Abnormalities in the expression of Lewis blood group-phenotype. They are residents of a rural Andean region of Nariño, Colombia, are the subject of this report. They were chosen because they had a histological diagnosis of intestinal metaplasia who are of the Lewis(a-b+) phenotype. The age of participating subjects at the time of the study each parameter was independently evaluated.

The evaluation of siabo- or sulfomucins was done blindly on October 19, 2017. © 1992 American Association for Cancer Research. cebp.aacrjournals.org Downloaded from
Le\textsuperscript{b} specificities. Monoclonal antibody 2.25LE (working dilution 1:40) reacts strongly with Le\textsuperscript{b} antigen and very weakly with Le\textsuperscript{a} antigen (18). Monoclonal antibody 7LE (working dilution 1:60) reacts with Le\textsuperscript{a} antigen and does not react with Le\textsuperscript{b} antigen (19–20). Monoclonal antibodies 252188 (Diagast; working dilution 1:150) and 16485G10 (Diagast; working dilution 1:70), have anti-A and anti-B specificities, respectively (21–22). Finally, monoclonal antibody A583 (Dako; working dilution 1:10) was used to recognize H type 2 structures. Only patients identified as Le\textsuperscript{b} by the above methodology are included in the present report.

The immunoperoxidase staining was performed on 3-\(\mu\)m sections, after routine deparaffinization and rehydration. The endogenous peroxidase was abolished after treatment for 15 min with 3\% hydrogen peroxide. The sections were successively incubated with (a) the working dilution of primary antibodies for 30 minutes; (b) peroxidase-conjugated rabbit anti-mouse immunoglobulin (P161; Dako), diluted 1:100, for 15 min; (c) peroxidase-conjugated swine anti-rabbit immunoglobulin (P399; Dako), diluted 1:100, for 15 min; and (d) a freshly prepared solution of 0.05\% 3-3’-diaminobenzidine tetrahydrochloride (D-5636; Sigma), 0.01\% hydrogen peroxide in phosphate-buffered saline, 0.01 M phosphate, and 0.15 M NaCl, at pH 7.2, for 10 min. The sections were faintly counterstained with Lillie’s hematoxylin and mounted with Permount (Fisher Scientific). Positive and negative controls were stained with every batch of samples. All the incubation steps were carried out at 37\(\degree\)C and followed by three washing steps with phosphate-buffered saline.

The ABH phenotype was determined on tissue samples by immunohistochemical staining of the gastric epithelium with anti-ABH monoclonal antibodies. The positive pattern of these reagents was used to establish the secretor status. Those cases that showed positive stain in the foveolar (surface) epithelium as well as deep glands were considered secretors; cases with negative reaction in the superficial epithelium were considered nonsecretors. The Lewis phenotype was determined by positive staining of the gastric surface epithelium with anti-Le\textsuperscript{a} or anti-Le\textsuperscript{b} monoclonal antibodies. Positive cases showed a diffuse homogeneous staining restricted to the surface gastric epithelium (23).

The anomalous presence of Le\textsuperscript{a} antigen was classified into three grades: 1, Le\textsuperscript{a} antigen in goblet cells only; 2, Le\textsuperscript{a} antigen abundant in goblet cells and weakly positive in columnar cells; 3, Le\textsuperscript{a} antigen abundantly present...
Table 1  Number of cases and proportional distribution of anomalous Lewis* presence in the first and second biopsies

<table>
<thead>
<tr>
<th>Second biopsy</th>
<th>Patterns 1 and 2</th>
<th>Pattern 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>24 (42.9)</td>
<td>0 (0.0)</td>
<td>33</td>
</tr>
<tr>
<td>Patterns 1 and 2</td>
<td>23 (41.1)</td>
<td>2 (0.3)</td>
<td>54</td>
</tr>
<tr>
<td>Pattern 3</td>
<td>9 (16.1)</td>
<td>9 (18.0)</td>
<td>18</td>
</tr>
<tr>
<td>Subtotal</td>
<td>56 (100.0)</td>
<td>11 (100.0)</td>
<td>117</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentages.

**Results**

Table 1 shows the abnormal expression Lea antigen in the first and second biopsies. Overall, the prevalence of abnormalities increased from 52% (61 of 117) in the first biopsy to 72% (84 of 117) in the second. Approximately 38% (below diagonal, 44 of 117) of subjects showed increased abnormalities (presumably progression of the precancerous process), 53% had no change (diagonal), and 9% (above diagonal, 11 of 117) regressed.

Table 2 compares the changes in Lea antigen expressed and the histological presence or absence of dysplastic changes in the first and second biopsy series. When the histological changes progressed from intestinal metaplasia to dysplasia, approximately one-half of the patients (14 of 27) showed progression of antigenic abnormalities; when the interval of follow-up was at least 5 years, no regressions of Lewis abnormalities were noted. When the histological lesions did not change over time, the Lewis alterations remained unchanged or progressed, with a small minority showing regression. After 5 years of follow-up there were no regressions of Lea antigen expression, with the exception of one case, which originally had a grade 1 alteration.

Fig. 3. Gastric dysplasia. A closely packed group of tubular glands replace the original gastric glands. They have reduced mucus secretion and large, hyperchromatic, overlapping nuclei. H&E.
Table 3 shows the time trends in the abnormal expression of Le\(^a\) antigen as related to the presence or absence of sulfomucins in the metaplastic cells in the first and second biopsy series. When sulfomucins appeared \textit{de novo}, so did the abnormal Le\(^a\) antigen in 72\% (13 of 18) of the cases. When sulfomucins were present in the first but not in the second biopsy, the Le\(^a\) antigen persisted in most cases (17 of 21). Separate analysis of the pattern of secretion of sulfated mucins showed that when the progression from negative to positive involved the goblet cells only, the proportion of progression of Lewis abnormalities was 60\%; when the sulfomucins appeared in columnar cells, the progression in Le\(^a\) antigen abnormalities was 87\%.

The estimated risks of histological changes are shown in Table 4. A significant and strong trend was detected for the new expression of cobonic metaplasia, indicating that Le\(^a\) expression is positively associated with the progression of the histological phenotype. No statistically significant trend was observed with the new expression of dysplasic histological phenotype or with regression patterns of such phenotype. A significant positive gradient of Le\(^a\) abnormality is observed with progression of sulfomucin expression and a significant negative gradient with loss of the sulfomucin phenotype, apparently indicating colinearity of the two markers.

### Sensitivity and Specificity

These parameters were studied in relation to new appearances (second biopsy only) of colonic metaplasia, sulfomucins, and dysplastic changes. In Table 5, only cases which at the time of the first biopsy were negative for the phenotypes under study were considered. It shows the number of cases in which both phenotypes remained negative, as well as those in which only one or both phenotypes made their appearance \textit{de novo} in the second biopsy.
Twenty-one of them showed colonic-type cells (no brush border) on hematoxylin-eosin stains on second biopsy. The odds of colonic metaplasia on morphological grounds is 6.9 if the Le\(^a\) antigen becomes abnormally expressed. The sensitivity of abnormal Lewis antigen as a marker of morphological colonic metaplasia is 76.2%, and its specificity is 68.2%.

Forty-seven subjects who had no dysplasia or Lewis\(^a\) antigen at first biopsy were examined for the new appearance of abnormal Le\(^a\) antigen and dysplastic changes in the second biopsy. The odds ratio of dysplasia in patients with abnormal Le\(^a\) antigen is 5.3. The sensitivity of the marker is 80% and its specificity 59%. Further analysis of this subgroup determined that 40 patients had small intestinal type of metaplasia at first biopsy, and for them there was a significant association of transformation to colonic metaplasia and dysplasia if the Le\(^a\) antigen was present in the second biopsy ($\chi^2 = 5.17; P = 0.02$). Only seven subjects had foci of colonic metaplasia at first biopsy, which prevented further statistical analysis of this group.

Table 6 shows a similar analysis of the simultaneous new appearance of both markers (Le\(^a\) antigen and sulfomucins) which are indicators of colonic metaplasia and dysplasia. The odds of new colonic metaplasia carried by the new appearance of both markers is 32.7 with a sensitivity of 87% and a specificity of 82%. The odds of new dysplastic changes carried by the new appearance of both markers is 13.0 with a sensitivity of 67% and a specificity of 87%. Table 7 summarizes the results of the value of the Lewis and sulfomucin markers as indicators of colonic metaplasia and dysplasia.

### Discussion

Extensive research on the natural history of the precancerous phenotype has been reported in gastric carcinoma and in precursors of gastric carcinoma. When the techniques are applied to biopsy material, as in the present report, a certain degree of sampling error is expected because the metaplastic and dysplastic lesions are known to be multifocal in nature. It is not known to what degree phenotypic abnormalities of the mucus secretions may regress, but that possibility should not be ruled out a priori. In the normal gastrointestinal tract of Le\(^a\) individuals, the Le\(^a\) antigen is expressed in the epithelial cells of intestine but never found in the stomach (24-25). Consequently, this Le\(^a\) epitope shows an intestinal organ specificity. Thus, the expression of Le\(^a\) antigen in gastric

### Table 5
Cases with new appearance of sulfomucins, colonic metaplasia, and dysplasia on second biopsy

<table>
<thead>
<tr>
<th>Lewis(^a) anomaly</th>
<th>Sulfated mucins</th>
<th>Colonic metaplasia</th>
<th>Dysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>–</td>
<td>12</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>+</td>
<td>21</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>19</td>
<td>27</td>
</tr>
</tbody>
</table>

### Table 6
Simultaneous new appearance of abnormal Lewis\(^a\) antigen and sulfomucins in cases with and without colonic metaplasia and dysplasia in the second biopsies

<table>
<thead>
<tr>
<th>Colonic metaplasia</th>
<th>Le(^a) Su(^a)</th>
<th>Le(^a) Su(^+)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>14</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>+</td>
<td>3</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Subtotal</td>
<td>17</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>Dysplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>13</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>+</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Subtotal</td>
<td>15</td>
<td>6</td>
<td>21</td>
</tr>
</tbody>
</table>

### Table 7
Odds ratio, sensitivity, specificity, and predictive values of Lewis\(^a\) abnormalities and sulfomucin expression as markers with colonic metaplasia and dysplasia

<table>
<thead>
<tr>
<th>Lewis(^a)</th>
<th>Sulfomucin</th>
<th>Colonic metaplasia</th>
<th>Dysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le(^a)</td>
<td>6.6</td>
<td>5.8</td>
<td>4.9</td>
</tr>
<tr>
<td>Su(^a)</td>
<td>6.6</td>
<td>5.8</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Le\(^a\), positive for abnormal Lewis\(^a\) antigen; Su\(^a\), positive for sulfomucins; CM, colonic dysplasia; OR, odds ratio; CI, confidence interval; PV, predictive value.
columnar cells can be regarded as ectopic. Such ectopic antigen expression associated with the precancerous process in the stomach (26) as well as in the colon (27–28) has often been reported in the literature (25).

The value of the abnormal expression of Le\(^a\) antigen as a marker of the progression of the gastric precancerous state is tested in this prospective study of a population at high risk of gastric cancer. The ratio of individuals positive at first and second biopsy is one measure of the changes over time in Le\(^a\) antigen status. In the group under study there were 61 and 84 individuals positive at first and second biopsy, respectively. The odds ratio, which measures the risk of an individual being positive on second biopsy versus unit risk on first biopsy, was 2.43. This ratio may be biased by uncontrolled factors. For example, persons are older at second biopsy and thus carry an excess risk by virtue of their more advanced age. Or people tested at an earlier date may have a longer period of surveillance in which to develop anomalies and progress histologically. The odds ratio when adjusted to control for each of these factors is virtually unchanged, indicating that age and duration of observation need not be controlled in the analysis.

From the data as of first and second biopsy, the Le\(^a\) status was classified as progression, no change, or regression. Groups were identified for comparative purposes and tested for statistical significance trends in Le\(^a\) status. Evidence of progression was implied by the de novo appearance of colonic metaplasia or dysplasia. Our data indicate that the abnormality increases in frequency proportionally to the length of follow-up as well as to the incidence of colonic metaplasia and dysplasia. The value of the marker was compared with another alteration of the mucin secretions of the gastric epithelium, i.e., the expression of sulfomucins which have been more extensively studied (29–32). It seems clear from the data presented that in this high-risk population the abnormal expression of Le\(^a\) antigen tends to appear earlier than that of the sulfomucins and for that reason tends to be less specific but more sensitive as an index of progression. The expression of both markers (sulfomucins and abnormal Le\(^a\)) conveys the highest relative risks and the highest specificity of the indexes of progression.

The appearance of abnormal mucins and Lewis antigen abnormalities in more advanced stages of the gastric precancerous process lends support to the multistage model of carcinogenesis for this organ. An increasing armamentarium of biomarkers is becoming available to monitor the progression of the neoplastic process in clinical research.

References

Prospective study of Lewis antigen alterations in the gastric precancerous process.

J Torrado, P Correa, B Ruiz, et al.