Lymphoid Hyperplasia

A Major Prognostic Feature in 519 Cases of Colorectal Carcinoma

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The size of the regional lymph nodes, germinal center, and paracortical areas, and the degree of perivascular lymphocyte cuffing (PLC) at the edges of 519 carcinomas of the large bowel have been analyzed microscopically and assessed quantitatively. Hyperplasia of these lymphoid areas, defined as relative or absolute size exceeding the median for the tumor stage, has been related to cancer-specific survival data for each of Dukes' Stages A, B, and C, and for disseminated disease commonly referred to as Stage D. Germinal center hyperplasia was associated with a major survival advantage in Stage B ($P = 0.003$) and in Stage C ($P = 0.041$) if present in tumor-involved lymph nodes. Paracortical hyperplasia related favorably to survival in Stages B and C; in Stage C such hyperplasia was most favorable if present in tumor-involved lymph nodes ($P = 0.009$). PLC related to favorable survival data only in Stage B. Lymphoid hyperplasia showed no correlation with survival in Stages A and D. (Am J Pathol 1980, 100:469-480)

COLORECTAL CARCINOMA is the second most common cause of death from malignant disease in Victoria, Australia.[1] Survival figures have not improved during the last 25 years in spite of earlier diagnosis and treatment.[2] It is therefore important to define risk categories and to diagnose postoperative recurrences at the earliest possible stage so that the best available treatment modalities can be instituted promptly.[3]

The aim of the present paper is to demonstrate that lymphoid hyperplasia, which is more common in "localized" tumor stages,[4] is of major prognostic importance also within Dukes' Stages B and C and that its absence is associated with a high death rate due to recurrent carcinoma.

Materials and Methods

The series consists of a total of 519 patients with confirmed adenocarcinomas of the colon and rectum who had their tumors resected between 1966 and June 1978 and examined histologically in this department of pathology. The patients were selected only insofar as they survived at least 3 months and that follow-up information, including cause of death, when applicable, was available. The mean age was 60 ± 12 years, and 287 of the patients

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were male. Follow-up (range 3–155 months) information was recorded at outpatient visits, generally at 1, 3, 6, and 12 months postoperatively followed by annual visits.

Tumor Staging

The operative specimens were examined fresh. Blocks of tumor tissue and all available lymph nodes (mean 5.2 ± 4.0) were fixed in formalin and processed for histologic examination according to routine. All slides were subsequently reexamined by one pathologist (E.P), applying conventional criteria for tumor staging [4,5] "blindly," ie, without knowledge of survival data. The latter were subsequently compiled by an independent worker. Stages A, B, and C did not include any tumors where macroscopic deposits extended beyond the operation area, locally or in the form of spread to distant organs (palliative resections). The latter group and cases with tumor invasion of adjacent organs were referred to as Stage D. The number of cases in Stages A, B, C, and D was 71, 213, 163, and 72, respectively.

Quantitative Assessment of Lymphoid Tissue

Two regional lymph node compartments, the germinal centers (Figure 1) and the paracortical areas (Figure 2) defined elsewhere,[6] and the total lymph node area measured on the histologic sections were assessed quantitatively.[4] Briefly, a microscope equipped with a 25-intersection standardized square eyepiece graticule (Zeiss, Oberkochen) was calibrated so that the square at ×4 objective magnification corresponded to a section area of 8.1 sq. mm. The number of intersections falling on germinal centers and paracortex in relation to the total counted is then proportional to their relative sizes.[7,8] These two lymph node compartments were expressed quantitatively as percentages of the total sectioned area of all lymph nodes in each case and as absolute area in square millimeters per "mean" sectioned lymph node. We assessed lymph nodes with tumor deposits separately, ignoring the actual tumor areas in the calculations.

The diameter of a hypothetical uninvolved "mean" lymph node, in each case assumed to be spherical, was calculated from the total lymph node counts and the above-mentioned quantitative relations. This diameter is hereafter referred to as size.

Perivascular lymphocyte cuffs (PLCs) (Figure 3), ie, the area occupied by sheaths of small, dark lymphocytes in the muscle, and pericolic/rectal fat at the deep tumor edge were assessed in 512 cases; in 7 the sections did not include the deep edge. We counted a minimum of 200 intersections (64.8 sq. mm), using the ×4 objective. Tissue within the tumor stroma or outside a hypothetical vertical line parallel to the lateral tumor edges was not assessed. PLC data were expressed as a percentage rounded off to the nearest whole number.

Statistical Evaluation of Lymphoid Hyperplasia and Cancer-Specific Survival

The quantitative lymph node and PLC data were evaluated separately within each tumor stage and the median established and used as a cut-off point for each of these parameters (Tables 1–4). When the percentages (relative sizes) for or absolute sizes of these compartments exceeded the median for the stage, the "mean" lymph node in each case was considered hyperplastic for germinal center or paracortical areas, respectively. Mean lymph node cellular proliferation or size exceeding the median for the tumor stage was considered as "hyperplastic," or "large." Values on or below the median were considered "hypoplastic," or "small." A PLC exceeding the median was considered "hyperplastic." Lymph node and PLC data as defined above were related to survival, ie, time from resection to death of cancer recurrence (uncensored data), or time to last observation in patients who did not die of cancer recurrence (censored data). The survival data were plotted by the method of Kaplan and Meier.[9] Differences between survival curves were

evaluated statistically by the Wilcoxon test according to Gehan [10] and were considered significant at the $P < 0.05$ level.

Results

No lymph node or PLC measurements related to survival data in Stages A and D (Tables 1 and 4).

Table 1—Stage A: Tumor and Regional Lymph Node Features in Relation to Cancer Survival (SUR)

Feature (median)	Died/total	SUR*	P†
Lymph nodes			
Germinal centers (0.5 sq mm)			
Hyperplastic	2/33 = 0.06	24	0.3
Hypoplastic	1/33 = 0.03	30	
Paracortex (2.5 sq mm)			
Hyperplastic	1/33 = 0.03	28	0.3
Hypoplastic	2/33 = 0.06	25	
Size (4.1 mm)			
Large	1/31 = 0.03	26	0.3
Small	2/35 = 0.06	25	
Perivascular lymphocyte cuffs (2%)			
Hyperplastic	2/35 = 0.06	26	0.3
Hypoplastic	1/35 = 0.03	28	

* Months at 95th percentile.
† *P* values refer to generalized Wilcoxon comparison of differences between survival curves.

Table 2—Stage B: Tumor and Regional Lymph Node Features in Relation to Cancer Survival (SUR)

Feature (median)	Died/total	SUR*	P
Lymph nodes			
Germinal centers (0.7 sq mm)			
Hyperplastic	12/99 = 0.12	77	0.003‡
Hypoplastic	24/105 = 0.23	36	
Paracortex (2.4 sq mm)			
Hyperplasic	17/102 = 0.17	55	0.025‡
Hypoplastic	19/102 = 0.19	38	
Size (4.5 mm)			
Large	14/100 = 0.14	NR†	0.057
Small	22/104 = 0.21	40	
Perivascular lymphocyte cuffs (3%)			
Hyperplastic	12/83 = 0.14	55	0.040
Hypoplastic	27/127 = 0.21	41	

* Months at the 75th percentile.
† Seventy-fifth percentile not reached.
‡ *P* value exceeding 0.05 when median based on relative (%) size.

Table 3—Stage C: Tumor and Regional Lymph Node Features in Relation to Cancer Survival (SUR)

Feature (median)	Died/total	SUR*	P
Lymph nodes			
I Normal			
Germinal centers (0.4 sq mm)			
Hyperplastic	19/64 = 0.30	NR†	0.4
Hypoplastic	21/73 = 0.29	NR	
Paracortex (1.6 sq mm)			
Hyperplastic	16/66 = 0.26	NR	0.047
Hypoplastic	24/71 = 0.32	36	
Size (4.0 mm)			
Large	21/67 = 0.31	45	0.4
Small	19/70 = 0.27	NR	
II Tumor-involved			
Germinal centers (0.1 sq mm)			
Hyperplastic	18/59 = 0.31	NR	0.041
Hypoplastic	28/60 = 0.47	27	
Paracortex (0.2 sq mm)			
Hyperplastic	16/59 = 0.27	NR	0.009
Hypoplastic	30/60 = 0.50	27	
Perivascular lymphocyte cuffs (2%)			
Hyperplastic	16/53 = 0.30	48	0.2
Hypoplastic	46/107 = 0.43	39	

* Median in months.
† Median not reached.

Lymph Node Size

Enlarged normal lymph nodes tended to be associated with longer survival in Stage B only, but this association did not reach statistical significance (Table 2).

Table 4—Stage D: Tumor and Regional Lymph Node Features in Relation to Cancer Survival (SUR)

Feature (median)	Died/total	SUR*	P
Lymph nodes (normal)			
Germinal centers (0.5 sq mm)			
Hyperplastic	21/30 = 0.70	15	0.09
Hypoplastic	24/30 = 0.80	9	
Paracortex (1.3 sq mm)			
Hyperplastic	17/27 = 0.63	16	0.09
Hypoplastic	28/33 = 0.85	9	
Size (4.0 mm)			
Large	17/26 = 0.65	15	0.5
Small	28/34 = 0.82	13	
Perivascular lymphocyte cuffs (2%)			
Hyperplastic	22/25 = 0.88	9	0.2
Hypoplastic	33/47 = 0.70	14	

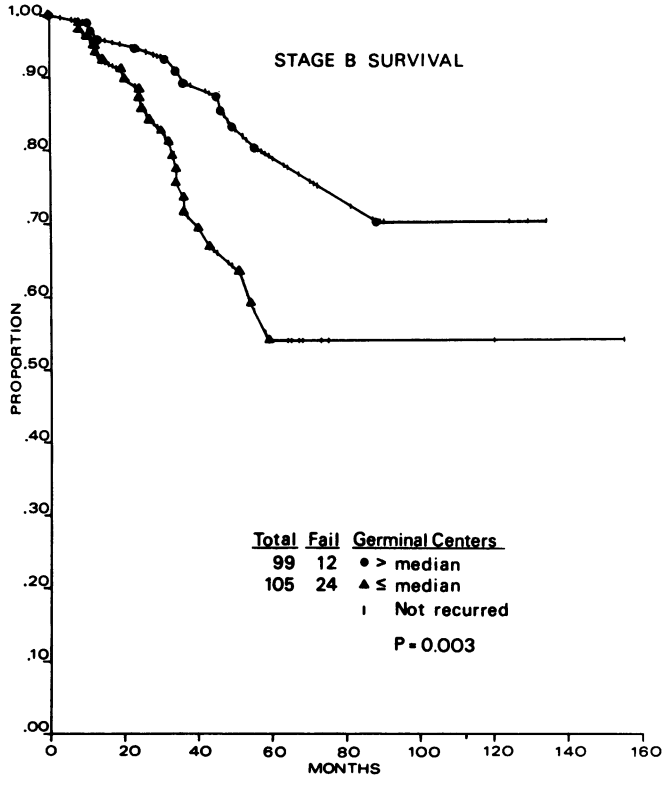
* Median in months.

Germinal Centers

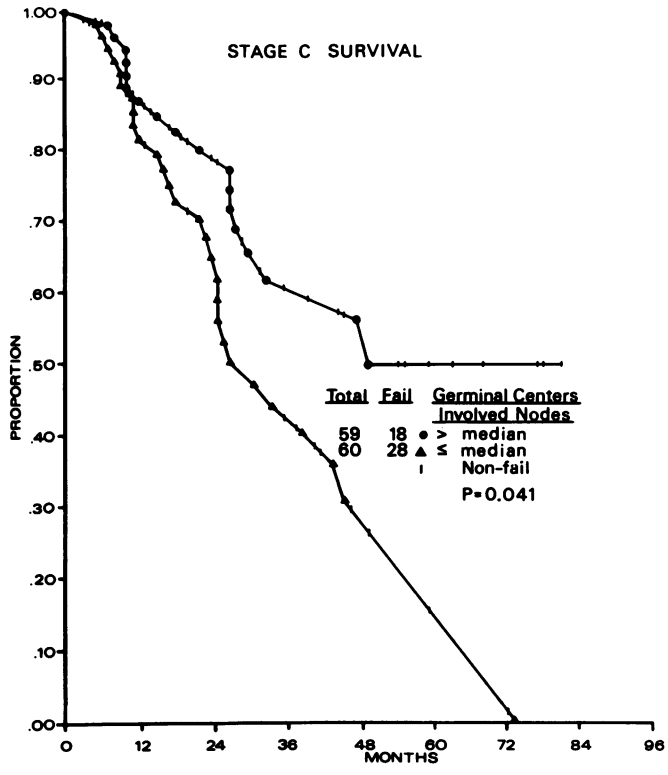
Germinal center hyperplasia was associated with a highly significant prolongation of survival in Stage B (Text-figure 1; Table 2), with absolute size as a criterion. There was no significant correlation between relative size (median 5%) and survival. Germinal center hyperplasia in tumor-involved lymph nodes (Stage C) related to significantly longer survival (Text-figure 2; Table 3).

Paracortical Areas

Hyperplasia (absolute values) of the paracortex in normal lymph nodes was associated with significantly longer survival in Stages B and C (Tables 2 and 3). Paracortical hyperplasia in tumor-involved lymph nodes (Stage C) was also a highly significant ($P = 0.01$) favorable prognostic feature (Text-figure 3; Table 3).



TEXT FIGURE 1—Survival time from surgery to death due to recurrent cancer in relation to germinal centers in Stage B. Patients with germinal center hyperplasia (*upper curve*) survive significantly longer.



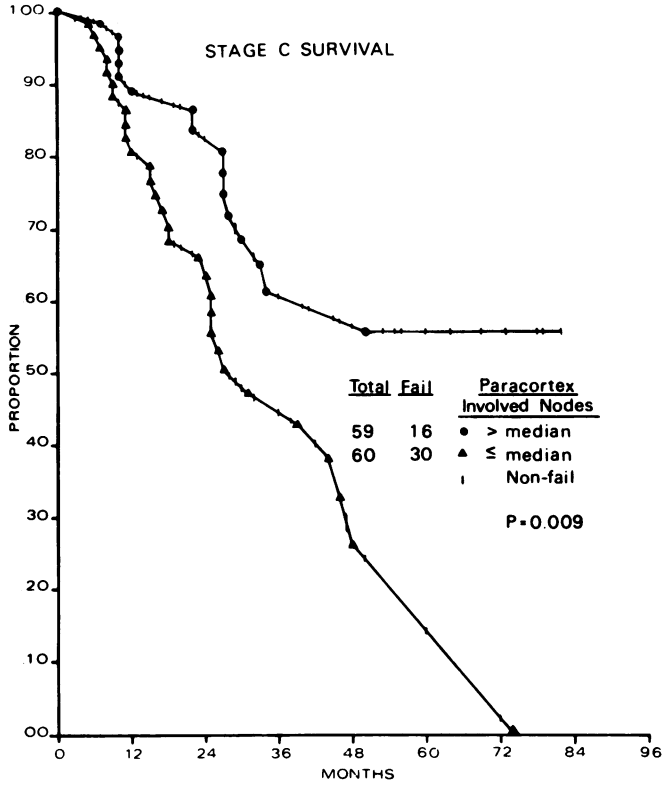
TEXT FIGURE 2—Survival in Stage C in relation to germinal centers in tumor-involved lymph nodes. Lymph nodes capable of hyperplastic germinal center response in the presence of tumor deposits are associated with significantly better survival (*upper curve*).

PLC

Hyperplasia of perivascular lymphocyte cuffs at the tumor edge was of prognostic importance only in Stage B and was associated with significantly ($P = 0.040$) longer survival (Table 2).

Discussion

The size of the germinal center and paracortical areas in sections of lymph nodes regional to 519 cases of colorectal carcinoma were analyzed morphometrically and expressed quantitatively. The size of uninvolved lymph nodes and the extent of PLCs at the deep tumor edge were similarly assessed. Numerical values of these parameters exceeding the median for the tumor stage were defined as hyperplasia of the corresponding lymph node areas or PLCs or as lymph node enlargement, respectively. Germinal center hyperplasia of normal, uninvolved regional lymph nodes



TEXT FIGURE 3—Survival in relation to paracortical response in tumor-involved lymph nodes. Paracortical hyperplasia is associated with a highly significant survival advantage (*upper curve*). At the time of the last death due to recurrent cancer in patients with paracortical hyperplasia, the percentage of survivors was 56, in comparison with 0 for those without (*lower curve*).

in Stage B and of metastatic nodes in Stage C was associated with significantly longer survival. Paracortical hyperplasia of normal regional lymph nodes was a favorable prognostic feature in Stages B and C only when related to absolute lymph node measurements. Furthermore, paracortical hyperplasia in tumor-involved (Stage C) lymph nodes was a highly significant, favorable prognostic factor. Perivascular lymphocyte cuffing was a favorable prognostic feature in Stage B only. Lymphoid hyperplasia was not of prognostic significance in Stages A and D.

The validity of morphometric lymph node data and of PLC data has been discussed elsewhere.[4] Briefly, germinal centers are not subject to interpretation error, unlike the paracortex; the lymph node diameter is likely to be underestimated. Absolute measurements are more reliable than relative (%) figures. The sampling error and the underestimation of lymph node size are compensated for by quantitative measurements of a

very large number of cases and the use of both relative and absolute measurements. Furthermore, our criteria of lymphoid hyperplasia are objectively statistically defined and hence reproducible. No attempts have been made to evaluate the possible prognostic significance of sinus histiocytosis [11,12] because of the lack of generally accepted [13] quantitative criteria.

The biologic significance of our findings is that hyperplasia of both PLCs and B and T lymphocyte areas [6] is of favorable prognostic significance in colorectal cancer Stages B and C, especially when present in tumor-involved lymph nodes in Stage C. Our data do not permit any interpretation of the specificity of lymphoid hyperplasia, ie, whether the corresponding antigens are tumor-associated or relate to secondary factors such as, eg, necrosis or infection. Whatever the explanation, lymphoid hyperplasia at the edge of colorectal carcinomas and in regional lymph nodes reflects prognostically favorable immunoreactivity.

We have previously reported on stage-related lymphoid hyperplasia in 509 of the colorectal carcinomas included in this study.[4] Hyperplasia of regional lymph node germinal centers and paracortex and of PLCs was significantly more common in the "localized" stages, A and B, than in Stages C and D. The data presented here show that such lymphoid hyperplasia is of major prognostic significance also within the individual tumor Stages B and C. Furthermore, our previously reported findings in a different series of patients of favorable prognostic implications of paracortical and PLC hyperplasia in Stage B colorectal carcinoma [14] have been confirmed by objective quantitative measurements and extended to include assessment also of germinal centers.

We have not found any reports in the literature of quantitative morphometric studies concerning the relationship between hyperplasia of regional lymph node compartments and cancer-specific survival in colorectal carcinoma. The prognostic value of subjective assessment of lymph node areas is equivocal.[12,14,15] House and Watt [16] reported significantly better survival in patients with >10 lymphocytes per high power microscopy field at the edge of the tumor. This finding is in agreement with our previous report [14] and present confirmation of PLC as a favorable feature in Stage B.

The data presented could be of importance not only for providing a supplement to the Dukes' staging [17] of colorectal carcinomas, making possible the identification of poor risk groups within Stages B and C, but also in the design of therapeutic trials and in the evaluation of the effects of chemoimmunotherapeutic regimens with selective effects on lymphoreticular cell populations.

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Acknowledgments

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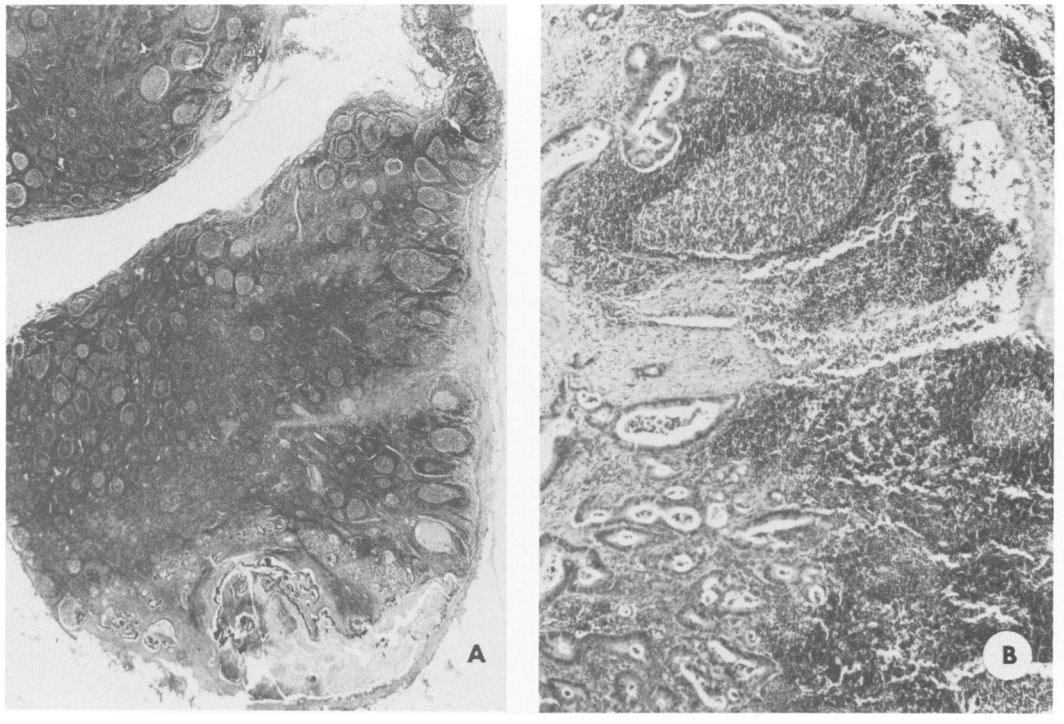
Figure 1A—Germinal center hyperplasia (14%, 5.3 sq mm/node section) in a tumor-involved lymph node regional to moderately well differentiated adenocarcinoma (Stage C) of the sigmoid colon. The patient, a 68-year-old man, is alive and healthy at 32 months. Hematoxylin alone was used for all photomicrographs. (×8) **B**—Secondary tumor deposits in the paracortical area (34%, 3.1 sq mm/node section) and near the prominent germinal center (*top*; 13%, 1.0 sq mm/node section). The patient, a 47-year-old woman, had moderately well differentiated carcinoma of the sigmoid colon resected and is alive without signs of recurrence at 82 months. Both normal lymph nodes (4/5) and one tumor-involved lymph node showed germinal center and paracortical hyperplasia (median for Stage C). (×50)

Figure 2A—Hyperplastic paracortex (left; 30%, 3.3 sq mm/node section). The patient had well differentiated adenocarcinoma (Stage B) of the colon removed at 68 and is still well 11 years later. (×50) **B**—Higher magnification of same lymph node as in Figure 1B, showing a secondary tumor deposit in the paracortical area at the prominent germinal center (*top*). (×130)



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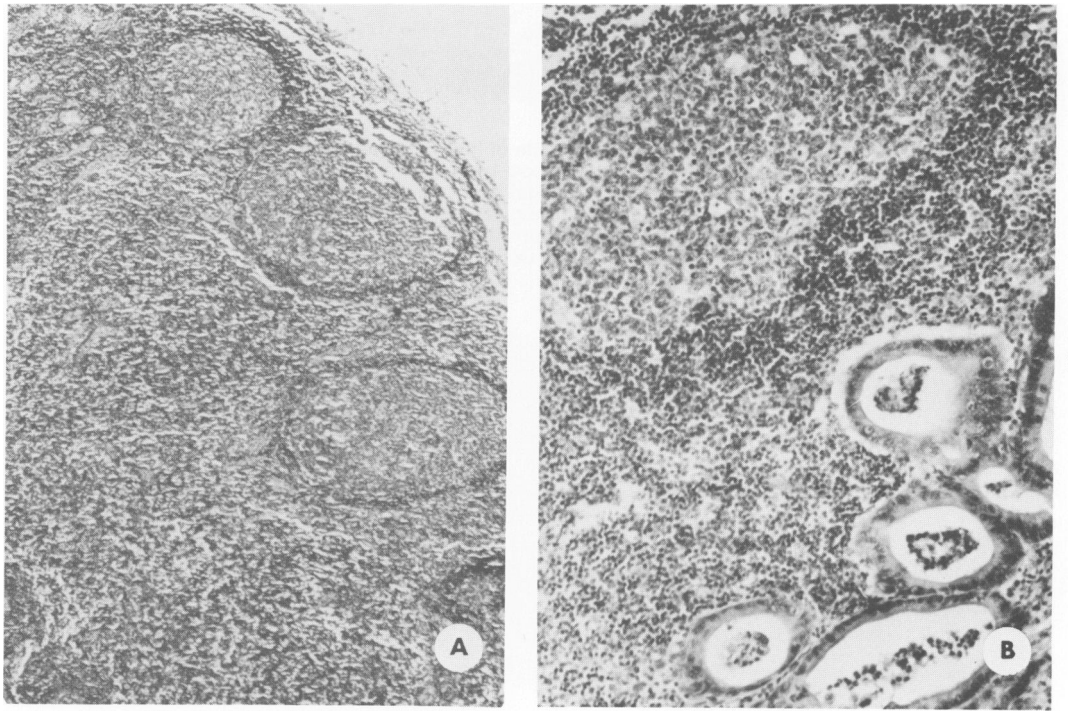

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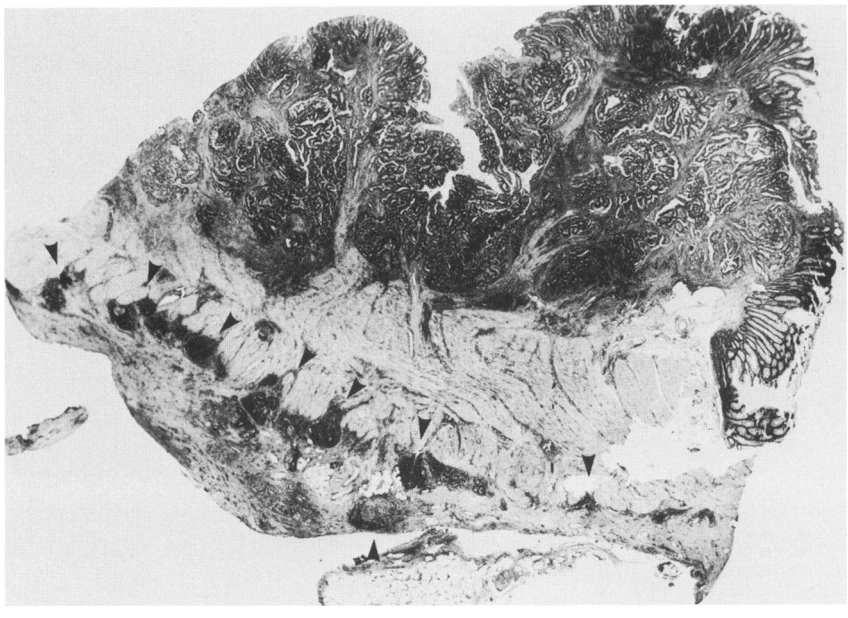

Figure 3—Well-differentiated adenocarcinoma of the colon, which in one area (not illustrated here) invaded through the outer muscle layer (Stage B). Regional lymph nodes showed paracortical (25%, 3.2 sq mm/node) and germinal center (7%, 0.9 sq mm/node section) hyperplasia. Prominent perivascular lymphocyte cuffing (19%; *arrows*) is seen at the vascular plexus. The tumor recurred at 62 months and led to the patient's death at 88 months at the age of 83 years. (×8)