the likelihood of metachronous adenomas *(41)*. Most recently, a double-blind, placebo-controlled intervention trial *(42)* showed a statistically significant 15%–20% reduction in the incidence of metachronous colorectal adenomas.

Other diet- and food-related variables. Some other food and dietary-behavior variables are, to varying degrees, associated with increased risk of colorectal neoplasia (e.g., egg consumption, sugar intake, and frequency of eating), and some are associated with possibly decreased risk of colorectal neoplasia (e.g., complex carbohydrate, vitamin D, and vitamin E). *See* references *(14,15)*.

Physical Activity and Body Mass

Physical activity. The relationship between physical activity and a reduced risk of colon cancer is among the most consistent findings in the epidemiologic literature, reported in studies of occupational activity, leisure activity, and total activity. Again, *see* references *(14,15)* for original citations and greater detail. Of nine cohort studies, only two have reported no substantial association. Case–control studies were also consistent: Of 11 studies, only one study noted an increased risk of colon cancer with higher total activity; the remaining studies showed inverse associations. Individuals with high levels of activity throughout their lives were found to have the lowest risk, whereas those who reported high levels of activity were more recently reported to show weaker inverse associations. There is little evidence that physical activity modifies rectal cancer risk.

Body mass. Four cohort studies and eight case–control studies have found that men who are in the highest quintile for body size, classifiable as obese, have as much as a twofold increased risk of colon cancer *(14,15,18)*. In contrast, one cohort and four case–control studies have shown no association between body mass index (BMI), i.e., weight in kg/height in m^2, and colon cancer risk in men *(14,15)*. Data on women are more inconsistent. Two cohort studies have reported no association between BMI and risk of colorectal cancer. However, the Iowa Women's Health Study showed that subjects who were in the highest quintile of BMI had a statistically significant 40% higher risk than those who were in the lowest quintile. Three case–control studies have also reported inconsistent findings for women.

A large U.S. multicenter case–control study (approximately 2000 case patients and 2400 control subjects) noted that, while BMI was not associated with elevated risk at high levels of long-term vigorous physical activity, risk appeared to be related both to total energy intake and to BMI at lower levels of such activity. The OR for those who were least active, had the highest energy intake, and the highest BMI was 3.4 (95% CI = 2.1–5.4) compared with the opposite extreme. The association was explained solely by the findings for men, in whom the OR for a comparison of the extremes was 7.2 (95% CI = 3.4–5.2); there was little association in women *(43)*. Waist-to-hip ratio was associated with increased risk in men *(44)* but not in women *(45)*.

Overall, the evidence suggests that obesity may increase the risk of colon cancer (particularly in men) but, as with physical activity, obesity does not appear to influence risk of rectal cancer.

Reproduction and Exogenous Hormone Use

In 1969, Fraumeni et al. *(46)* noted that nuns experienced an excess not only of known hormone-associated cancers but also of colon cancer. Several case–control studies in the 1970s noted, but did not explain, a higher risk of colon cancer among nulliparous women. In 1980, we *(5)* presented a hypothesis (based on these studies, on the known sex differences in colon cancer incidence, on international changes in fertility, and on animal data) that higher parity, early age at first birth, and use of oral contraceptives would each be associated with a reduced risk of colon cancer, largely as a result of changes in lipids and bile acids that occur with changes in the hormonal milieu. There is now an additional hypothesis to explain the role of hormones in colon cancer *(47,48)*, which will be discussed later in the section entitled "Somatic and Genetic Changes and Mechanisms—Integrating the Data."

More than 20 epidemiologic studies have reported on reproduction and colon cancer. Overall, it appears that age at first birth is not associated with colon cancer risk; the conservative interpretation of the parity data is similar, especially given that all the cohort studies show no association; *see* reference *(15)* for original citations and greater detail. Nevertheless, the differences between the findings of the cohort and population-based, case–control studies remain to be explained, as does the role of age at clinical presentation *(37)*.

The first investigation of a relationship between hormone replacement therapy (HRT) and colorectal cancer risk was published in 1981 by Weiss et al. *(49)*, who reported a null association with colon cancer. In 1983, we *(50)* reported a statistically significant lower risk of colon cancer with use of the high-estrogen oral contraceptives then available and a marginally (and nonsignificantly) decreased risk (OR = 0.8; 95% CI = 0.4–1.5) with non-oral contraceptive hormone use. Since that time, there have been 15 other studies *(45,51–64)*. Findings among these studies are not entirely consistent; among the 12 that focused on colon cancer or provided separate data on colon cancer, seven studies *(57,58,60–64)* showed statistically significant lower risk with HRT or a less well specified hormone variable, two studies *(45,50)* showed nonsignificantly lower risks, two studies *(55,59)* were null, and one study *(56)* showed elevated risks among users.

The papers by Newcomb and Storer *(62)* and Kampman et al. *(63)* provide typical findings. Consistent with other recent observations, these papers reported an approximate halving of risk with recent HRT use. This degree of risk reduction was maintained for about 10 years after cessation of use. Data on duration of use suggest that longer use is associated with lower risk. We have shown *(48)*, as have others *(65,66)*, that a similar pattern of association exists between HRT and risk for adenomatous polyps of both the colon and the rectum.

Family History

Individuals with a family history of colorectal cancer are themselves at increased risk, not only as a result of the rare high-risk syndromes *(10,12)* but also more generally in the population, with a risk approximately twofold that of individuals without a family history of colorectal cancer *(9,67–71)*. For a discussion of known genetic syndromes with a markedly elevated risk of colorectal cancer, *see* the section below entitled "Genetic Predisposition—Inherited Syndromes." Inherited en-

zyme polymorphisms may also carry an excess risk—*see* "Genetic Predisposition—High-Prevalence Polymorphisms"—but probably do not contribute to a positive family history (Slattery ML, Edwards SL, Samowitz W, Potter JD: manuscript submitted for publication).

Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

NSAIDs, including aspirin, have been consistently associated with a reduced risk of colorectal cancer. At least seven case–control studies of aspirin *(72–78)* have shown a lower risk of colorectal cancer; four cohort studies have reported lower risk *(79–81)* or lower mortality *(82).* An inverse association with adenomatous polyps was also seen with regular aspirin use *(74,80,83,84).* Sulindac was reported to induce regression of adenomas in patients with FAP *(85).* In minor contrast, one cohort study *(86)* and one low-dose aspirin intervention study, analyzed both as an intervention trial *(87)* and at later follow-up *(88),* showed null results. In rodents, aspirin *(89),* indomethacin *(90,91),* sulindac *(92),* piroxicam *(93),* and celecoxib (a specific cyclooxygenase-2 [COX-2] inhibitor) *(94)* inhibited carcinogenesis.

Smoking

Smoking cigarettes has not often been associated with an elevated risk of colorectal cancer, although greater risk associated with smoking cigars and pipes has been described *(95,96).* Several studies *(97–100),* however, have noted a higher risk for colon cancer among cigarette smokers, especially among those with very long smoking histories. Furthermore, essentially every study that has examined the association between cigarette smoking and adenomatous polyps has reported an elevated risk *(97,98,101–113).* Most recently, interest has focused on the possibility that risk associated with smoking is modified by polymorphisms in metabolizing enzymes (*see* section below entitled "Genetic Predisposition—High-Prevalence Polymorphisms").

Alcohol

In 1957, Stocks *(114)* first reported a marginally elevated risk of colorectal cancer among daily beer drinkers compared with abstainers. Subsequently, this association with cancers of the large bowel has been explored in many other studies. *See* references *(14,15)* for original citations.

Six cohort studies of alcoholics have compared their cancer mortality experience with the cancer mortality experience of the appropriate general population. None of these studies found statistically significant elevations in colon or rectal cancer mortality. Five general population cohort studies have reported on colon cancer; four showed statistically significant associations with alcohol consumption, as did each of the three that explored rectal cancer risk and two of the three studies that reported on colorectal cancer. Eighteen case–control studies have examined alcohol consumption and colon cancer; alcohol consumption was associated with increased risk in nine. Nine of 17 studies of rectal cancer reported similarly elevated risks.

Studies of both colon and rectal cancers have shown a more consistently elevated risk among men than among women, perhaps because of the generally lower consumption of alcohol among women. There is no strong evidence to suggest that one source of alcohol (e.g., beer) is more associated with risk than alcohol as a whole. Indeed, the WCRF report *(14)* concluded, "high alcohol consumption probably increases the risk of cancers of the colon and rectum" and the association is likely to be "related to total ethanol intake, irrespective of the type of drink." As already noted, there are studies showing both increased risks and no association, but there are essentially no studies that show a reduced risk with higher intake. The inconsistencies may be the consequence of small sample sizes; of differences in control groups, study methods, or preferred beverages between the sexes and across countries; or, possibly, of population differences in susceptibility to, and metabolism of, alcohol.

Genetic Predisposition—Inherited Syndromes

Familial Adenomatous Polyposis (FAP)

FAP is a rare autosomal dominant syndrome *(10)* caused by an inherited mutation in the *APC* gene. Localization of the gene was independently accomplished in 1987 by Leppert et al. *(115)* and Bodmer et al. *(116);* the gene was mapped to chromosome 5q and subsequently cloned and sequenced *(117).* The population frequency of truncated forms of *APC* has been estimated at one in 10 000, and lifetime penetrance was, until recently, thought to approach 100%. In the mouse, however, there is clear evidence of modification of penetrance by other genes (*see below*); it remains to be established whether this is true in humans. The disease is characterized by the development, sometimes from childhood, of multiple colorectal adenomas, numbering from a few polyps to several thousand. There are also extracolonic manifestations. Left untreated, one or more of the polyps will progress to cancer at a mean age of 44 years—approximately 20 years earlier than the appearance of cancers among unaffected individuals. The mutations described so far in FAP families are at different sites within *APC,* but almost all lead to stop codons and thus a truncated APC protein *(118,119).* A nontruncating polymorphism of *APC* has been shown to be more prevalent among the Ashkenazim and to carry a modestly elevated risk of colorectal cancer *(120).* Mutations at the *APC* locus are a common and early somatic event in polyps and cancer *(121);* i.e., for some individuals the first hit is the germline mutation, whereas for others it is a somatic event. *APC* can also be silenced by hypermethylation *(122).*

There are several variants of FAP. They include the following: Gardner's syndrome, in which specific extracolonic manifestations are expressed, e.g., osteomas, skin fibromas, and epidermoid cysts *(123);* Turcot's syndrome, which includes medulloblastoma *(124);* and the attenuated form of FAP, in which many fewer polyps are found *(125).* There are correlations between *APC* mutation sites and the variant phenotypes, e.g., attenuated FAP where the mutations are at the 5′ end of the gene *(126),* the profuse polyposis syndrome where the inherited mutation normally occurs between codons 1285 and 1465 *(127),* the appearance of congenital hypertrophy of the retinal pigment epithelium (CHRPE) with mutations at codons 542–1309 *(128),* and a multiplicity of extracolonic manifestations with mutations at codons 1465, 1546, and 2621 *(128).* Nonetheless, the same mutation can be associated with different phenotypes, even within a single family *(129).*

This variation in the phenotype raises issues about other modifiers—both genes and environment. For carriers of a mutated *APC,* there are data to support other genetic as well as environmental influences on the phenotype. Backcrosses using the MIN (multiple intestinal neoplasia) mouse (*APC*+/−) pro-

vided evidence of the gene *Mom-1* (modifier of min) that attenuates the phenotype *(130).* (Although the human homologue of the gene—a secretory phospholipase—has been identified, there is no clear link to the manifestation of polyps in FAP families or to colon cancer more generally.) These experiments with *APC* and *Mom-1* were done using standard mouse genetics. More recently, three other genetic modifiers have been identified in studies using transgenics. Both DNA methyltransferase *(131)* and *COX-2* knockouts *(132)* crossed with *APC*+/− mice show markedly attenuated phenotypes; in contrast, the cross with the *Smad4* knockout shows a more severe phenotype *(133).* These experiments also provide some evidence for the importance of DNA methylation (which is markedly abnormal in both inherited and sporadic colon cancers and can be influenced by dietary factors, especially folate) and of the role of COX-2 (which can be inhibited by aspirin and other NSAIDs). Indeed, there is, as noted above, a large body of evidence to show that aspirin and other NSAIDs are associated with a reduced risk of sporadic colon cancer and can reduce the number of polyps in the rectum of patients with FAP.

In general, this finding suggests that, although inheriting a mutated copy of *APC* is associated with a highly penetrant phenotype, there are, nonetheless, both genetic and environmental influences that modify that penetrance. Even if surgery remains the only treatment for FAP patients for the foreseeable future, such findings have implications both for the management of FAP patients following colectomy (the rectum remains prone to polyp formation) and for the prevention of the sporadic disease. Some of the known functions of the APC protein will be discussed below in the section entitled "Somatic and Genetic Changes and Mechanisms—Integrating the Data."

Hereditary Nonpolyposis Colorectal Cancer (HNPCC)

In 1913, Warthin *(134)* described several different family cancer syndromes. In one of these families, family G, the initial history was almost entirely of gastric and uterine cancers. This family, particularly in the most recent generations, has a very high incidence of colorectal cancers and is archetypical of HNPCC families.

HNPCC is an inherited autosomal dominant syndrome *(135),* with a much less well defined phenotype than FAP, not easily distinguished from "sporadic" polyposis and cancer on physical examination (there is no tendency to extensive polyposis). Although there were initial claims that it accounts for about 15% of colorectal cancer cases, recent studies *(136,137)* suggest that this proportion may be around 2% or less. The most clearly distinguishing features of the family history are the tendency to early onset and a pattern of other cancers—particularly those involving the endometrium, urinary tract, stomach, and biliary system *(138).* There have been several attempts to provide a sensitive and specific definition of the syndrome. The Amsterdam criteria *(139)* (Table 1) are, on the basis of molecular diagnosis, overly restrictive. A National Cancer Institute workshop *(140)* expanded these clinical criteria (Bethesda criteria; Table 1). The clinically defined history can be confirmed by examining the tumor for microsatellite instability or by testing for germline mutations in a family of genes that are involved in DNA mismatch repair (MMR) (*see below*). Microsatellite instability, while common in HNPCC tumors, is nonetheless neither sensitive nor specific. It is unclear whether survival of HNPCC patients is better than *(141)* or essentially similar to *(142)* that of patients with sporadic tumors.

Table 1. Criteria for the clinical diagnosis of hereditory nonpolyposis colorectal cancer (HNPCC)

Amsterdam criteria (1991)
1) Three cases of familial colorectal cancer in which two of the affected individuals are first-degree relatives of the third.
2) Colorectal cancers occurring across two generations.
3) One colorectal cancer diagnosed under age 50 years.

Bethesda criteria (1997)
1) Amsterdam criteria individuals.
2) Individuals with two HNPCC-related cancers: synchronous/metachronous colorectal cancers; endometrial, ovarian, gastric, hepatobiliary, small intestine, or renal tract transitional cell cancers.
3) Individuals with colorectal cancer and a first-degree relative with one or more of the following:
   (a) Colorectal cancer diagnosed under 45 years
   (b) HNPCC-related cancer diagnosed under 45 years
   (c) Adenoma diagnosed under 40 years
4) Individuals under 45 years of age with colorectal or endometrial cancer.
5) Individuals with proximal cancer of undifferentiated type.
6) Individuals under 45 years of age with signet-ring cancer.
7) Individuals under 40 years of age with adenomas.

The DNA MMR system identifies and repairs, in a strand-specific manner, errors that result from the activity of DNA polymerase during replication. Runs of repeats, e.g., dinucleotide repeats such as $(CA)_n$, are prone to slippage during replication. The MMR system (involving a complex set of proteins) recognizes the mismatch, binds to it, excises the mismatched region, and facilitates the resynthesis of the correct sequence *(143).* Microsatellite instability is a characteristic feature of genomes that show defects in MMR from bacteria to humans and was recently recognized in colorectal cancers *(144,145).* The human homologue of one of the known bacterial MMR genes (*MutS*) was identified on chromosome 2p *(146–148).* It was the first gene shown to be mutated in the germline of an HNPCC family (*hMSH2*—a human *MutS* homologue). Subsequently, a homologue of the bacterial *MutL* gene (*hMLH1*) was identified on chromosome 3q *(149,150).* Mutations in these two genes account for a large majority of the HNPCC families identified to date *(151).* There are several other homologues of the *MutL* and *MutS* genes, two of which (*MutL* homologues: *hPMS1* and *hPMS2*) account for a small proportion of HNPCC families. One other *MutS* homologue, *GTBP/hMSH6*, has been reported to be mutated in HNPCC *(152);* others (e.g., *hMSH3*) have not.

The loss of MMR has several consequences, most crucially loss of proofreading and correction of small deletions and insertions *(153).* Furthermore, colorectal cell lines deficient in MMR show generally higher accumulation of other mutations and deletions *(154).* As with the other major gene *APC,* somatic mutation *(155)* or hypermethylation *(156,157)* of MMR genes is also a pathway to colorectal cancer.

The steps in the molecular pathogenesis of colorectal cancer that involve either germline or somatic silencing of *APC* or MMR genes will be discussed in the section below entitled "Somatic and Genetic Changes and Mechanisms—Integrating the Data."

Genetic Predisposition—High-Prevalence Polymorphisms

N-Acetyltransferases (*NAT1, NAT2*) and Cytochrome P450 (*CYP*) Enzymes

Meat consumption is associated with increased risk of cancer, and perhaps this is particularly true of heavily cooked meat. Heterocyclic amines may be colorectal carcinogens. Together, these observations provoked the question as to whether the metabolism of heterocyclic amines, which may be altered by genetic variability of at least three relevant enzymes (NAT1, NAT2, and CYP_{1A2}) might influence the risk of colorectal neoplasia *(158,159).* The evidence is somewhat mixed, with the earlier small studies suggesting that the genetic polymorphisms themselves *(160)* may be associated with risk or that there may be an interaction with meat consumption *(161).* Not all of the mechanisms and genetic data are consistent *(162).* The larger and more recent studies do not support an independent role for *NAT2* with cancer *(163–166)* or polyps *(113,167)* and, although suggestive of an interaction with meat or tobacco smoke *(163,164),* are not consistent *(113,166).* Results for *NAT1* are also not consistent and suggest that there is no independent association with genotype *(163,168–170).* Combinations of rapid *NAT1, NAT2,* and perhaps CYP_{1A2} genotypes may be associated with elevated risk in the presence of tobacco smoking or high intake of meat *(163).* One important problem is that, although CYP_{1A2} is phenotypically variable, the locus of the genetic variation remains to be identified.

Methylenetetrahydrofolate Reductase (MTHFR)

Vegetables are associated with a decreased risk of colorectal neoplasia. One of the postulated mechanisms is that folate, central to methyl-group metabolism, may influence both methylation of DNA and the available nucleotide pool for DNA replication and repair. As noted above, there is some evidence, albeit inconsistent, that folate and vitamin B_{12} (a cofactor in this pathway) are associated with a reduced risk of colorectal neoplasia *(28–31).* There is a growing body of evidence to suggest that MTHFR, a polymorphic enzyme, influences that association such that those at highest risk for both adenomas and cancer have the variant (TT) genotype and low intakes of folate and vitamin B_{12} [*(171,172);* Ulrich CM, Kampman E, Bigler J, Schwartz SM, Chen C, Bostick R, et al.: manuscript submitted for publication].

Nonetheless, the complete story is more complex than initially thought because those with the variant (TT) genotype in the presence of an adequate folate and B_{12} intake have, if anything, a somewhat reduced risk of colorectal neoplasia [*(171);* Ulrich CM, Kampman E, Bigler J, Schwartz SM, Chen C, Bostick R, et al.: manuscript submitted for publication]. Perhaps the fact that MTHFR is at the "crossroads" between methyl-group transfer and the manufacture of nucleotides ensures that risk of carcinogenesis increases only when both a deficiency of folate and a variant genotype are present (Ulrich CM, Kampman E, Bigler J, Schwartz SM, Chen C, Bostick L, et al.: manuscript submitted for publication). This combination results in limited transfer of methyl groups as well as abnormalities of DNA repair, including uracil misincorporation. Conversely, under conditions characterized by high intakes of folate/B_{12}, among individuals with a variant genotype, methionine synthase functions at full capacity and effectively utilizes the 5-methyltetrahydrofolate that is the product of MTHFR, thus resulting in adequate quantities of *S*-adenosylmethionine (SAM), an increased pool of 5,10-methylenetetrahydrofolate, and enhanced nucleotide production. The overall result is plausibly a decreased risk of colonic neoplasia (Ulrich CM, Kampman E, Bigler J, Schwartz SM, Chen C, Bostick R, et al. manuscript submitted for publication). The final complexity arises because both general hypomethylation of DNA and hypermethylation of specific gene promoter regions are characteristics of colorectal cancer (*see below*).

Somatic and Genetic Changes and Mechanisms—Integrating the Data

Microarchitecture of the Large Bowel

The microarchitecture of the colon is characterized by crypts that are approximately 50 cells deep. In the small bowel, crypts and villi provide a very large surface area for nutrient absorption. In contrast, there is no need for a large surface area in the large bowel where little other than water is reabsorbed. Other pressures almost certainly account for the evolutionary persistence of colonic crypts. The most likely explanation is protection of the crypt progenitor cells from the very mutagenic environment of the colonic lumen. The structure of the crypt and the dynamics of cell replication ensure that, under normal circumstances, both the crypt stem cells and the immediate daughter cells replicate in the lowest one third, giving rise as they divide and then migrate to all cells that line the crypt *(173).* This location, coupled with the generally outward/upward pressures exerted by secreted mucus, ensures that interaction between colonic contents and replicating cells is essentially nonexistent. By the time that normal crypt cells reach the surface, they are not replicating, are differentiated, and may be beginning to undergo apoptosis (i.e., programmed cell death). Therefore, any mutagenic events in these cells have essentially no impact on the integrity of the crypt cell population.

This pattern of events has two consequences. First, in order that there is a population of mutated cells that subsequently gives rise to an adenoma, a stem (or very early daughter) cell must undergo the first hit. This first hit, because of the microarchitecture, is most plausibly blood-borne rather than luminal. A mutation in a progenitor cell means that there is a replicating population of cells with an abnormal phenotype, thus increasing the odds that one will undergo a second hit. The second probable consequence of the microarchitecture is that, in order for the colonic contents to play a role in colon carcinogenesis, there must be replicating abnormal cells either protruding into the lumen, i.e., a polyp, or at least in potential contact with the fecal stream, i.e., a microadenoma. It is now known that a single hit, namely, a mutation in *APC,* creates all the conditions—abnormalities of adhesion, migration, and replication—to grow a polyp (*see below*). Fig. 1 illustrates the relevant steps.

The molecular steps have been elucidated, but evidence to support the importance of blood-borne agents first and luminal agents later is currently circumstantial because organotypic cultures of colon cells have been, at best, only partially successful *(174).* It is known that aberrant crypt foci or microadenomas occur frequently *(175,176)* and that individual adenomas are premalignant but only relatively rarely develop into cancers—even in FAP patients.

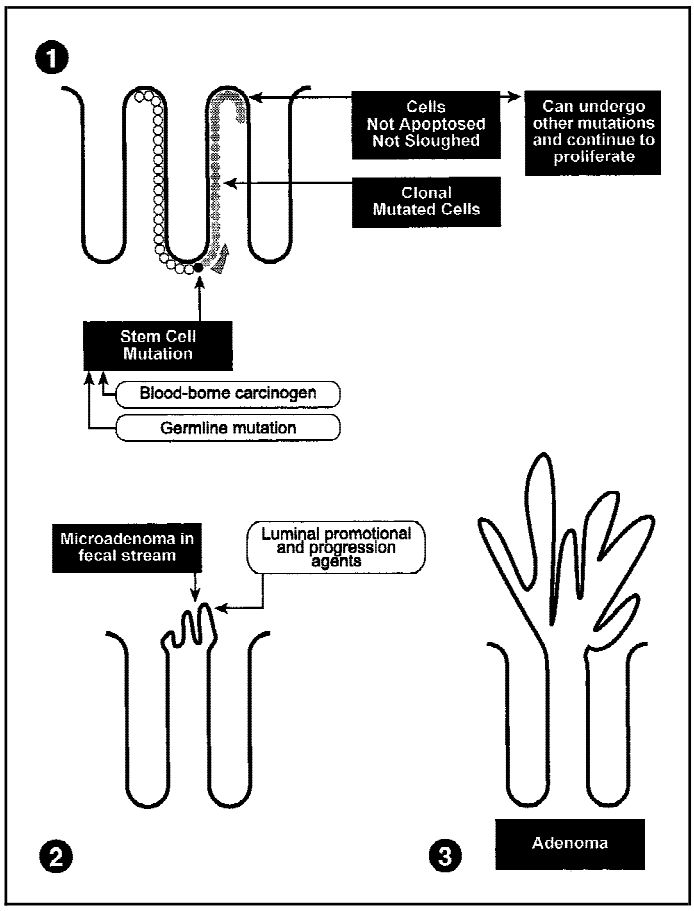

Fig. 1. Interactions of colon crypt cells with blood-borne and luminal agents. **1.** Mutation of *APC* (adenomatous polyposis coli gene) in stem cell, as a result of blood-borne agents or germline mutation, produces abnormalities in cell proliferation, migration, and adhesion. **2.** Abnormal cells accumulate at the top of the crypt; an aberrant-crypt focus (ACF) forms and begins to protrude into the fecal stream. **3.** Other mutations are more likely with the contact of proliferating cells with fecal mutagens, and an adenoma forms by sequential clonal expansion.

The above seems, at first glance, little better than a description of already established events with no predictive power in the absence of an organotypic model. What makes it more plausible is that, in ulcerative colitis, which carries an approximate 20-fold elevated risk of cancer, *APC* mutations are found in fewer than 10% of cases. Instead, the early event appears to be a *p53* mutation *(177).* In ulcerative colitis, there is no need to grow a polyp in order to provide a target for multiple hits; because of the destruction of the epithelium, the colonic contents are readily able to interact with proliferating stem and early daughter cells and provide the first hit. What appears necessary for the development of cancer from ulcerative colitis dysplasia is a loss of a cell cycle checkpoint.

A germline mutation of *APC* represents one kind of field defect—a widespread inherited predisposition to malignant change. Ulcerative colitis, particularly pancolitis, represents another. It may be that individuals without such inherited or acquired abnormalities nonetheless have, as a result of other inherited susceptibilities or of prolonged exposure to specific agents or a growth-promoting metabolic milieu, a similar kind of defect resulting in an increased tendency to polyp formation or dysplasia. The presence of such a genetic or acquired field defect has not been demonstrated but does hold promise for early detection.

Pathways to Colorectal Cancer

The evidence suggests that there are at least four pathways from normal cell to colorectal cancer. However, some of the molecular machinery appears to be common (Fig. 2).

The archetypal pathogenic pathway (the adenoma–carcinoma sequence) was first described by Morson, Hill, and colleagues *(178,179).* This process is now accepted as central to the majority of cancers and an early key event is the mutation (germline or somatic) of *APC (117).* Loss or mutation of *APC* induces polyp formation as a result of the loss of orderly cell replication, adhesion, and migration; some known functions of the APC protein are shown in Table 2 *(180–182).* The pathway involves β-catenin (which both binds E-cadherin and activates transcription) and *Tcf* (T-cell factor), a downstream transcriptional activator gene. A mutant *APC* or β-*catenin* results in failure of proper adhesion–migration of cells and the transcription of a proliferative signal *(183–185)* that can operate via c-*MYC (186).* Mutations in β-*catenin* can substitute functionally for loss of *APC (184,187)* (Fig. 2, A).

The important finding is that the first hit induces a change in the crypt architecture—the heaping up of a replicating microadenoma into the fecal stream. This change, in turn, makes a second hit and subsequent hits more likely.

On the basis of the allelotypes of a series of tumors, Vogelstein and colleagues *(188–192)* showed that the molecular steps after the activation of the *APC*–β-*catenin–Tcf* pathway involve an accumulating (but not linear) series of specific chromosomal and genetic changes that accompany the transition from normal colonic mucosa to metastatic carcinoma. These include mutation of K-*ras* (a proto-oncogene), changes in methylation patterns, and mutation or loss of *p53* (a tumor suppressor gene controlling entry into the cell cycle). Other important losses include *DPC4/Smad4 (193)* or possibly *DCC (191)* on chromosome 18 and the Peutz-Jeghers gene *(194–196)* on chromosome 19.

Following the identification of the HNPCC genes as being DNA MMR genes, it was postulated that these mutations might make mutations of *APC,* K-*ras, p53,* etc., more likely. It has since become clear that other genes are damaged or lost in HNPCC—particularly the transforming growth factor (TGF) β receptor II gene (*TGFβRII*) (in a growth-controlling pathway) *(197), BAX* (apoptosis) *(198),* and even the MMR genes themselves *(154).* Indeed, there is some evidence *(199–203),* although not entirely consistent *(144,204),* that mutations of K-*ras, p53,* and *APC* are less common in HNPCC and in sporadic tumors with microsatellite instability. Certainly, the mutational spectrum of *APC* is different between the two pathways *(204).* The signaling pathway from TGFβ proceeds via Smad transcription factors; loss of *Smad4/Dpc4* is a common event in sporadic tumors *(193)* and has been shown to exacerbate the *APC* mutant phenotype in mice *(133).*

In the absence of an inherited *APC* mutation, the likelihood of developing an adenoma in an individual with a DNA MMR defect may not be greatly different from that in the general population. However, once an adenoma develops, its progression to carcinoma is more rapid in the individual with a DNA MMR defect as the colonic environment induces irreparable damage. Evidence of the importance of the environment and of the quantitatively (rather than of the qualitatively) different risk in HNPCC patients compared with the general population comes from the comparison of the tissue distribution of HNPCC tumors in Warthin's family G at the turn of the century *(134)* and in the

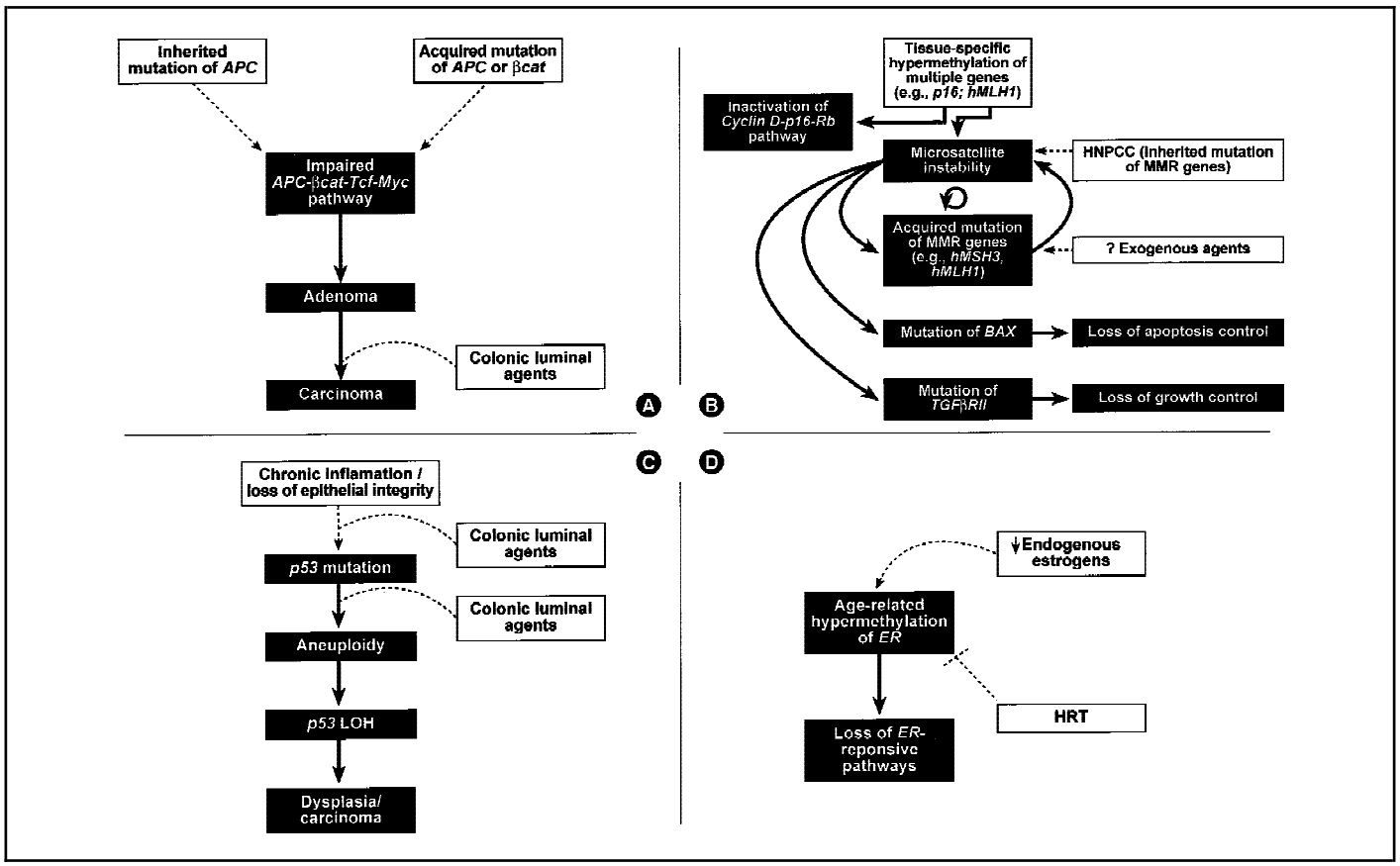

Fig. 2. Four pathways to colorectal cancer. At least four separate molecular pathways to colorectal cancer exist: **A)** *APC–β-catenin–Tcf– MYC* pathway associated with the adenoma–carcinoma sequence; **B)** the HNPCC pathway characterized by loss of DNA mismatch repair (by inherited or acquired mutation or methylation) that results in microsatellite instability in the tumors; **C)** the ulcerative colitis dysplasia–carcinoma sequence that is usually not associated with *APC* mutation or polyp formation; **D)** hypermethylation silencing of the estrogen receptor gene, which may be part of a wider pattern of gene-specific hypermethylation—common in sporadic tumors. APC = adenomatous polyposis coli; βcat = β-catenin; Tcf = T-cell factor; LOH = loss of heterozygosity; HNPCC = hereditary nonpolyposis colorectal cancer; MMR = mismatch repair; TGF = transforming growth factor; ER = estrogen receptor; HRT = hormone replacement therapy.

Table 2. Functions of adenomatous polyposis coli (APC) protein

- Regulation of β-catenin-induced signaling
- Regulation of cell adhesion via β-catenin and E-cadherin
- Regulation of cell migration via interaction with microtubules
- Cell cycle block perhaps by direct inhibition of cell cycle components

subsequent follow-up *(205)*. Initially, over three generations, the family showed five stomach cancers, 10 uterine cancers, and only two, perhaps three, "intestine" cancers *(134)*. When Lynch et al. *(205)* described the family in 1981, there were then 10 or 11 colorectal cancers in the third generation (plus 15 endometrial cancers and two stomach cancers) and 23 colorectal cancers in the fourth generation (plus two endometrial cancers and one stomach cancer). This observation shows that the distribution of the disease in an HNPCC family essentially exaggerates the pattern seen over this century in the general population: stomach cancer more common around 1900 and subsequently declining, endometrial cancer more common later but now less so, and a recent higher risk of colorectal cancer.

It is possible to lose DNA MMR function not by somatic mutation but by hypermethylation. Hypermethylation of *hMLH1* now appears to be a very early event in many sporadic colorectal tumors *(156,206)*, an epigenetic event that leads, in turn, to a mutator phenotype. The cause of the hypermethylationsilencing has yet to be determined; speculation surrounds the possibility that there is a rapid switch of methylation patterns affecting whole regions of the genome, perhaps implicating a still earlier mutation in a gene that controls large-scale chromosome integrity *(207)*. Evidence that this methylation switch involves more than the loss of MMR comes from studies showing that there is a similar silencing of $p16^{ink4A}$, *TSP-1*, and *IGF2* *(207,208)*.

Thus, the initial hit is an inherited or acquired mutation in an MMR gene or hypermethylation specifically of *hMLH1*. At the heart of this pathway is the probability that there will now be a vicious cycle of increasing microsatellite instability involving the MMR genes themselves and additional losses of important controllers of DNA and cell integrity (Fig. 2, B).

The third pathway to colorectal cancer is via ulcerative colitis *(209)* (Fig. 2, C). Although this disease is a minor contributor to the overall population burden of colorectal cancer, individuals with ulcerative colitis have about a 20-fold excess risk. This pathway appears to involve a dysplasia–carcinoma sequence, more like Barrett's esophagus or stomach cancer. As noted earlier, there is no "need" to grow a polyp. The somatic molecular changes are much less well defined, but *APC* mutations are uncommon and *p53* loss can occur early, appearing even in diploid histologically normal tissue *(178)*. Microsatellite insta-

bility may occur in the absence of DNA MMR defects—again, even in normal-appearing tissue *(210)*—suggesting perhaps an overwhelming source of mutagenic activity consequent upon the exposure of proliferating cells to the colonic contents.

Fourth, there is evidence to show that almost all colon cancers arise from cells in which the estrogen receptor (ER) gene has been silenced *(47)* (Fig. 2, D). Unlike the switched methylation silencing of *MLH1* and *p16*, hypermethylation of *ER* is an age-related phenomenon; there are also other genes that show hypermethylation with age. It is not known whether this is an early crucial step; moreover, it is not established why loss of the ER protein is so critical to colonic epithelial cells—what estrogen-responsive genes are downstream?

Finally, loss of a mitotic checkpoint may be important. One of the characteristics of many colorectal tumors is a marked degree of chromosomal instability—obvious as widespread aneuploidy. It has recently been demonstrated *(211)* that this chromosomal instability is consistently associated with loss of function of a mitotic checkpoint. Furthermore, in at least some cases, the checkpoint was lost because of mutational inactivation of the human homologue of *BUB1*. In yeast, *BUB1* is part of the chromosomal segregation machinery. Aneuploidy may be important because it increases the loss of other tumor suppressor genes. Certainly, it adds weight to the evidence that a heightened degree of mutability of the genome is a characteristic of human tumors *(212)*. How this particular step interacts with other pathways to colorectal cancer is not yet established, but a transfected mutant *hBUB1* induced loss of the mitotic checkpoint in cells that were euploid but showed microsatellite instability *(211)*.

Where Do the Environmental Influences Interact With the Molecular Pathways?

Some of the "targets" in the molecular pathway to colon cancer are now clear—the *APC*–β-*catenin*–*Tcf* pathway, DNA MMR genes, *p53*, H-*ras*, *ER*, and perhaps *hBUB1*. Loss of these controls can be via small mutations, by CpG island hypermethylation, by large-scale losses of genomic material that itself can be facilitated by loss of some of these genes, and perhaps by extensive switched hypermethylation. It seems likely that some of these processes are secondary to events associated with selection for autonomous cells; this is almost certainly true of the large-scale genomic losses characteristic of the later stages of most cancers. However, smaller scale changes are more probably the consequence of changes in the microenvironment of the DNA and the cell, and these, in turn, may be influenced by diet, smoking, exercise, HRT use, etc.

One view is that ingested foods and host responses primarily determine and condition the "growth media" *(213)*—both tissue side and luminal side—in which the colonic cells are bathed. Risk factors may operate through a variety of physiologic cascades *(213,214)*. For example, on the tissue side of colonic cells, not only is there exposure to meat- and smoking-derived carcinogens and vegetable-derived anticarcinogens, but also there is the likelihood that crosstalk exists between fibroblasts and epithelial cells; this crosstalk may be particularly mediated by endogenous growth factors. On the luminal side—in the presence of a growing microadenoma/adenoma—ingested vegetables will increase the fiber content, which is fermentable by gut bacteria, thus producing volatile fatty acids. This result, in turn, influences cell replication, cell maturation, and apoptosis. Meanwhile, fiber and the higher bacterial mass increase stool bulk and reduce transit time—as does physical activity. Bioactive compounds, widespread in vegetables, may also influence growth of abnormal cells, directly or via metabolites. Some plausible interactions, summarized in Table 3, are listed below.

Vegetables, folate, fiber, and anticarcinogens. A key constituent of vegetables is folate. There is evidence that consumption of folate (which is also derived from other sources including supplements) has a relationship with risk of both adenomas and cancer. Furthermore, this risk may be modified by at least one genetically determined metabolic step in the folate pathway, i.e., that controlled by MTHFR. Folate and MTHFR may be important in influencing the availability of SAM—the universal methyl donor—and ultimately both DNA methylation and the nucleotide pool *(215)*. DNA hypomethylation is an early step in colon carcinogenesis *(216)*. Methylation is under genetic control, and the expression of the methyltransferase gene has been shown to be considerably increased in the normal mucosa of cancer patients and more so in polyp and cancer tissue *(217)*. Chronic methionine or choline deficiency results in alterations of DNA methylation and produces large numbers of a variety of tumors in rats and mice *(218)*. Folate deficiency may have related effects *(219)*. Major changes in methylation patterns are common in colorectal cancer and are clearly related to genetic instability *(207,220,221)*.

Fiber ferments in the large bowel to produce short-chain fatty acids. Butyrate is an important colonic fuel and induces apoptosis in colonic cell lines *(222)*.

More generally, vegetables contain a large number of substances—both micronutrients, such as carotenoids and ascorbate with antioxidant activity, and other bioactive compounds, such as phenols, flavonoids, isothiocyanates, and indoles with a variety of potent anticarcinogenic properties *(27,223)*. There are no data to show that any bioactive compound influences DNA repair; however, at many of the steps from initial exposure to a procarcinogen to the appearance of cancer, one or more known phytochemicals can alter the likelihood of carcinogenesis, usually in a favorable direction. The antioxidant compounds may be

Table 3. Epidemiology and biology of colorectal cancer—some links*

Population risk factors	Molecules
Family history	FAP → APC pathway HNPCC → microsatellite instability pathway ?Others
Meat and smoking	Nitrosamines and heterocyclic amines → ?*APC* mutation ?K-*ras* mutation
Alcohol	Acetaldehyde → DNA damage Effects via reduced folate
Vegetables	Antioxidants → reduced DNA damage Folate → DNA integrity Fiber → SCFAs → apoptosis
Physical activity/low body mass	Reduced growth stimulus Reduced transit time
NSAIDs	COX-2 inhibition
HRT	?Prevention of *ER* hypermethylation

*FAP = familial adenomatous polyposis; APC = adenomatous polyposis coli; HNPCC = hereditary nonpolyposis colorectal cancer; SCFAs = short-chain fatty acids; NSAIDs = nonsteroidal anti-inflammatory drugs; COX = cyclooxygenase; HRT = hormone replacement therapy; ER = estrogen receptor.

particularly important in ulcerative colitis-associated carcinogenesis, given the extensive flux of oxidation in the lumen.

Perhaps the most important observation, from a public health perspective, is the evidence that higher intake of plant foods is tightly linked to lower risks of almost all epithelial cancers. The public health recommendations that follow are obvious but far from being implemented *(14)*. Nonetheless, there is still a great deal to learn about mechanisms, not just to satisfy intellectual curiosity but also to provide insights into molecular pathways and thus into other possible approaches to risk reduction, especially among high-risk individuals. The best research strategy is likely to involve human experimental nutrition—"feeding studies"—using molecular tools to monitor the intermediate biology.

Meat and heterocyclic amines. Pursuing the argument that blood-borne carcinogens may well produce somatic mutations in *APC* and perhaps K-*ras*, important sources of known carcinogens with established links to risks to colorectal cancer include cooked meat (e.g., heterocyclic amines and nitrosamines). Sugimura and Sato *(224)* originally proposed that specific heterocyclic amines are important in the etiology of colon cancer. Several separate classes of these compounds have been identified *(225)* and have been shown to be carcinogenic in animals *(226,227)*, including having a direct effect on *APC* *(227)*. Nitrosamines are also plausible human colon carcinogens, the levels of which are related to dietary intake of meat *(228)*. An elevated risk may be exacerbated by genetically determined variations in relevant metabolic pathways but, as noted above, to date, the larger studies are less impressive.

The next research steps probably involve establishing all of the polymorphisms in the metabolizing enzymes involved in handling heterocyclic amines, polycyclic aromatic hydrocarbons (PAHs), nitrosamines, etc. Until this task is completed and the results are applied to studies of populations with well-measured intakes of meat, across a broad range of consumption and cooking practices, there will be tantalizing findings implicating, then not implicating, meat and showing intermittent interactions with metabolic profiles. The ideal population will include a sizable proportion of vegetarians, for the same reason that we include nonsmokers in studies of tobacco. The public health recommendation appears clear—both for coronary heart disease and cancer: "If eaten at all, limit intake of red meat to less than 3 ounces daily" *(14)*. In the United States, this recommendation is likely also to have a beneficial ecologic impact.

NSAIDs. NSAIDs clearly suppress COX-2 *(229)* and are capable of inhibiting polyp growth even in individuals with FAP. COX-2 inhibition produces effects on epithelial proliferation and apoptosis *(230)* and on angiogenesis *(231)*. Most recently, there is evidence that NSAIDs (including aspirin) may directly suppress the HNPCC-associated mutator phenotype by genetic selection for a subset of cells that do not express microsatellite instability *(232)*. These agents show considerable promise for protecting even those at high genetically influenced risk. Clinical trials to establish their efficacy more generally are under way.

Smoking. Tobacco smoke is a major source of a wide variety of carcinogens—including heterocyclic amines, polycyclic hydrocarbons, and nitrosamines. These are plausible blood-borne carcinogens and the, as yet incomplete, evidence that there are interactions between smoking and metabolic genotype reinforces the importance of this exposure. There is evidence from rat models that *APC* is a target for heterocyclic amines, but whether this is true in humans remains to be shown *(227)*. Many of the same questions about the carcinogens in meat and their genetically variable metabolism (*see above*) apply to those in tobacco smoke.

Alcohol. A local action of ethanol on tissue has been proposed through a solvent or cytotoxic effect, perhaps more relevant in the upper alimentary tract. Acetaldehyde is a potent adduct former, and alcohol is known to inhibit DNA repair *(233)*. Alcohol may exert its effect through associated deficiencies in nutrients, particularly folate *(29,234)*. As is true for understanding the role of plant foods in colorectal cancer, feeding studies with alcohol, in which both molecular and physiologic responses are monitored, are an important next step.

HRT. The evidence that *ER* hypermethylation increases with age and is a central feature of colon cancer suggests that perhaps declining levels of estrogen may be important. The inverse relationship between HRT and both polyps and cancer may be a consequence of replacing the declining endogenous estrogen levels and thus reducing the likelihood that the *ER* gene will be silenced by methylation *(235)*. Identifying the estrogen-responsive targets that are involved in colorectal carcinogenesis constitutes an important research question.

Physical activity and obesity. One hypothesis for the role of physical activity is that it stimulates colon peristalsis, thereby decreasing the time that colonic contents are in contact with the epithelium; however, transit time is not a well-established risk factor for colon neoplasia. Exercise has both acute and chronic hormonal effects and favorable effects on the immune system *(15)*. Furthermore, higher physical activity, especially in the presence of lower body mass, is associated with a general metabolic milieu (lower insulin, glucose, and triacylglycerol levels and possibly lower levels of other growth factors) that is less favorable to the growth of cancer in general and perhaps colon cancer in particular *(236)*.

This last point raises the more general issue of how various specific exposures and behaviors might jointly influence risk of colorectal cancer. After all, many of the risk factors tend to cluster in the lives of individuals and populations: physical inactivity, obesity, high consumption of meat, alcohol and tobacco use, and low intake of plant foods. Various subsets of this list are implicated as causal in a variety of metabolic disorders: diabetes mellitus, hyperlipidemias, and hypertension. These, in turn, are themselves associated with defined disease outcomes. It is not difficult to hypothesize from a variety of perspectives [sex differences in lipid and bile acid metabolism *(5,237)*, Syndrome X *(236)*, and hyperinsulinemia and diabetes mellitus *(238)*] that large-bowel cancer is really one more metabolic disorder induced by energy imbalance. The phenotypic manifestations (ischemic heart disease, hypertension, diabetes mellitus, Syndrome X, and colon cancer) will be determined by underlying genotype plus the subtle variation in the pattern of excesses. From a research perspective, there are a variety of leads that allow us to begin to characterize the metabolic genotypes that shape the phenotypes: genes involved in lipid metabolism, in appetite regulation, in diabetes predisposition, and in pathways to obesity. From a public health perspective, however, the protective steps are already clear: Maintain regular physical activity and normal body weight *(14)*. How to do so in a sea of stimuli designed to achieve

exactly the opposite goal is a bizarre conundrum that we have inflicted on ourselves and increasingly on others.

CONCLUSION

The clinical and molecular evidence suggests that there are several pathways to colorectal cancer. Two of the somatic pathways essentially parallel the more rapid processes seen in those with inherited mutations in *APC* and MMR genes, respectively. The epidemiologic evidence shows that a variety of exogenous agents (e.g., tobacco smoke and meat) may increase the risk, and others (e.g., NSAIDS, vegetables, and HRT) may reduce the risk. The role that several of these agents play is becoming clearer. At the population level, the not entirely consistent associations and small relative risks are what might be expected if the agents operate to accelerate or interrupt only one or some of the pathways. On the other hand, there may be some underlying metabolic patterns that provide a milieu in which cancer is more or less likely to occur. As the heterogeneous nature of the pathways becomes clearer, several things will happen. First, the relative risks for specific host states and exogenous agents will become stronger as subsets of the population with specified susceptibilities are identified. Second, this identification of subsets will improve the ability to tailor preventive strategies, although the current evidence suggests that a postmenopausal woman who exercises vigorously, has a diet high in vegetables, and takes HRT and aspirin may well be at only a fraction of the risk seen in the general population. Third, clarification of pathways and their related agents should improve markedly the possibility of using early changes as screening markers.

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NOTE

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