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Tritiated Thymidine (ϕ_p,ϕ_h) Labeling Distribution as a Marker for Hereditary Predisposition to Colon Cancer[1]

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ABSTRACT

New analytical methods have been developed for measurement of the height distribution patterns of [^{3}H]dThd-labeled epithelial cells in colonic crypts of high- and low-risk population groups. Labeled cells were segregated with respect to crypt-height into 10 compartments of equal size, plus a lumenal surface compartment for each subject. Members of families prone to polyposis coli and to non-polyposis colon cancer were compared to subjects at lower risk. The latter included persons from polyp-free and cancer-free branches of the same families, normal controls, and patients with colon cancer from the general population. Significant differences were found between groups of patients with familial polyposis or familial colon cancer and subjects at low risk, when labeled cell distributions were compared over all the crypt height compartments ($p < 0.001$). Distributions of the occupancy fractions of labeled cells in the upper region (*i.e.*, 40%) of the crypt (ϕ_h), measured for the fraction of each population (ϕ_p), revealed a discriminant level that separated over 90% of low-risk subjects from a major fraction of those affected with familial colon cancer or polyposis and from close to one-half of the at-risk progeny as expected for an autosomal dominant trait. However, subjects with colon cancer in the general population had (ϕ_p,ϕ_h) distributions closer to the low-risk groups, although a subgroup of patients had abnormal ϕ_h values characteristic of hereditary disease. Thus, the (ϕ_p,ϕ_h) distribution appears to be a more precise measure of risk than previously used in discriminating populations with genetic susceptibility to colon cancer from those at lower risk and may be useful as a marker to identify individuals with the at-risk phenotype.

INTRODUCTION

Studies of cell proliferation have aided our understanding of the neoplastic transformation of colonic epithelial cells in hereditary conditions that predispose to cancer (1, 5–7, 10, 11, 13, 17, 19, 20). Special attention has been given to FP[3] and to its variant, Gardner syndrome, which eventually progress to cancer of the large intestine. Both in FP and in FCC, the patterns of disease transmission are consistent with an autosomal dominant mode of inheritance (2, 8, 14–16).

In flat colonic mucosa of FP, an abnormal pattern of incorporation of [^{3}H]dThd into DNA of colonic epithelial cells has been observed, in which increased numbers of epithelial cells in the upper (lumenal) portion of the colonic crypts continue to incorporate [^{3}H]dThd into DNA. These abnormally proliferative upper crypt epithelial cells tend to accumulate near the lumenal surface of the colonic mucosa and give rise to adenomas (1, 4–6, 10, 11, 13).

The present study describes new analytical methods that have been developed for measurement of the loci of [^{3}H]dThd incorporation in colonic epithelial cells of flat mucosa. Observations in symptomatic and in at-risk members of families with FP and FCC were compared to those in familial and general population control groups.

MATERIALS AND METHODS

Population Groups Studied

Familial Polyposis Coli

FP$_s$. Seventeen subjects with FP$_s$ were followed at Memorial Hospital and at the National Cancer Institute; 11 had undergone subtotal colectomy, all with ileorectal anastomosis, prior to testing; there were 5 males and 12 females, 12 to 62 years old.

FP$_a$. Eleven asymptomatic first-degree relatives of FP cases who were potential carriers of the polyposis gene were studied; there were 4 males and 7 females, 14 to 33 years old.

FP$_n$. Ten subjects from a 3-generation polyposis-free branch of a family with FP were studied; there were 6 males and 4 females, 14 to 70 years old.

Familial Colon Cancer without Polyposis

FCC$_s$. Eight colon cancer cases after curative segmental resections of the affected portion of the colon and reanastomosis were followed at Creighton University (15, 16); there were 4 males and 4 females, 32 to 73 years old.

FCC$_a$. The group included 22 clinically normal first-degree relatives of FCC$_s$ patients, who are believed to have about a 50% lifetime risk of developing colon cancer (15, 16); there were 16 males and 6 females, 14 to 71 years old.

FCC$_n$. Studied were 13 subjects from a 3-generation colon cancer-free branch of one of the FCC kindreds, 4 males and 9 females, 15 to 41 years old.

Colon Cancer Cases in the General Population

Seventeen subjects in the general population had colon cancer and were scheduled for surgical resection. The patterns of [^{3}H]dThd labeling

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[3] The abbreviations used are: FP, familial polyposis coli; FCC, familial colon cancer without polyposis; [^{3}H]dThd, [^{3}H]thymidine; FP$_s$, symptomatic polyposis coli; FP$_a$, at risk for familial polyposis coli; FP$_n$, not at risk for familial polyposis coli; FCC$_s$, symptomatic familial colon cancer without polyposis; FCC$_a$, at risk for familial colon cancer without polyposis; FCC$_n$, not at risk for familial colon cancer without polyposis.

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of colonic epithelial cells in these subjects were reported previously (17); there were 10 males and 7 females, 25 to 78 years old.

Normal Subjects in the General Population

Fifteen subjects from the general population had no evidence of colonic disease in their personal histories; there were 10 males and 5 females, 23 to 78 years old.

Biopsies of Colonic Mucosa

All subjects had a general medical history taken, a physical examination, and an air-contrast barium enema. All were given a liquid diet for 2 days, followed by tap water enemas on the morning of the study. Using a biopsy miniforceps during sigmoidoscopy, 2 to 3 specimens were removed from normal-appearing flat colonic mucosa at locations 4 to 8 cm above the level of the anus for each subject. In postcolectomy subjects, biopsies were removed from the remaining rectal segment.

Specimens of mucosa cut 1 mm thick were incubated in a Dubnoff shaker for 1 hr in Eagle's basic salt solution with 10% fetal calf serum, 1 μCi of [^{3}H]dThd per ml of solution (specific activity, >25.0 Ci/mmol), and 95% O_2-5% CO_2 was bubbled through the solution. The mucosal specimens from a given patient were pooled for a single measurement in 3-μm paraffin sections and processed for microautoradiography using NTB2 emulsion (Kodak). The slides were developed and stained with hematoxylin and eosin after 3 to 4 weeks of exposure time (4, 5).

Glands longitudinally sectioned from base to lumen were analyzed. In these glands, the entire length of the crypt was visible in the section, and the base of the crypt contacted the muscularis mucosa. The total number of cells per crypt column (a single column of cells on each side of the length of the crypt) and the number and position of [^{3}H]dThd-labeled cells were counted. The average numbers of crypts counted per individual were 35.5 ± 6.20 (S.E.) for FP, 30.6 ± 4.49 for FCC, and 36.7 ± 4.1 for controls. The average numbers of epithelial cells per crypt were 93.1 ± 3.1 for FP, 85.3 ± 1.5 for FCC, and 95.7 ± 2.3 for controls. All specimens of mucosa were histologically characteristic of the large intestine.

Height Distribution Patterns of [^{3}H]dThd-labeled Colonic Epithelial Cells

In order to standardize the position of labeled cells within the crypt columns, for each subject the heights of all [^{3}H]dThd-labeled epithelial cells were measured and expressed in a uniform manner. Each labeled cell was assigned a crypt coordinate (c) value between 0 and 1, equal to the fraction of the location of the cell above the base of its crypt column. By this method, a labeled cell that was located at the base of a crypt column would be assigned a height coordinate value of zero ($c = 0$), while a labeled cell located at the lumenal surface would be assigned a height coordinate value equal to 1 ($c = 1$). A labeled cell located 47% of the way up from the base of a crypt column would be assigned a height coordinate value of $c = 0.47$.

To summarize information on the frequency distribution of an individual's labeled cells in all of the standardized crypts, the labeled cells were segregated into height compartments within the crypts. Accordingly, the labeled cells for the individual were segregated by height-in-crypt or c value into 10 compartments of equal size, ranging from $0.0 \leq c < 0.1$, $0.1 \leq c < 0.2$, ... $0.8 \leq c < 0.9$, and $0.9 \leq c < 1.0$, plus an 11th "compartment" corresponding to c values equal to 1 (lumenal surface); these 11 compartments were referred to by ordinal numbers from 0 to 10. The fraction of all of the individual's labeled cells that fell into a given compartment (*e.g.*, the ith compartment) was calculated and described by an additional number, ϕ_i. The overall height-distribution pattern of an individual's [^{3}H]dThd-labeled epithelial cells within the crypt was defined by the distribution of the occupancy fractions ϕ_i over the 11 height compartments.

The labeled cell data for each population group were summarized with reference to the crypt height compartments. Thus, the total number of labeled cells found in a given compartment, *e.g.*, the ith compartment, for all the individuals in a group, defined a frequency of occurrence f_i. From these data, population occupancy fractions were computed by taking the ratios of the various frequencies f_i to their total. A comparison was then made of the population fractions of labeled cells for the combined low-risk groups (FP_n, FCC_n, and normal subjects), and the high-risk groups FP_s and FCC_s.

Further Comparison of Labeled Cell Frequencies and Consolidation of Crypt Height Compartments

Further comparisons of labeled cell frequencies between high- and low-risk groups were then carried out by graphically displaying χ_i intermediate data derived in the following manner. For any given crypt height compartment i, a χ_i value was defined as a normalized difference between the labeled cell frequency f_i for the high-risk population and a corresponding frequency F_i associated with the low-risk population taken as a reference. F_i results when all of the labeled cell frequencies for the low-risk population are scaled by a common factor to bring their total into agreement with that of the labeled cell frequencies for the high-risk population; the normalization of each frequency difference ($f_i - F_i$) is carried out with reference to $F_i^{1/2}$. Thus, the normalized frequency differences χ_i are defined by

$$\chi_i = (f_i - F_i)/F_i^{1/2}$$

The values χ_i, in the role of components of a generalized vector the squared length of which equals χ^2 are used here to facilitate graphic display of the direction in which any 2 populations differ in labeled cell frequency *versus* crypt height compartment number. In this role as a vector component or indicator of the direction of population differences, the χ_i values contain polarity information *versus* compartment number, as shown below.

This analysis enables a consolidation of individual crypt height compartments where maximum differences between high- and low-risk populations are observed, thus facilitating the development of risk markers. Based on the results of the χ_i comparisons between high-risk and low-risk populations, the 5 crypt height compartments numbered 6 through 10 were empirically consolidated to comprise a "high-crypt region," consisting of the upper 40% of the height of the crypt. When applied to the data for each individual studied, the fraction of an individual's [^{3}H]-dThd-labeled cells located in the high-crypt region was defined as a quantity ϕ_h ("high-fraction," or upper crypt occupancy fraction), where $\phi_h = \phi_6 + \phi_7 + \phi_8 + \phi_9 + \phi_{10}$, the sum of the individual's labeled cell occupancy fractions for the crypt height Compartments 6 through 10 inclusive.

In presentation of the data, the fraction of the individuals in a group whose value of ϕ_h equaled or exceeded a specified ϕ_h value was denoted by ϕ_p ("population fraction"), a function of the ϕ_h value specified in its definition. The graph of ϕ_p as a function of ϕ_h furnishes a useful statistical characterization of the distribution of ϕ_h values for the individuals comprising a given population and serves as a referent permitting comparison of different populations. The ϕ_p *versus* ϕ_h graph for a given population represents the complemented integrated probability distribution of the ϕ_h values for the population. The ordinate ϕ_p therefore has a convenient meaning as a general fractile coordinate and defines the "fractile axis" for the (ϕ_p,ϕ_h) graph; that graph can be termed a fractile distribution of the ϕ_h values for the individuals comprising a given population. The (ϕ_p,ϕ_h) graphs also show all of the individual data points for all of the individuals in each population group, together with the number of points greater or less than any given ϕ_h value, and the degree of overlap of the data in each population group. Graphs of ϕ_p *versus* ϕ_h were plotted from the empirical labeled cell data for various groups tested.

RESULTS

Height Distribution Patterns for Labeled Cells in High-Risk and Low-Risk Groups. Chart 1*A* is a histogram displaying the

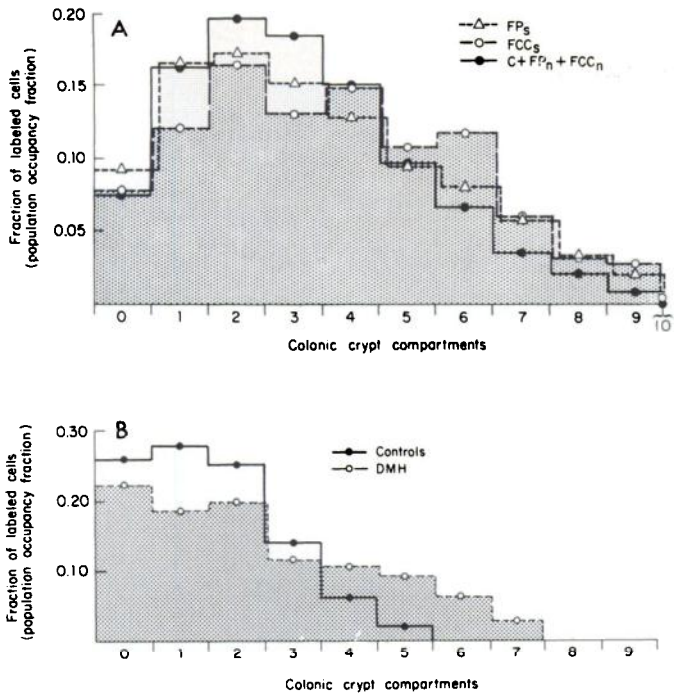

Chart 1. *A*, comparison of population occupancy fractions of [^{3}H]dThd-labeled epithelial cells in colonic crypt compartments of the high-risk group FP$_s$, the high-risk group FCC$_s$, and the combined low-risk groups [FP$_n$, FCC$_n$, and normal (*C*)]. *Abscissa numbers*, colonic crypt height compartments. *Ordinate*, fraction of a given population's labeled cells that occupy any specified height compartment in the colonic crypts. ●, data for the combined low-risk groups; ○, data for FCC$_s$; △, data for FP$_s$. *B*, histograms of population occupancy fractions of [^{3}H]dThd-labeled epithelial cells in colonic crypt compartments of CF-1 mice. *Ordinate* and *abscissa* as in *A*. ●, normal mice; ○, mice after 6 injections of 1,2-dimethylhydrazine (*DMH*) (20 μg/mouse) (data from Ref. 18).

height distribution patterns (population occupancy fractions) for the 2 high-risk groups (FP$_s$ and FCC$_s$) compared to those for the combined low-risk group (FP$_n$, FCC$_n$, and normal subjects). The 2 high-risk groups had larger fractions of their [^{3}H]dThd-labeled cells located in the upper crypt height Compartments 6 through 10 and smaller fractions of their labeled cells in crypt height compartments below the midregion. These findings indicate a shift of the proliferative cell compartment toward the lumenal surface in the high-risk groups. As shown in Chart 1*B*, a similar pattern of [^{3}H]dThd labeling of colonic crypt epithelial cells occurred in mice exposed to an experimental carcinogen, 1,2-dimethylhydrazine (18). In each of these comparisons, high-risk populations revealed an expansion of the proliferative compartment towards the lumenal region of the colonic crypt.

Comparison of Crypt Compartment Frequency Distribution of Labeled Cells between High-Risk and Low-Risk Groups. Using the data presented in Chart 1*A*, χ^2 analyses comparing the distributions of labeled cells over all of the crypt height compartments showed highly significant differences between each of the high-risk groups FP$_s$ and FCC$_s$ and the combined low-risk groups (FP$_n$, FCC$_n$, and normal subjects) ($p < 0.001$ for each comparison).

To provide a further measure of differences between the cell-labeling patterns for various pairs of high-risk and low-risk groups, the χ_i differences between pairs of groups were determined from the labeling frequencies by the method previously described. For this analysis, the high-risk group FP$_s$ was compared to the combined low-risk groups (FP$_n$, FCC$_n$, and normal subjects) and to its low-risk kindred FP$_n$; FCC$_s$ was compared to the low-risk groups and to its low-risk kindred FCC$_n$. For each of the comparisons, the resulting χ_i values were plotted *versus* *i*, the crypt height compartment number. As shown in Chart 2, a common trend of differences between high- and low-risk groups was observed, with each histogram serving as a generalized vector describing the direction in which its underlying pair of population patterns differs. Findings are summarized as follows.

For both high-risk groups, FP and FCC, the proportion of labeled cells was consistently greatest in crypt height Compartments 6 through 10, thus indicating the largest contribution to the differences observed and serving as an obvious discriminant for comparing the high-risk *versus* the low-risk groups. Based on these results, data from the 5 crypt height Compartments 6 through 10 were consolidated, and their combined labeled cell occupancy fraction, ϕ_h, was studied as a discriminant for risk.

The proportion of labeled cells in the basal Compartment 0 tended to be greater for the high-risk groups compared to the low-risk groups so that the basal compartment may be a potential further marker for risk discrimination.

In crypt height Compartments 2 and 3, the proportion of labeled cells was consistently smaller for the high-risk groups compared to the low-risk groups and tended to be smaller also in Compartment 4.

The proportion of labeled cells in Compartment 5 was similar among the high- and low-risk groups; Compartment 5 thus served as a transition region between the negative zone composed of Compartments 2 to 4 and the positive zone of Compartments 6 to 10.

In Compartment 1, the proportion of labeled cells was equivocal between the high-risk and the low-risk groups, so that Compartment 1 served as a transition region between the positive Compartment 0 and the negative Compartments 2 to 4.

Comparative Distribution of the Upper-Crypt Labeled Cell Occupancy Fraction (ϕ_h) for High-Risk and Low-Risk Groups. The labeled-cell occupancy fraction in the upper 40% of the crypt

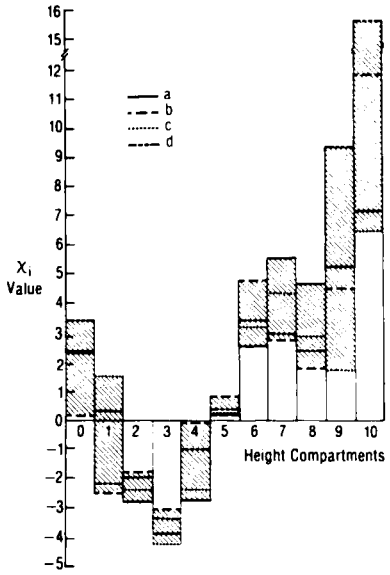

Chart 2. χ_i histograms showing comparisons between 4 pairs of high-risk and low-risk groups. *a*, the high-risk group FP$_s$ *versus* the combined low-risk groups (FP$_n$, FCC$_n$, normal subjects); *b*, the high-risk group FCC$_s$ *versus* the same combined low-risk groups; *c*, the high-risk group FP$_s$ *versus* its low-risk kindred FP$_n$; *d*, the high-risk group FCC$_s$ *versus* its low-risk kindred FCC$_n$.

(ϕ_h) was studied to determine its distribution in a given population. In this analysis, the population fraction ϕ_p was plotted *versus* ϕ_h, displaying the fractile distribution of the ϕ_h data. Chart 3 shows a clear separation between the (ϕ_p,ϕ_h) distributions for patients with FP_s and patients with FCC_s and subjects in the combined low-risk group (FP_n, FCC_n, and normal subjects). In FP, the distributions for the FP_s and FP_a groups were separated from the FP_n group (Chart 4*A*). In FCC, the patterns for the FCC_s and FCC_a groups were similarly distinguishable from those of the FCC_n group (Chart 4*B*).

These comparisons reveal different distributions for high-risk and low-risk populations. For example, in Chart 3, a ϕ_h value of 0.225 is equaled or exceeded by 75% of the individuals in FCC_s and by 29% of the individuals in FP_s (sensitivity, 75 and 29%, respectively), as compared to only 8% of the individuals in the combined low-risk groups. Thus, for both FP_s and FCC_s, a large percentage of high-risk individuals are selected by a cutoff point that excludes 92% of the low-risk subjects (specificity). It is realized, however, that these numerical estimates are tentative and require larger numbers of observations.

Similarly, in Chart 4*A*, if a ϕ_h value of 0.225 is again taken as a dividing line, 45% of the individuals with FP_a and 29% of the individuals with FP_s have a value of ϕ_h that falls on or to the right of the dividing line, whereas 90% of the individuals in FP_n fall to the left; and in Chart 4*B*, 75% of FCC_s individuals and 59% of FCC_a individuals fall on or to the right of the line, whereas 92% of the FCC_n individuals fall to the left. Thus, in all 3 comparisons, a cutoff value of ϕ_h exists that separates sizable percentages of the symptomatic and at-risk individuals from the majority of not-at-risk individuals.

Thus, in FP, a dividing line set at $\phi_h = 0.225$ (Chart 4*A*) accepts 45% of the asymptomatic at-risk individuals and rejects 90% of the known not-at-risk individuals. In FCC, the same dividing line (Chart 4*B*) accepts 59% of the asymptomatic at-risk individuals and rejects 92% of those not at risk. From a genetic point of view, the proportions of asymptomatic at-risk individuals in FP and FCC families who are in an abnormal range are close to 50%, which is consistent with the proportions of offspring expected to inherit the autosomal dominant gene for FP or FCC.

In contrast, patients with colon cancer drawn from the general population (Chart 5, Group CC) have a pattern virtually indistinguishable from that of the normal population. However, the skewed distribution of upper-crypt labeled-cell occupancy fractions containing high ϕ_h values suggests the presence of a subgroup of patients who share the cellular defect associated with familial predisposition to colon cancer.

Since colon cancer risk is greater in males than in females and in older than in younger age groups, we examined these covariables. The only trend was a higher fraction of labeled cells in the upper-crypt region of subjects older than 18 years of age in the FP_a group. There was no age effect seen for the corresponding FCC_a group. Since colectomy could affect results of the assay in the polyposis group, we compared pre- and postcolectomy

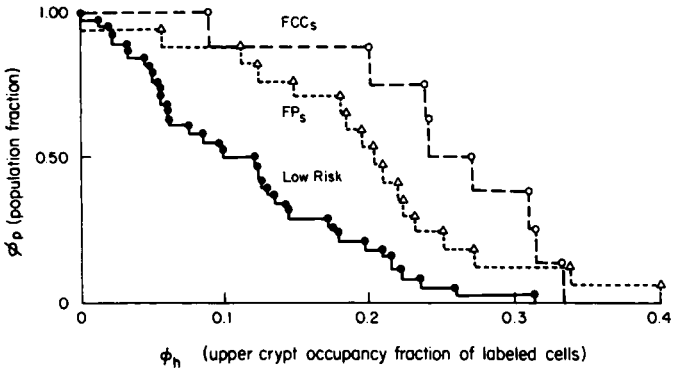

Chart 3. Comparisons of the fractile distributions of ϕ_h between the high-risk populations FP_a and FCC_a and the combined low-risk populations (FP_n, FCC_n, and normal subjects). *Abscissa*, ("high-fraction" or upper-crypt occupancy fraction), fraction of an individual's [^{3}H]dThd-labeled epithelial cells that are found in the upper 40% of the crypt, including the lumenal surface; *ordinate*, fraction of all the individuals in a given population whose measured ϕ_h values equal or exceed *abscissa* value.

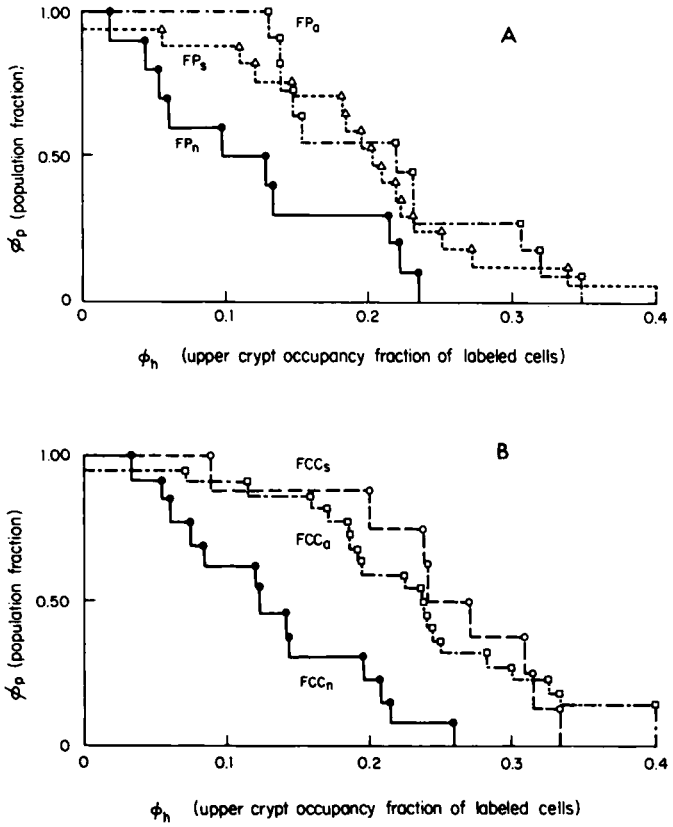

Chart 4. *A*, comparisons of the fractile distributions of ϕ_h between the high-risk groups FP_s and FP_a and the polyposis-free family branch FP_n. *Ordinate* and *abscissa* as in Chart 3. *B*, comparisons of the fractile distributions of ϕ_h between the high-risk groups FCC_s and FCC_a and the colon cancer-free family branch FCC_n. *Ordinate* and *abscissa* as in Chart 3.

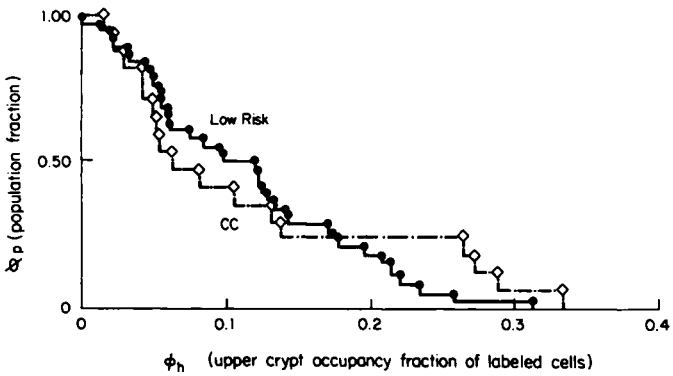

Chart 5. Comparison of the fractile distributions of ϕ_h between subjects with colon cancer (*CC*) in the general population and the combined low-risk groups (FP_n, FCC_n, and normal subjects). *Ordinate* and *abscissa* as in Chart 3.

patients; no differences in age or sex or labeling pattern were found.

The above findings were accompanied by increases in the percentage of cells that were [^{3}H]dThd labeled (labeling index) in the upper 40% of the crypts in the high-risk groups compared to the controls; however, labeling indices over the entire crypt did not uniformly parallel the risk status. Thus, in the upper 40% of the crypt, labeling indices per crypt column ranged from 2.4 to 3.6% in the high-risk groups, compared to 2.1% in controls and 1.9% in the colon cancer group. Over the entire crypt, labeling indices ranged from 5.7 to 8.7% in the high-risk groups compared to 7.4% in controls and 9.1% in the colon cancer group.

DISCUSSION

Previous studies of FP have documented that the proliferative region of the colonic crypts expands from the basal zone toward the lumenal surface (1, 4–6, 10, 11, 13). Similar findings have been noted in actively renewing precancerous states involving gastric and cervical epithelium (14, 19, 20). The abnormality is characterized by a failure of epithelial cells to repress DNA synthesis during maturation, as cells migrate to the lumenal end of the mucosal layer that lines these organs. The same kind of lesion has been observed in the colonic mucosa of rodents following administration of 1,2-dimethylhydrazine (3, 18) and in cervical epithelium after methylcholanthrene (9). Thus, in organs lined by layers of actively renewing epithelial cells, an ectopic expansion of the proliferative compartment of the cells appears to represent a phenotypic marker associated with the development of a precancerous state.

The present analysis involved a larger number of subjects at varying risk of colon cancer than in previous studies and used new methods providing greater precision in measuring the proliferative compartment of the colonic crypts. The measurements were carried out under standardized conditions previously reported (4, 5, 17), and the current results are in agreement with earlier findings (1, 4–6, 10, 17). The reproducibility of the method is indicated by a current series, in which duplicate measurements were made on multiple biopsies removed from each of 66 subjects. Using the above ϕ_h criterion of 0.225, the risk status was the same in the duplicate measurements in 88% of the subjects and differed in 12% of the subjects.

In FP, both symptomatic and at-risk subjects displayed a greater expansion of the proliferative compartment than in an unaffected family branch. Similar findings were seen in the setting of FCC. Colon cancer patients from the population at large did not demonstrate the same expansion of the proliferative compartment, but possible bimodality in the crypt-height distribution of labeled cells suggested a subpopulation of patients who share the lesion seen in groups with genetic predisposition to colon cancer.

In this study, we segregated the [^{3}H]dThd-labeled cells into a greater number of crypt compartments, enabling additional observations on the distribution of labeled cells within the colonic crypts of high- and low-risk subjects. The analysis revealed that any natural subdivision of the crypt must have dividing lines falling into the present Compartments 1 and 5, which are in the approximate vicinity of the 15 and the 55% height points in the crypt.

Findings also revealed the lowest region of the colonic crypt, Compartment 0, to be a positive zone of particular interest and a potential marker for risk discrimination; the latter was separated from the adjacent compartments which were negative zones. In addition, identification of the polarity of the adjacent Compartments 2 to 5 facilitated identification of Compartments 6 to 10, which were shown to be the major discriminant for comparison of the high- and low-risk groups.

Although the measurements in this study furnished statistical dividing lines on the basis of which FP_s and FCC_s patients could be characterized, the percentage of subjects showing an expansion of the proliferative compartment was less for FP_s than for FCC_s. In patients with polyposis, a substantial proportion of the mucosal surface has already developed into adenomas; this should partially exhaust the abnormal regions of flat mucosa detectable by [^{3}H]dThd cell labeling and may account for the observed difference, since only flat mucosa is selected for analysis.

This assay system characterizes the cell-labeling patterns of different populations and has potential utility in identifying carriers with genetic susceptibility to colon cancer. In FP but not FCC, clinical characteristics of the syndrome aid in identifying the carrier state (2, 14). Since the estimated frequency of FCC is greater than that of FP (14), there is a special need for laboratory markers to screen at-risk familial members. In the technique under study, it is encouraging that the measured values of ϕ_h (upper-crypt occupancy fraction of labeled cells) in 59% of at-risk subjects in the FCC_a group and in 45% of those of the FP_a group fall above a dividing line that excludes 92% of subjects known to be at low risk. The first 2 percentage values correspond approximately to the proportion of at-risk individuals expected to develop polyposis or FCC, because each condition has an autosomal dominant mode of inheritance.

Using this test system, the expression of an inherited defect in humans resembles the effect of a chemical carcinogen in laboratory animals (12). This is consistent with the role of host-environmental interactions, a multistage process of colon carcinogenesis, and with the concept that a modified gene may predispose cells to the action of endogenous or exogenous carcinogens or tumor promoters. Although the data in this study do not distinguish among the mechanisms by which genetic and environmental influences operate in the development of colon cancer, the methods developed can facilitate further analyses designed to explore this problem.

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