Sources of Variability in Estimating Ornithine Decarboxylase Activity and Polyamine Contents in Human Colorectal Mucosa

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Abstract

The activity of ornithine decarboxylase (ODC), the first enzyme in polyamine synthesis, is elevated during epithelial carcinogenesis. Since this enzyme is a target for colon and other cancer chemoprevention strategies, we sought to identify sources of variability affecting the measurement of tissue ODC activities and polyamine contents. Multiple colorectal biopsies were obtained from 39 patients undergoing colonoscopy. Biopsy size affected polyamine but not ODC values. Spermidine (spd)/spermine (spm) ratios varied less than the contents of the individual amines. Bowel preparation methods did not affect any of the measurements. ODC activities and spd:spm ratios did not vary with bowel location. Lab assay methods contributed to sources of error. Variability was greatest for polyamine content measurements but was reduced when polyamine contents were analyzed as spd:spm ratios. Intrapatient variability of these parameters was as great or greater than interpatient variability. When measured in apparently unaffected colorectal mucosa, none of these parameters were significantly correlated with prior polyp history, number of prevalent polyps found at current colonoscopy, or polyp size. Thus, neither ODC activity nor polyamine contents of normal mucosa appear to be discriminatory markers of colorectal carcinogenesis. However, spd:spm ratios, which show the least variability among measures of polyamine contents, should be a good marker of the consequence of polyamine synthesis inhibition in chemoprevention trials.

Introduction

A variety of intracellular processes requisite for cellular proliferation and differentiation require the polyamines spermidine, spermine, and their precursor, putrescine (1). In mammalian cells, the first step in polyamine synthesis is catalyzed by ODC (2-4). Intracellular polyamine concentrations and ODC activity are highly regulated (1, 5-8). For example, ODC activity is low in quiescent cells but can be increased manyfold within a few hours after exposure to trophic stimuli, including food, growth factors, hormones, and drugs, and after tissue injury during regenerative growth (9-13). In addition, diurnal oscillations in ODC activity have been demonstrated in various organs (14, 15).

Several lines of evidence suggest that ODC may play an important role in neoplastic transformation. ODC activity and polyamine contents are elevated in a variety of human precancers and cancers, relative to adjacent normal tissue specimens of the same organ system (16, 17). In experimental animal models of carcinogenesis, many tumor-promoting agents induce ODC activity in target tissues (18-21), and inhibitors of this enzyme suppress cancer development (22-24). Recently, several groups have reported that ODC overexpression may be sufficient to induce neoplastic transformation (25, 26).

ODC activities and, to a lesser degree, polyamine contents, have been measured in human normal and neoplastic colorectal tissues by a number of groups. We and others find that generally ODC activity and/or polyamine concentrations in colorectal cancer and benign neoplastic polyp tissue tend to be increased, relative to uninvolved normal appearing mucosa (21, 27-38). Some groups have reported that the estimation of these parameters in apparently normal colorectal mucosa may stratify patients by neoplasia status, in that individuals with cancers or adenomatous polyps have higher ODC activities or polyamine contents than those without neoplasia (28, 30, 33, 37, 39-41). Other groups have not found these measurements in flat mucosa to be discriminatory (39, 42-46). Nonetheless, ODC and polyamine contents have been used as efficacy endpoints in short-term intervention trials with the hypothesis that reduction in these parameters might diminish biological susceptibility to neoplastic transformation (47-49).

Measurements of ODC activity and polyamine levels, or any other biological parameter, are associated with technical errors and biological variability. Because a relatively high degree of intrapatient variability will obscure true differences between individuals (interpatient variability) and true biological changes in response to an intervention within an individual, we attempted to identify and quantify sources of intrapatient variability in ODC activities and polyamine contents in human colorectal biopsies. We then compared the magnitude of these variances to differences between patients.

Materials and Methods

Patients

Study subjects consisted of 39 patients scheduled to undergo colonoscopy for standard clinical indications at the Tucson Veterans Affairs Medical Center. Patients were predominately male (38 male, 1 female) with a mean age of 61/4 years. Of these, 33 patients met the criteria for colorectal neoplasia, and 6 were considered to be apparently normal (21).

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3 The abbreviations used are: ODC, ornithine decarboxylase; spd, spermidine; spm, spermine.
of 67 ± 11 years (range, 34–90). Thirty-eight of 39 patients had a complete colonoscopy to the cecum. Patients were categorized by the most significant neoplastic lesion found at previous or current examinations: 1 patient had a malignant polyp; 17 had one or more large (≥1 cm) adenomatous polyps; 11 patients had one or more adenomatous polyps <1 cm, and 9 were polyp free (one patient was not categorized because he had never had a complete colonoscopy).

**Procedural Methods**

Colonoscopy was performed between 8 and 12 a.m. with an Olympus Model CF1T10L colonoscope (Olympus Corp., Lake Success, NY) after bowel purgation with polyethylene glycol solution. Mucosal biopsies were obtained in all subjects with a “large” biopsy forceps (Olympus Model FB-13U); additional biopsies were performed in selected patients with a standard or “regular” size forceps (Olympus Model FB-24E). Patients were included in 5 different subgroups in this protocol. In one group of 10 patients, a single large biopsy was taken during the insertion of the colonoscope from the rectum (10 cm from the anus), transverse colon, and cecum, with a second biopsy of the rectum on completion of the examination. In another group of 15 patients, single large biopsies were obtained from each of 4 quadrants at the same level of the rectum (10 cm from the anus) on withdrawal of the colonoscope. In 5 of these patients, a second set of 4-quadrant large biopsies were taken 1 cm distal to the first biopsies and pooled for each patient. In another group of 10 patients, 6 adjacent biopsies were taken from one wall of the rectum within a 3- × 3-cm area on withdrawal of the colonoscope; 3 biopsies were large and 3 were regular size. Ten patients underwent flexible sigmoidoscopy 7–21 days prior to colonoscopy with an Olympus Model GIF 1T10 endoscope. Examination was initially performed without bowel preparation, and a single large biopsy was obtained from the distal rectum. A 500-mL water enema was subsequently administered, and after evacuation, a second large biopsy was obtained from the same level within the rectum. A final group of 10 patients underwent flexible sigmoidoscopy between 30 and 60 days after colonoscopy, and large biopsies were obtained from the four quadrants of the rectum after water enema preparation.

All biopsies were snap-frozen in liquid nitrogen and stored individually except the pooled set of 4 quadrants biopsies in 5 patients. Histological interpretation of any previous and present colonoscopic findings were recorded. The protocol was approved by the University of Arizona Institutional Review Board.

**Laboratory Methods**

For ODC analysis, tissue samples were thawed and kept on ice during processing. The samples were minced and homogenized in 0.05 M sodium-potassium phosphate buffer, pH 7.2, containing 0.1 mM EDTA and 1.0 mM dithiothreitol at 1 mg tissue weight/50 μL buffer. The soluble fraction was clarified at 2000 × g for 5 min in a refrigerated microfuge, and ODC activity was measured in duplicate as described previously (50). One unit of ODC activity is defined as 1 nmol 14CO2 released from 14C-labeled ornithine/h.

Polyamines were analyzed by reverse-phase, ion-paired high performance liquid chromatography using the method of Seiler and Knodgen (51) as applied to colorectal tissue by us (48). After ODC analysis, the residual soluble fraction was made 0.2 M HClO4, and acid-soluble and acid-insoluble fractions were obtained by centrifugation at 12,000 × g. After addition of diaminohexane as an internal standard, up to 200 μl of the acid soluble fraction was analyzed by high performance liquid chromatography for polyamine contents. Using this method, the limit of detection is ~0.01 nmol for the amines putrescine, spermidine, spermine, and their acetyl derivatives. For the studies conducted here, in which tissue samples generally were prepared at a concentration which corresponded to ~1 mg/200 μl, the limit of polyamine detection was ~0.01 nmol polyamine/1 mg soluble protein. Protein contents of various fractions were determined using either the Bradford (52) or the BCA assay according to the manufacturer’s recommendation (Pierce). Acid-insoluble fractions were solubilized in 0.5 M NaOH prior to analysis for protein contents.

**Statistical Methods**

The data were analyzed in the context of the general linear model. When analyzing the data for the presence of systematic differences between the means, measurements from multiple biopsies were averaged for each patient and then compared using the paired t test and repeated measures analysis of variance. Correlations between the clinical variables and the average of each patient’s biopsy measurements were tested in the context of a linear regression model. In order to stabilize the variance, the regressions were performed on logarithmic transformations of the variables. The adequacy of these models was investigated using residual analysis.

In order to estimate the variation in each of the ODC activity or polyamine measurements, a variance components analysis was performed. Variance components were estimated by the analysis of variance method, and those estimates were used to compute the intraclass correlation.

**Results**

**Systematic Effects Due to Biopsy Procedure**

Subsets of patients were studied to assess any systematic trends in the ODC activity and polyamine measurements that were associated with biopsy techniques.

**Biopsy Size.** Six adjacent biopsies (3 large, 3 regular) were taken from one wall of the rectum in a subgroup of 10 patients and assayed in the same laboratory run. The ODC and polyamine measurements for the large biopsies versus the regular biopsies were compared using repeated measures analysis of variance. Table 1 contains the average measurements across the 10 patients for each of the ODC and polyamine measurements by biopsy size. The polyamine measurements were significantly higher in the large biopsies. The differences in the ODC measurements (P = 0.74) and spd:spm ratio (P = 0.08) were not statistically significant.

In a second subgroup of five patients, eight biopsies were taken (two adjacent biopsies from each quadrant). For each patient, four biopsies (one from each quadrant) were assayed individually, and four biopsies were combined and four measurements made from the combined lysate. As seen in Table 2, ODC and putrescine measurements were significantly higher when the biopsies were combined. The differences in spermidine and spermine measurements,
transverse colon measurements to have higher putrescine without bowel preparation and after two bowel preparations.

Colon Preparation. A single rectal biopsy was obtained from each of three different colonic regions in 10 patients and differences among the colon locations sampled. Table 3 contains the results of a component of variation analysis applied to these data. Such an analysis at-tempted to quantify the degree to which various sources of error contribute to the variability of a measurement. We assumed a model in which the measurement of the true ODC activity or polyamine content for a given patient was

Table 1 ODC activities* and polyamine contents* by biopsy size

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Large</th>
<th>Regular</th>
<th>Difference</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODC activity</td>
<td>0.858 ± 0.158</td>
<td>0.902 ± 0.127</td>
<td>0.044 ± 0.136</td>
<td>0.745</td>
</tr>
<tr>
<td>Putrescine</td>
<td>0.042 ± 0.014</td>
<td>0.016 ± 0.006</td>
<td>0.026 ± 0.011</td>
<td>0.023</td>
</tr>
<tr>
<td>Spermidine</td>
<td>0.485 ± 0.030</td>
<td>0.358 ± 0.020</td>
<td>0.127 ± 0.041</td>
<td>0.003</td>
</tr>
<tr>
<td>Spermine</td>
<td>1.149 ± 0.051</td>
<td>0.882 ± 0.029</td>
<td>0.267 ± 0.086</td>
<td>0.003</td>
</tr>
<tr>
<td>Spd:spm</td>
<td>0.420 ± 0.016</td>
<td>0.401 ± 0.008</td>
<td>0.019 ± 0.011</td>
<td>0.077</td>
</tr>
</tbody>
</table>

* Mean ± SEM for n = 10 patients; ODC activities are given in units (as defined in “Materials and Methods”) /1 mg soluble protein.
* Polyamine contents are given in nmol polyamine /1 mg soluble protein.
* P value from paired t test of equality of means.

Table 2 ODC activities* and polyamine contents* by combination of biopsies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Individual</th>
<th>Combined</th>
<th>Difference</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODC activity</td>
<td>1.397 ± 0.181</td>
<td>1.728 ± 0.167</td>
<td>0.332 ± 0.085</td>
<td>0.017</td>
</tr>
<tr>
<td>Putrescine</td>
<td>0.121 ± 0.012</td>
<td>0.191 ± 0.024</td>
<td>0.071 ± 0.020</td>
<td>0.023</td>
</tr>
<tr>
<td>Spermidine</td>
<td>0.611 ± 0.064</td>
<td>0.719 ± 0.078</td>
<td>0.108 ± 0.121</td>
<td>0.419</td>
</tr>
<tr>
<td>Spermine</td>
<td>1.200 ± 0.104</td>
<td>1.620 ± 0.144</td>
<td>0.420 ± 0.157</td>
<td>0.055</td>
</tr>
<tr>
<td>Spd:spm ratio</td>
<td>0.495 ± 0.021</td>
<td>0.460 ± 0.060</td>
<td>0.035 ± 0.053</td>
<td>0.542</td>
</tr>
</tbody>
</table>

* Mean ± SEM for n = 5 patients; ODC activities are given in units (as defined in “Materials and Methods”) /1 mg soluble protein.
* Polyamine contents are given in nmol polyamine /1 mg soluble protein.
* P value from paired t test of equality of means.

Table 3 ODC activities* and polyamine contents* by colon location

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rectum</th>
<th>Rectum</th>
<th>Transverse</th>
<th>Cecum</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODC activity</td>
<td>0.435 ± 0.103</td>
<td>0.669 ± 0.169</td>
<td>0.502 ± 0.112</td>
<td>0.495 ± 0.057</td>
<td>0.336</td>
</tr>
<tr>
<td>Putrescine</td>
<td>0.004 ± 0.004</td>
<td>n.d.</td>
<td>0.030 ± 0.016</td>
<td>0.052 ± 0.016</td>
<td>0.006</td>
</tr>
<tr>
<td>Spermidine</td>
<td>0.700 ± 0.120</td>
<td>0.678 ± 0.055</td>
<td>0.418 ± 0.072</td>
<td>0.336 ± 0.023</td>
<td>0.001</td>
</tr>
<tr>
<td>Spermine</td>
<td>1.609 ± 0.287</td>
<td>1.508 ± 0.119</td>
<td>0.943 ± 0.087</td>
<td>0.829 ± 0.028</td>
<td>0.001</td>
</tr>
<tr>
<td>Spd:spm ratio</td>
<td>0.443 ± 0.028</td>
<td>0.457 ± 0.033</td>
<td>0.424 ± 0.036</td>
<td>0.407 ± 0.026</td>
<td>0.235</td>
</tr>
</tbody>
</table>

* Mean ± SEM for n = 10 patients; ODC activities are given in units (as defined in “Materials and Methods”) /1 mg soluble protein.
* Polyamine contents are given in nmol polyamine /1 mg soluble protein.
* During insertion of colonoscope.
* During withdrawal of colonoscope.
* P value from repeated measures of analysis of variance.
* n.d., none detected (see “Materials and Methods” for limits of detection).

although tending to be higher in the combined biopsies, did not achieve statistical significance, nor did the differences in spd:spm ratios.

Biopsy Location. Single large biopsies were obtained from each of three different colonic regions in 10 patients and assayed in the same laboratory run. Clearly evident from the results shown in Table 3 was a tendency for the cecum and transverse colon measurements to have higher putrescine and lower spermidine and spermine measurements. The spd:spm ratio was less consistently lower in the cecum and transverse colon, and differences in the ratio means were not statistically significant. The ODC measurements showed still greater variability with no consistent differences observed among the colon locations sampled.

Colon Preparation. A single rectal biopsy was obtained without bowel preparation and after two bowel preparation regimens (water enema and polyethylene glycol lavage) in a subgroup of 10 patients. A single patient had biopsies obtained after no preparation and water enema only. The biopsies were assayed for ODC and polyamine measurements. As seen in Table 4, a repeated measures analysis of variance failed to show statistical significance among the preparation regimens with respect to either ODC or polyamine measurements.

Source of Variation

Single large biopsies from each quadrant of the rectum 10 cm above the anus were obtained at endoscopy from 25 patients following a water enema. Fig. 1 displays the ODC and polyamine measurements from these biopsies, along with similar measurements obtained from 10 patients in the biopsy size study. Immediately apparent is the large amount of variation between blocks of patients, whose biopsies were assayed on different dates. Table 5 presents the average measurements across patients for each laboratory run. Analysis of variance was performed to test the equality of the distributions for ODC and polyamine measurements across the five laboratory runs. All variables were logarithmically transformed to adjust for the increasing variance of the measurements as the magnitude of the measurement increased. A significant effect due to laboratory run was observed for ODC and all of the polyamine measurements, but not for the spd:spm ratio.

Table 6 contains the results of a component of variation analysis applied to these data. Such an analysis attempts to quantify the degree to which various sources of error contribute to the variability of a measurement. We assumed a model in which the measurement of the true ODC activity or polyamine content for a given patient was
Variability in ODC and Polyamine Measurements

Table 4. ODC activities* and polyamine contents† by colon preparation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>None</th>
<th>Water</th>
<th>PEG*</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODC activity</td>
<td>0.321 ± 0.083</td>
<td>0.355 ± 0.088</td>
<td>0.435 ± 0.103</td>
<td>0.500</td>
</tr>
<tr>
<td>Putrescine</td>
<td>0.002 ± 0.002</td>
<td>n.d.</td>
<td>0.004 ± 0.004</td>
<td>0.376</td>
</tr>
<tr>
<td>Spermidine</td>
<td>0.666 ± 0.111</td>
<td>0.781 ± 0.107</td>
<td>0.700 ± 0.120</td>
<td>0.150</td>
</tr>
<tr>
<td>Spermine</td>
<td>1.630 ± 0.301</td>
<td>1.636 ± 0.229</td>
<td>1.609 ± 0.287</td>
<td>0.866</td>
</tr>
<tr>
<td>Spd:spm ratio</td>
<td>0.425 ± 0.032</td>
<td>0.478 ± 0.027</td>
<td>0.433 ± 0.028</td>
<td>0.204</td>
</tr>
</tbody>
</table>

*Mean ± SEM; ODC activities are given in units (as defined in "Materials and Methods")/mg soluble protein.
†Polyamine contents are given in nmol polyamine/mg soluble protein.
*= Polyethylene glycol lavage.
†P value from repeated measures analysis of variance.

subject to errors due to laboratory assay date, measurement error within a laboratory run, and biological variation among biopsies from the same patient. Of interest in this analysis was the intraclass correlation. This parameter represents the relative size of the variation among the patients' true values compared to the total variability of the measurements. A value close to 0 suggests that the patients' true values are masked by the noise of the measurement, whereas a value close to 1 suggests that the measurement easily separates the patients with respect to their true values.

From Table 6, it is clear that none of the measurements were free of noise. The polyamine measurements had a very low intraclass correlation. The ODC activity and spd:spm ratios had moderate intraclass correlations, with most of the noise coming from the variation among biopsies within a laboratory run rather than the variation due to laboratory run.

The interpatient variability estimated in the analysis presented in Table 6 was based on biopsies obtained at a single time. We expected the ODC and polyamine meas-
Spd:spm ratio 0.004 0.003 0.000 0.493
Spermine -4.368 29.670 60.063 -0.05 1
ODC Activity 0.344 0.171 0.099 0.560

tained at 3 different clinic visits separated by several weeks, but all biopsies were assayed in the same laboratory run. Such a sampling scheme precluded our ability to simultaneously estimate the variance components due to between patient variability, within patient variability over time, between biopsy variability within a patient at a single time, and variability due to laboratory run. We could, however, use the data from the multiple endoscopy sessions to estimate the relative magnitude of the true inter- and intrapatient variability over time. Using this approach, we estimated that the true interpatient variability in ODC activities and spd:spm ratios were 22.0 and 29.2%, respectively, of the combined inter- and intrapatient variability over time.

**Associations with Clinical Variables**

Linear regression analysis of the full data set was performed which modeled the logarithmically transformed ODC and polyamine measurements as a function of polyp status and age while adjusting for the effects due to laboratory runs. None of these analysis showed statistically significant associations between ODC or polyamine measurements and polyp history or age (P > 0.20 in all models).

**Discussion**

Elevated ODC activities and polyamine contents are generally associated with increased cell growth (1, 5). Drugs which block polyamine synthesis have potent anticarcinogenic effects in experimental animal models (22–24). These findings have been the rationale for further investigation of ODC and polyamine measurements as indicators of cancer risk and intermediate markers of the antitumor effects of preventive interventions (53).

There have been few reports in the literature addressing quality assurance issues related to these measurements in colorectal mucosa. It has been reported that the measurement of ODC assay in this tissue is sensitive to minor technical factors (54, 55). Age, gender, bowel preparation, and biopsy location (proximal versus distal colon) have also been reported to seemingly affect ODC activity (56–58).

Our studies suggest a need for standardization of a biopsy technique when measuring colorectal tissue ODC activities and polyamine contents. We found a tendency for larger or pooled biopsies to result in higher values of ODC activities and polyamine contents. Spd:spm ratios, however, were relatively unaffected by biopsy size. While we found a significant difference in polyamine levels by location in the colon, spd:spm ratios were less affected by location, although there did appear to be a nonsignificant trend toward lower ratios in the more proximal colon. The ODC measurements showed no clear trend by location. Colon preparation methods, including no prep, water enema, or polyethylene glycol lavage did not affect ODC or polyamine measurements.

A highly significant effect of technical factors on the ODC and polyamine measurements was evident in this study. The use of different protein assays for different laboratory runs appeared to account for much of the variation between batches, although other factors might also have been contributory (45, 56). In direct comparisons, the BCA assay detected greater protein contents in rectal mucosal samples compared to the Bradford assay. When ODC and polyamine contents are assayed on different dates, such as might happen in a clinical study over a protracted period of time, normalization to tissue protein contents can be a source of considerable error, especially when more than one method is used. The assessment of spd:spm ratios does not depend on measurements of polyamine content, nor is it affected by other technical errors, such as volume measurements during sample processing. The high intraclass correlation (Table 6) for spd:spm ratios signifies that the true values of this parameter are least masked by measurement noise compared to other measures of polyamine content.

Since most of the random noise in those measurements was ascribed to interbiopsy variation within a patient, the intraclass correlation could be improved by individually assaying multiple biopsies. If the measurements of k biopsies, in which each biopsy is assayed separately and not pooled, are averaged, the contribution of the between biopsy variation to the total variability is proportionately decreased by a factor of 1/k. Thus, if the results from 4 biopsies were used to assess ODC activities or polyamine contents, the intraclass correlation would be estimated at 0.708 and 0.750 for ODC and spd:spm ratio, respectively.

**Table 5** ODC activities and polyamine contents by date of assay

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>Fourth</th>
<th>Fifth</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODC activity</td>
<td>0.510 ± 0.066</td>
<td>0.408 ± 0.166</td>
<td>1.396 ± 0.167</td>
<td>1.140 ± 0.618</td>
<td>0.858 ± 0.158</td>
<td>0.008</td>
</tr>
<tr>
<td>Putrescine</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.120 ± 0.012</td>
<td>1.767 ± 0.542</td>
<td>0.042 ± 0.045</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spermidine</td>
<td>0.644 ± 0.063</td>
<td>0.087 ± 0.008</td>
<td>0.611 ± 0.064</td>
<td>8.512 ± 1.301</td>
<td>0.485 ± 0.030</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spermine</td>
<td>1.430 ± 0.097</td>
<td>0.222 ± 0.020</td>
<td>1.200 ± 0.104</td>
<td>19.864 ± 2.281</td>
<td>1.149 ± 0.051</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spd:spm ratio</td>
<td>0.459 ± 0.030</td>
<td>0.398 ± 0.021</td>
<td>0.485 ± 0.021</td>
<td>0.429 ± 0.020</td>
<td>0.420 ± 0.016</td>
<td>0.141</td>
</tr>
</tbody>
</table>

* Mean ± SEM; ODC activities are given in units (as defined in "Materials and Methods")/1 mg soluble protein.

* Polyamine contents are given in nmol polyamine/1 mg soluble protein.

* P value from repeated measures analysis of variance.

* n.d., none detected.

**Table 6** Sources of variation for ODC activity and polyamine content (variance components and intraclass correlation for n = 35 patients)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>ODC Activity</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
<th>Spd:spm ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>0.344</td>
<td>0.134</td>
<td>-0.229</td>
<td>-4.368</td>
<td>0.004</td>
</tr>
<tr>
<td>Biopsy</td>
<td>0.171</td>
<td>0.305</td>
<td>5.400</td>
<td>29.670</td>
<td>0.003</td>
</tr>
<tr>
<td>Assay date</td>
<td>0.099</td>
<td>0.486</td>
<td>10.875</td>
<td>60.063</td>
<td>0.000</td>
</tr>
<tr>
<td>Intracl. correlation*</td>
<td>0.560</td>
<td>0.145</td>
<td>-0.014</td>
<td>-0.051</td>
<td>0.493</td>
</tr>
</tbody>
</table>

* Ratio of between patient variability to total variability.
The variance components estimated in Table 6 can be used to estimate 95% prediction intervals for the difference between two ODC or polyamine measurements when the true underlying values are equal. Because some of the sources of error can be attenuated by repeated sampling, we consider in Table 7 the 95% prediction intervals for the mean of $k = 1, 2, 4, 8$ biopsies. The width of the prediction intervals will also be affected by whether the comparison is made between measurements assayed in the same laboratory run (such as would occur when comparing different patients) or in different laboratory runs (such as would occur when following a single patient over time). Thus, it is not unusual to see differences of $\pm 1.84$ units in ODC measurement based on single biopsies and assayed within the same laboratory run, even when the true ODC activity levels are equal. If we compare biopsies assayed in different laboratory runs, that prediction interval increases to $\pm 2.03$. When the comparison is based on the average of four biopsies, the prediction intervals shrink to $\pm 1.55$ and $\pm 1.77$ for comparisons within or across laboratory runs, respectively. From Table 7 it is apparent that the polyamine measurements are affected much more by differences between laboratory runs, and the spd:spm ratio is affected less.

Spd:spm ratio measurements are not reliable as an indicator of cancer risk. We were unable to demonstrate an association between polyamine history or age and the spd:spm ratio in the small study reported here. In a larger study to be reported elsewhere, we did find a statistically significant inverse relationship between spd:spm ratios and patient age.

In summary, this study identifies a number of technical and biological factors affecting the measurement of ODC activity and polyamine contents in human colorectal mucosal tissue. First, our results show that standardization of biopsy procedures, including maximization of biopsy size, is necessary in order to minimize variability in measurements of these end points. Second, none of these parameters of polyamine metabolism appears to be a reliable marker of cancer risk. Finally, spd:spm ratios seem to be the most reliable marker of human colonic mucosal polyamine contents when reliability is defined in terms of reproducibility. This final conclusion has implications for certain proposed clinical cancer prevention trials. Inhibitors of ODC activity, such as $\alpha$-difluoromethylornithine, suppress putrescine and spermidine, but not spermine, contents in cells and tissues (6, 48, 49). Thus, measurement of spd:spm ratios in target tissues may be the optimal method of assessing the short-term efficacy of ODC inhibitors in human chemoprevention studies.

References
Sources of variability in estimating ornithine decarboxylase activity and polyamine contents in human colorectal mucosa.

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