Microsatellite Instability Is a Favorable Prognostic Indicator in Patients With Colorectal Cancer Receiving Chemotherapy

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Background & Aims: Adjuvant 5-fluorouracil (5-FU)-based chemotherapy is standard treatment for stage C colorectal cancer (CRC). Approximately 12% of CRCs are characterized by microsatellite instability (MSI), a hallmark of a DNA mismatch repair defect. We investigated the safety of adjuvant 5-FU-based chemotherapy for MSI CRC and compared the prognosis of MSI CRC and MSI CRC patients receiving adjuvant therapy. Methods: Previously, a prospective series consisting of 1044 consecutive CRCs has been collected and the MSI status of each sample determined. Patients with stage C cancer who had received adjuvant chemotherapy (n = 95) were followed up for 7–63 months (median, 31 months) after surgery. Results: No unexpected or serious adverse effects were observed when 5-FU-based chemotherapy was used as adjuvant treatment for MSI CRC. Three-year recurrence-free survival was 90% and 43% in the MSI (n = 11) and MSI (n = 84) groups, respectively (P = 0.020). Conclusions: Adjuvant 5-FU-based chemotherapy is feasible for both MSI CRCs and MSI CRCs, and patients with MSI CRC who receive adjuvant therapy have an excellent prognosis.

Colorectal cancer (CRC) is the second or third most common cancer in Western societies. At diagnosis, approximately one fourth of all cases are stage C, i.e., regional lymph node metastases are present but no detectable metastatic lesions are found in other organs (International Union Against Cancer [UICC] stage III, Dukes stage C, Australian Clinicopathological Staging [ACPS] stage C). Patients with stage C CRC are generally considered to benefit from adjuvant 5-fluorouracil (5-FU)-based chemotherapy, which is often given in combination with leucovorin or levamisole.

Of all cancer types, the molecular changes leading to malignant growth are perhaps best known in CRC. According to recent evidence, there are 2 alternative ways cells can acquire mutations in tumor-suppressor genes and oncogenes while becoming increasingly malignant. Traditional chromosomal instability causes aberrant karyotypes, aneuploidy, and “macroscopic instability,” whereas the “mutator phenotype” results in single base alterations, slippages, and small loops at DNA repeat loci. The mutator phenotype is evident in tumors from hereditary nonpolyposis colorectal cancer (HNPCC) families and in a subgroup of sporadic CRCs. There are well documented histologic, pathologic, and clinical characteristics associated with MSI-positive (MSI) CRCs: proximal predominance, DNA diploidy, poor histologic differentiation, mucinous growth, large primary tumor size, and, perhaps paradoxically, more favorable stage distribution.

Mismatch repair enzymes normally repair replication errors DNA polymerases make during cell division. When both alleles of a mismatch repair gene are damaged, no functional enzyme is produced, and a mutator phenotype is seen. The resulting changes can be most easily observed at microsatellite repeat loci, and the phenotype is thus called microsatellite instability (MSI, also known as MIN, MI, or replication error phenomenon, RER). Colorectal, endometrial, and many other cancers observed in HNPCC families are MSI, but the phenomenon is not restricted to HNPCC. Instead, 12%–15% of all CRCs are MSI, and HNPCC accounts for only 16% of these cases. The remaining 84% are sporadic cancers caused by somatic biallelic inactivation of a mismatch repair gene, most often hypermethylation of the MLH1 gene. Thus, hereditary and sporadic MSI cases are similar on the molecular level, because both the maternal and the paternal mis-

Abbreviations used in this paper: 5-FU, 5-fluorouracil; CRC, colorectal cancer; HNPCC, hereditary nonpolyposis colorectal cancer; MSI, microsatellite instability; MSI, MSI positive; MSI, MSI negative.
match repair gene are inactive. Other cancer types besides CRC also commonly exhibit MSI.\textsuperscript{19}

To our knowledge, it has not been investigated if MSI\textsuperscript{+} and MSI-negative (MSI\textsuperscript{−}) cancers are equally sensitive to adjuvant therapy in vivo. In view of their different molecular backgrounds, it is not unexpected that some in vitro data suggest that MSI\textsuperscript{+} cancer cells respond differently to certain chemotherapeutics. Although resistant to many agents, MSI\textsuperscript{−} cells are reported to have an increased vulnerability to camptothecin and halogenated thymidine radiation sensitizers,\textsuperscript{20–22} and seem to retain their sensitivity to oxaliplatin, topotecan, and irinotecan.\textsuperscript{23–25} Both irinotecan and oxaliplatin have been successfully used in combination with 5-FU and leucovorin for treating metastatic CRC.\textsuperscript{26–28}

The most widely used drug in chemotherapy of CRC is 5-FU.\textsuperscript{1} Aebi et al.\textsuperscript{29} used a cytotoxicity assay to evaluate the efficacy of 5-FU on 2 MSI\textsuperscript{+} cell lines and reported no reduction of effect. Carethers et al.\textsuperscript{30} used 1 of these 2 lines for an enrichment assay in the presence of 5-FU and found that MSI\textsuperscript{+} cells had increased by 21% in comparison with MSI\textsuperscript{−} cells after 5 days of incubation. They also investigated the colony-forming ability of MSI\textsuperscript{+} cells and found them 28-fold compared with MSI\textsuperscript{−} cells after 10 days of incubation in 5-FU. Although the reports come to opposite conclusions with regard to the sensitivity of MSI\textsuperscript{+} cells to 5-FU in vitro, on closer assessment the results may not be completely contradictory. In the former study, a reduction of sensitivity to 5-FU was not noted for MSI\textsuperscript{+} cells. Considering that the enrichment assay used in the latter study is very sensitive to relative growth advantages, it is not unlikely that the relative increase in MSI\textsuperscript{+} cells in comparison with MSI\textsuperscript{−} cells after incubation in 5-FU reflects a low level in vitro resistance to the drug, but that in vivo 5-FU retains its effect on MSI\textsuperscript{+} as well as MSI\textsuperscript{−} cells.

It has been suggested in retrospective analyses that MSI\textsuperscript{+} cancers have better prognosis than MSI\textsuperscript{−} cases.\textsuperscript{4,7,10,12,31–35} Some of these series had been collected with HNPCC studies in mind, and thus the proportion of young individuals may have been overrepresented. Because sporadic MSI\textsuperscript{+} tumors are well known to have a more favorable stage distribution,\textsuperscript{4,9–12} univariate analysis without stratification for stage becomes biased. Bubb et al.\textsuperscript{32} used the Cox proportional hazards method, allowed for various variables including stage, and found that MSI\textsuperscript{+} patients had better survival with a hazard ratio of 0.39. However, they grouped stages C and D together, which makes interpretation of the results more difficult, because stage D MSI\textsuperscript{+} is rare.\textsuperscript{4,9–11} A recent report suggests that hereditary CRCs associated with HNPCC (and thus probably MSI\textsuperscript{+}) have better than average prognosis.\textsuperscript{36} The cases in this study were not analyzed according to stage but rather divided into local and nonlocal disease. In another report, a significant survival advantage was not found for 85 HNPCC cases.\textsuperscript{37} Lukish et al.\textsuperscript{34} studied young patients with sporadic MSI\textsuperscript{+} CRC, some of whom had received adjuvant therapy. Although not statistically significant, the results suggest that MSI\textsuperscript{+} patients had improved prognosis, which was even better if 5-FU had been administered. However, adjuvant therapy had not been randomized and the stage distribution of MSI\textsuperscript{+} cases was more favorable. Curran et al.\textsuperscript{38} studied 159 stage B CRC patients, and found no association between survival and MSI status. In a retrospective series of 213 patients with stage C CRC who had not received adjuvant chemotherapy, MSI\textsuperscript{+} cases were found to have a more favorable prognosis.\textsuperscript{35} The study was published in abstract form, and the investigators suggest that adjuvant therapy may not benefit stage C patients with MSI\textsuperscript{+} tumors because their prognosis is already better. Chen et al.\textsuperscript{33} studied stage B and stage D CRCs. Survival seemed better for MSI\textsuperscript{+} cases in both groups, but was not statistically significant. Using multivariate analysis, Gryfe et al.\textsuperscript{12} found improved survival for young MSI\textsuperscript{+} CRC patients even when allowing for differences in stage distribution. According to the survival curves, good prognosis is suggested for MSI\textsuperscript{+} cases in all stages, but statistical significance is not reported for individual stages.

In conclusion, these retrospective studies seem to suggest that MSI\textsuperscript{+} CRC cases have a better prognosis than MSI\textsuperscript{−} cases, but few studies have considered the more favorable stage distribution of MSI\textsuperscript{+} cases. Furthermore, the safety of 5-FU for MSI\textsuperscript{+} CRC patients has not been studied, or the prognosis of patients receiving 5-FU–based chemotherapy for MSI\textsuperscript{+} CRC in comparison with MSI\textsuperscript{−} CRC. This study was designed to answer these questions; to avoid possible bias generated by heterogeneous tumor stages or treatments, we restricted this prospective, population-based series to patients with stage C CRC who had received 5-FU–based chemotherapy.

**Materials and Methods**

**Patients**

To obtain enough material for useful statistical analysis, we took advantage of a previously collected series.\textsuperscript{11,39,40} In these studies, fresh frozen tissue samples from 1044 consecutive CRCs were obtained in a prospective manner from 9 large
regional hospitals in southeastern Finland. Patients with CRC were diagnosed and underwent surgery routinely without the surgeon knowing the MSI status. Written informed consent for genetic analysis of the tumor sample was obtained from each patient according to the Human Investigations and Eth-ical Committee–approved research proposal. In this study the focus was on patients who had stage C CRC based on surgical, pathologic, and clinical staging and who received adjuvant chemotherapy after surgery. Patient characteristics are given in Table 1.

### MSI Analysis

The MSI status was determined in previous studies. Briefly, the first 236 CRCs were analyzed with 7–14 radioactively labeled markers, the next 273 CRCs with 7–16 fluorescently labeled markers, and the last 535 with 2 polyA markers (BAT26 and TGFβRII). According to the National Cancer Institute criteria, all cases of MSI in these reports are MSI-high. Of the total 1044 patients included in this series, 282 had stage C tumors, 12% of which were MSI+ (33/282). After exclusion of patients who did not receive adjuvant therapy within 3 months of surgery, a total of 95 stage C cases remained. Eleven (12%) were MSI+ and 84 (88%) were MSI-.

### Adjuvant Treatment

The decision to give adjuvant treatment to the patients with CRC was made by an oncologist together with the patient. Sometimes, the surgeon discussed the matter with the patient, and an oncological consultation was not done if the patient was reluctant. Because the MSI status of the CRC was not known at the time of surgery or decision to give chemotherapy, it could not affect these decisions. Furthermore, the MSI analysis was performed at a physically distant location by personnel other than the physicians responsible for patient care. In all cases, the adjuvant therapy was 5-FU based. Overall, the most common combination was 5-FU and leucovorin (n = 69). Alternatives were 5-FU + methotrexate (n = 14), 5-FU + levamisole (n = 4), and 5-FU alone (n = 8). A similar distribution was seen in the MSI+ group. Usually the adjuvant chemotherapy was begun within 6 weeks of surgery; 3 months was set as the latest time for the chemotherapy to be considered adjuvant. After recurrence of the disease, irinotecan, oral karmofur, or low-dose 5-FU were the most common second- and third-line chemotherapeutics.

### Follow-up

For stage C cases, all surgical and oncological case records were collected from the date of the surgery onwards and studied by one of the authors (A.H.). Routine controls were usually performed by the surgeon, and the oncologist was consulted after recurrence of the disease. As a criterion for recurrent disease, cytologic biopsy evidence was preferred. However, in a few cases relapse was diagnosed on the basis of computerized tomographic, ultrasound, or visual laparotomy evidence without cytologic confirmation. In addition to the 2 sets of case records, the status of each patient as dead or alive was checked on June 1, 1999, at the population register center. Because the health care system in Finland is highly centralized, it was rare to have the hospital case records interrupted or be incomplete with regard to patient follow-up.

### Statistical Analysis

Survival analysis was performed using a BMDP computer program (BMDP Statistical Software; Department of
Biomathematics, University of California Press, Los Angeles, CA). Survival was estimated with the product-limit method, and comparison of survival between groups was done with the log-rank test. When computing recurrence-free survival, survival was calculated from the date of surgery to the date of recurrence or death from any cause, and patients who were alive without recurrence were censored. The relative importance of risk factors was assessed with Cox’s stepwise proportional hazard model (BMDP 2L). Frequency tables were analyzed using the Fisher exact test. Age distributions were compared using the Mann–Whitney U test. All P values are 2-tailed.

Results

Adjuvant Therapy

While the majority of stage C cases were referred to an oncologist, adjuvant therapy was decided on in 95 of 282 cases (34%) (Table 2). The most common reasons for choosing not to give adjuvant therapy to the patient were, in order from more to less frequent: (1) lack of consent by the patient, (2) severe systemic disease, (3) complications or death due to surgery. A combination of reluctance on the part of the patient and systemic disease was common. The median age was 61 years (range, 30–81) (Table 1). There was no difference in the age distribution between the MSI+ (n = 11; median, 59; range, 32–76) and MSI– patients (n = 84; median, 62; range, 30–81; P = 0.53, Mann–Whitney test). Eighty–two percent of the MSI+ cases were in the proximal colon compared with 36% of the MSI– cases (P = 0.0066; Table 1). There were no cases of rectal cancer in the MSI+ population, while there were 36 cases (43%) of rectal or rectosigmoid cancer among the MSI– cases (Table 1). These figures are similar to those in previous reports.7–10

Most cases were histologically moderately differentiated in both groups (64% and 69% for the MSI+ and MSI– groups, respectively), and no association between differentiation and MSI status was found (P = 0.68 when tested well differentiated vs. moderately and poorly differentiated). MSI status was not associated with gender (P = 0.69, Table 1).

No unexpected serious adverse effects of adjuvant therapy were recorded in either study group. The most common side effects consisted of leukopenia, diarrhea, other abdominal complaints, and mucositis, as expected for 5-FU–based chemotherapy.

Follow-up and Prognosis

The median follow-up time of the patients alive was 31 months (range, 7–63 months). During the follow-up, 44 patients had disease recurrence (MSI+, n = 1; MSI–, n = 43) and 33 patients died (MSI+, n = 1; MSI–, n = 32). The 3-year overall survival was 90% among MSI+ and 62% among MSI– patients (P = 0.10, log-rank test) (Table 2). As shown in Figure 1, the 3-year recurrence-free survival was 90% in the MSI+ group, but only 43% in the MSI– group (P = 0.020, log-rank test).

In addition to the MSI status, age was significantly associated with poor survival. Patients older than the median age of 61 years had an average 33% 3-year recurrence-free survival; for patients younger than 61, the figure was 64% (P = 0.0045). Gender (P = 0.27), cancer location in the cecum or the ascending colon vs. the transverse or descending colon or the rectum (P = 0.37), or the histologic grade of differentiation (P = 0.59) were not significantly associated with recurrence-free survival. When age at diagnosis (the median was set as the cutoff value) and the MSI status were entered into Cox’s stepwise multivariate model, both age (P = 0.005) and MSI status (P = 0.014) had an independent influence on the risk of cancer recurrence.

Survival in Stage D MSI+ CRC

The aim of this study was to investigate stage C cancer, but because patients with stage D cancer are commonly treated with similar agents as used for adjuvant treatment, we also looked briefly at stage D (Table 3). MSI+ stage D CRC is very rare, and there were only 8 cases in this series of 1044 CRCs. Five of 8 had received chemotherapy, which was 5-FU based in all cases. Four of 8 patients were alive at least 2 years after surgery, and 6 of 8 at least 12 months. For the patients who received chemotherapy, the median survival time was 17 months.
but because of the small patient numbers, the confidence intervals are wide (Brookmeyer–Crowley 95% confidence interval, 7–43 months).

**Discussion**

In this study, we took advantage of a prospectively collected series of 1044 consecutive cases of CRC whose MSI status was known. Age was not used as an exclusion criteria, but the patients had to be fit for surgery. Only 95 of 282 (34%) patients with stage C cancer underwent adjuvant 5-FU–based chemotherapy, a small if realistic proportion considering the study began in 1993 and the National Consensus Statement in support of adjuvant treatment was only given in 1998. These demographic data may not be available elsewhere, but it is possible that this figure reflects the true frequency of the use of adjuvant chemotherapy in Western countries. Eleven of the 95 (12%) stage C patients who received adjuvant treatment had MSI CRC. This is similar to previous reports on the frequency of MSI among unselected CRC.

There are little data on the in vivo efficacy or toxicity of 5-FU for patients with MSI CRC. This has raised concern because MSI tumors account for a significant portion of CRC and have a distinct molecular background. No evidence of unexpected side effects was reported in the MSI CRC study groups. This was not very surprising because most MSI CRC cases are sporadic, and even the hereditary cases have a functional allele of each mismatch repair gene in each normal (necancer) cell, which means each normal cell has a functioning mismatch repair system, except for rare cases of dominant negative HNPCC.

The MSI cancers were associated with excellent survival. Both recurrence-free and overall survival were 90% at 3 years, while in the MSI group 3-year recurrence-free survival was 43% ($P = 0.020$) and overall survival 62% ($P = 0.10$). Only a single patient in the MSI CRC group had recurrent cancer. The large primary tumor was classified T3N3M0, and affected nodes were evident as a continuous palpable tract along the ileocolic artery all the way up to the mesenteric artery. The ascending colon was attached to the posterior visceral peritoneum in many places, and had to be manually mobilized. According to the surgeon, the resection margins to the healthy tissue were small. There were no relapses among the other 10 cases of stage C MSI CRC, but there were 43 recurrences among the 84 cases that were MSI CRC.

In Finland, the 3-year cancer-specific survival of stage A and B CRC is 94% and 75%, respectively. In comparison, MSI CRC with adjuvant therapy seems to have a more favorable prognosis than MSI CRC, although case selection may slightly confound this comparison. These results suggest that MSI CRC is often curable even when locoregional lymph node metastases are present, although longer follow-up is needed to confirm the long-term survival advantage. It is interesting that MSI CRCs seem to have a better prognosis, because they are often less differentiated and larger than MSI CRCs, features usually suggesting high proliferation and low survival. The reasons for the putative survival advantage are not known, but plausible explanations include a self-destructive effect of numerous mutations accumulating in the cell genome, possibly mutating genes necessary for the viability of the malignant clone. Resulting mutant proteins may also be transferred to the cell membrane to evoke an immune reaction against the tumor. It is not impossible that the Crohn's-like lymphoid reaction attributed to many MSI CRCs is associated with activated immune defenses. However, MSI CRCs...

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Surgery</th>
<th>Chemotherapy</th>
<th>Recurrences</th>
<th>Follow-up endpoint</th>
<th>Follow-up (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>9/94</td>
<td>5-FU</td>
<td>5/95 pelvic peritoneum</td>
<td>Alive 6/99</td>
<td>57</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>8/93</td>
<td>5-FU + MTX, FEM</td>
<td>2/95</td>
<td>Dead 3/97</td>
<td>43</td>
</tr>
<tr>
<td>3</td>
<td>82</td>
<td>3/95</td>
<td>No</td>
<td>No</td>
<td>Dead 7/95</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>68</td>
<td>9/95</td>
<td>No</td>
<td>Carcinosis at surgery</td>
<td>Dead 12/97</td>
<td>27</td>
</tr>
<tr>
<td>5</td>
<td>77</td>
<td>8/95</td>
<td>No</td>
<td>Carcinosis at surgery</td>
<td>Dead 6/98</td>
<td>34</td>
</tr>
<tr>
<td>6</td>
<td>64</td>
<td>11/95</td>
<td>5-FU + MTX, 5-FU</td>
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<td>Dead 11/96</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>76</td>
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<td>Dead 9/97</td>
<td>17</td>
</tr>
<tr>
<td>8</td>
<td>57</td>
<td>11/96</td>
<td>5-FU, irinotecan</td>
<td>2/97, liver</td>
<td>Dead 6/97</td>
<td>7</td>
</tr>
</tbody>
</table>

MTX, methotrexate; FEM, 5-FU, epirubisin, and methotrexate.

*Died of bleeding from tumor.*
cells often seem to lack expression of β-microglobulin, which could reflect avoidance of T-cell surveillance.

Most published studies suggest that MSI+ CRC generally has a more favorable outcome, although not all reports agree. To our knowledge, our study is the first analysis based on a prospective series, and the analysis restricted to stage C only, eliminating the confounding effect of the unequal stage distribution between MSI+ and MSI− populations. Furthermore, unlike in earlier studies, we restricted the analysis to patients treated with 5-FU–based adjuvant chemotherapy, because adjuvant therapy has a substantial effect on outcome and needs to be taken into account when assessing prognosis. Because adjuvant therapy was not randomized in this trial, the chemosensitivity of MSI+ CRC to 5-FU cannot be reliably assessed. Comparison with the group that did not receive adjuvant therapy would not be meaningful because patients with better prognosis are more likely to receive chemotherapy in a clinical setting. In addition to a prospective randomized trial, this important question could be addressed with retrospective analysis of the MSI status of cancers in patients who have participated in clinical adjuvant chemotherapy trials randomized for 5-FU, or comparison of MSI status with 5-FU treatment response in metastatic disease. This study sets the stage for such and further studies, because we now show that 5-FU is safe for MSI+ CRC and that the prognosis for MSI+ CRC seems to be very good when treated with adjuvant 5-FU–based chemotherapy.

Interestingly, in other cancer types MSI+ does not necessarily result in a favorable prognosis. No survival advantage was found in a series of 126 cases of gastric cancer, 13% of which were MSI+. MSI+ has been associated with poor prognosis in sporadic endometrial carcinoma and breast cancer.

The presence of distant metastases at the time of diagnosis (stage D) is rare in the MSI+ subgroup of CRC; we found only 8 cases in the present series (Table 3). However, interestingly, 4 of these 8 patients lived 2 years, when normally the average 2-year survival is 16%. Furthermore, the median survival time was 17 months for patients who received 5-FU–based chemotherapy, which may compare favorably with that of patients with advanced CRC treated with 5-FU–based chemotherapy in recent trials, despite selection criteria applied in such trials. Although the number of cases is too small to make any firm conclusions, this finding suggests that MSI+ CRC may have a relatively good prognosis even after overt distant metastases have developed. This would not be completely surprising because metastasis to local lymph nodes or to more distant sites can be, from the biological viewpoint, considered related forms of the same disease.

In summary, 5-FU–based adjuvant treatment is safe for patients with MSI+ CRC. With treatment for adjuvant 5-FU–based chemotherapy after surgery, the prognosis of MSI+ stage C CRC is significantly better than that of patients with MSI− tumors. CRCs with MSI have a different molecular background than cases without MSI, and randomized clinical trials are needed to find out whether adjuvant 5-FU–based chemotherapy benefits equally MSI+ and MSI− cases, or if MSI+ CRCs show a greater sensitivity to other chemotherapeutics such as irinotecan or oxaliplatin, as suggested by in vitro findings. However, the considerable survival difference between MSI+ and MSI− cases after adjuvant 5-FU–based therapy supports the hypothesis that also MSI+ tumors are sensitive to 5-FU. Otherwise, such a large difference would have been unlikely considering that adjuvant 5-FU increases the survival of stage C patients not grouped by MSI status, and thus mostly MSI−.

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Received January 10, 2000. Accepted June 28, 2000.
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The authors thank Drs. Albert de la Chapelle and Reijo Salovaara; Siv Lindroos, Sinikka Lindh, Tuula Lehtinen, and Kirsi Pylvänäinen for help and advice; and the physicians and staff at other participating institutions: Kuopio University Central Hospital and Central Hospitals of Joensuu, Mikkeli, Lappeenranta, Kajaani, Kotka, and Savonlinna.

Child of the Child classification of liver impairment

Charles Gardner Child, III (1908–1991) was born into a New England medical family that settled in the dignified professional milieu of metropolitan New York. After graduation from Yale in 1930, he enrolled at the Cornell Medical College where, inspired by his surgical mentor George Heuer, he was imbued with Halstedian ideals. Early on he served briefly on the New York boxing commission but was summarily dismissed when he “learned too much about profound electrolyte disturbance in boxers attempting to control pre-fight weight restrictions.” Thereafter, his entire professional career was as a university surgeon, first at Tufts, then at Michigan where he became chairman of the department and was recognized as a world authority on the surgical management of portal hypertension and chronic pancreatitis. In the 1960s he served as editor of The Journal of Surgical Research and chairman of the American Board of Surgery. Aside from his many professional accomplishments, he is best remembered by students and colleagues in the warmth of his family home on the shore of Barton Pond in Ann Arbor.

—Contributed by WILLIAM S. HAUBRICH, M.D., Scripps Clinic and Research Foundation, La Jolla, California