Hepatic Catalase Activity in Advanced Human Cancer

T. OHNUMA² G. MALDIA³, AND J. F. HOLLAND¹

Summary

Hepatic catalase activity in unanesthetized man has been determined by measuring the disappearance rate of hydrogen peroxide spectrophotometrically in homogenates from needle biopsy specimens of liver. Ten healthy male prisoners, 50 patients with advanced cancer, and 60 cadavers shortly after death with cancer were studied.

There is a wide range of catalase activity in normal human liver. Catalase activity per gm of liver nitrogen in patients dead with cancer was approximately 40% of the normal mean. In serial biopsy studies, advancing cancer with progressive clinical deterioration was associated with decreasing catalase activity which occurred independently from weight change. Response to chemotherapy, surgery, or radiation therapy evidenced by objective regression of tumor masses was associated with a rise in liver catalase activity. This pattern of catalase response was found in all the broad classes of cancer studied. In the presence of profound systemic drug toxicity, catalase elevation was sometimes not seen despite tumor regression. When survival of those who responded and those who failed to respond to their cancer treatments were compared, there is a clear survival advantage for those who sustained response. This advantage was anticipated by higher liver catalase activity after response to treatment.

Introduction

Depression of hepatic catalase activity in tumor-bearing animals has been recognized as one of the most nearly consistent effects of cancer on the host. Catalase depression has also been reported following a great variety of other stimuli. In a recent comprehensive review of the mechanisms of liver catalase depression, Kampschmidt has stressed the multiplicity of possible factors which may be active under experimental conditions.

A paucity of data pertain to human studies, despite the fact that the initial observation of catalase depression was made by Blumenthal and Brahn (2) in man dead with cancer. We have been unable to find reports of studies in unanesthetized patients or in normal subjects. Repeated observations in the same patient have not heretofore been described.

The use and relative safety of needle liver biopsy suggested to us the feasibility of studying hepatic catalase activity in man throughout the course of advanced cancer. We expected to encounter instances of progressive clinical decline and of therapeutically induced regression of neoplasms which could provide alterations in the course of cancer suitable for study.

We have found that hepatic catalase activity is readily quantified repeatedly; that in the course of advanced cancer it is progressively depressed below normal; and that objective tumor regression in the patients we have studied is accompanied by prolongation of survival when compared to nonresponders, and by rise in catalase activity. These observations form the basis of the present communication. Portions of this work have been reported in preliminary form elsewhere (10, 11). (Certain values previously reported have been recalculated in this paper according to the Appendix.)

Materials and Methods

Fifty patients, 30 men and 20 women who died with cancer, were studied. A liver biopsy was done within 6 hr after death. To obtain normal liver catalase activity, liver biopsies were done on 10 male volunteer prisoners. Their ages ranged from 30 to 60 years. All were clinically well and none had a history of liver disease.

A total of 141 observations of catalase activity were made on 59 patients with cancer, 22 men and 37 women. In 39 of these patients, 2 to 8 biopsies were performed. One patient with Hodgkin's disease and uremia was apparently well after biopsy, but died 4 hr later. No anatomic cause was recognized at autopsy. One healthy volunteer sustained a right hemothorax with hypotension. He recovered uneventfully. No other complications were encountered in the other biopsies.

Liver specimens were obtained by Vim-Silverman needle biopsy through a transthoracic approach. Patients were postabsorptive for 5-15 hr. Ordinarily, there was no premedication. Rarely, alphaprodine hydrochloride (Nisentil) or meperidine was administered. Local anesthesia was achieved with 1% Xylocaine. The local anesthetic was not injected into the liver substance. Specimens were immediately placed on ice and assayed within 30 min after biopsy. The piece of cylindrical liver tissue was inspected and, if not grossly homogeneous, it was discarded. If it was homogeneous, it was divided into 3 parts; the middle portion was fixed in formalin and stained with hematoxylin and eosin. If tumor cells were seen microscopically in the center specimen, the data obtained were not used. Approximately 10-15 mg of tissue remained for chemical study.

The weighed end cylinder specimens were homogenized by 50...
 strokes in a Potter-Elvehjem homogenizer with a glass pestle in approximately 1.5 ml of 0.05 M potassium phosphate buffer, pH 7.0. The homogenizer was rinsed 3 times with buffer. Cadaver specimens obtained by the same needle technic were diluted to 5 ml, and 1 ml was used for assay. Clinical specimens were diluted to 5 ml, and 200 µl were used for assay. Later, all specimens were diluted to approximately 2.8 mg of liver/ml in order to obtain a concentration of about 400 µg of protein/ml of homogenate. To test if the 50-stroke homogenization method was effective in releasing all intracellular catalase, some homogenates were further subjected to 2.5- or 5-min homogenization in an Omnimizer (Ivan Sorvall Co.) immersed in ice, and other specimens to 5 min omni-mixing with the phosphate buffer to which had been added 1% Triton X-100 detergent by volume (Rohm and Haas Co.). The homogenates were utilized for catalase assay, for protein determination by the method of Lowry et al. (9), and for other determinations. The protein value as determined by the Lowry procedure was multiplied by 16% to obtain the nitrogen concentration in the sample used.

Liver catalase activity was determined spectrophotometrically by the method of Beers and Sizer (1). An approximately 5 x 10-2 M solution of hydrogen peroxide was prepared by freshly diluting 0.15 ml of 30% hydrogen peroxide (Superoxol, Merck) with 25 ml of 0.05 M potassium phosphate buffer, pH 7.0. A silica cuvette in the optical path of a Beckman DU spectrophotometer served as the reaction vessel. The blank cuvette contained 2.8 ml of phosphate buffer and 0.2 ml of liver homogenate. The reaction vessel contained 1.8 ml of buffer and 1.0 ml of 5 x 10-2 M hydrogen peroxide solution. At zero time, 0.2 ml of liver homogenate was delivered rapidly and forcefully into the reaction cuvette and the absorbance at 240 nm was determined at 10-sec intervals from 10 to 70 sec. Duplicate reactions were run on all specimens and the average absorbance calculated at each point. All procedures were performed at 0°-4°C except for spectrophotometric readings, which were accomplished at room temperature with the refrigerated reagents. The velocity of catalase reaction is only slightly affected by temperature in this range (1).

The disappearance of hydrogen peroxide follows the equation for 1st order reaction kinetics:

$$\log_{10} A = \log_{10} A_0 - kt/2.3$$

where $A_0$ = absorbance due to hydrogen peroxide at 0 time, $A$ = absorbance due to hydrogen peroxide at time $t$, and $k$ = rate constant. Therefore, the logarithm of the absorbancy was plotted against time and $k$ was calculated from the slope of the resulting straight line. Thus each catalase unit specifies the relative logarithmic disappearance rate of hydrogen peroxide/sec and is expressed per gm of liver nitrogen.

### TABLE 1

<table>
<thead>
<tr>
<th>Cadaver no.</th>
<th>Time (hr)</th>
<th>0.5</th>
<th>3.5</th>
<th>17</th>
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<tbody>
<tr>
<td>1</td>
<td>280</td>
<td>287</td>
<td>255</td>
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<tr>
<td>2</td>
<td>305</td>
<td>306</td>
<td>386</td>
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<table>
<thead>
<tr>
<th>Homogenization technique</th>
</tr>
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<tbody>
<tr>
<td>Potter-Elvehjem</td>
</tr>
<tr>
<td>Omnimix</td>
</tr>
<tr>
<td>50 strokes</td>
</tr>
<tr>
<td>2.5 min</td>
</tr>
<tr>
<td>5 min</td>
</tr>
<tr>
<td>5 min + 1% Triton</td>
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</tbody>
</table>

### TABLE 2

<table>
<thead>
<tr>
<th>Cadaver no.</th>
<th>Homogenization technique</th>
<th>50 Strokes as % of Maximum Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Potter-Elvehjem</td>
<td>Omnimix</td>
</tr>
<tr>
<td>1</td>
<td>382</td>
<td>416</td>
</tr>
<tr>
<td>2</td>
<td>355</td>
<td>353</td>
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<tr>
<td>3</td>
<td>261</td>
<td>312</td>
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<tr>
<td>4</td>
<td>438</td>
<td>460</td>
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<tr>
<td>5</td>
<td>332</td>
<td>294</td>
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<tr>
<td>6</td>
<td>518</td>
<td>416</td>
</tr>
<tr>
<td>7</td>
<td>282</td>
<td>339</td>
</tr>
<tr>
<td>8</td>
<td>259</td>
<td>312</td>
</tr>
<tr>
<td>9</td>
<td>172</td>
<td>195</td>
</tr>
<tr>
<td>10</td>
<td>210</td>
<td>229</td>
</tr>
<tr>
<td>11</td>
<td>342</td>
<td>350</td>
</tr>
</tbody>
</table>

### Results

Catalase activity was estimated serially postmortem in several refrigerated cadavers. Specimens were obtained by needle technic. In 2 instances observations were continued for 17 hr. No significant change in activity was found (Table 1).

Catalase activity of cadaver livers determined by 50-stroke Potter-Elvehjem homogenization and by more vigorous mechanical homogenization is presented in Table 2. Fifty-stroke recovery was 100 ± 15% of the homogenized catalase. Comparison of the ordinary 50-stroke technic with homomixing in 1% Triton is also shown. The 50-stroke method accomplished 89% ± 7 recovery.

In biopsies from patients the 50-stroke method produced 76 ± 6% recovery of that obtained from homomixing in 1% Triton (Table 3). Repeat specimens during the course of observation of Patients 1 and 2 show concordant changes in the activities for the 2 homogenization technics.

The remainder of the data of the study were collected using the 50-stroke homogenization technic before the experiments in Tables 2 and 3 were performed. They provide a close approximation to the maximum catalase activity detectable in cadaveric tissue. In cancer patients the activity measured represents about 0.75 of the activity recoverable with Triton and more vigorous recovery.
TABLE 3
CATALASE ACTIVITY IN PATIENTS WITH CANCER, AS INFLUENCED BY HOMOGENIZATION TECHNIC (UNITS/GM OF LIVER NITROGEN)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis and Remarks</th>
<th>Homogenization Technique</th>
<th>50 Strokes as % of Maximum Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50 strokes</td>
<td>Omnimix +1% Triton</td>
</tr>
<tr>
<td>1a</td>
<td>Metastatic embryonal carcinoma of testis</td>
<td>484</td>
<td>653</td>
</tr>
<tr>
<td>1b</td>
<td>Regression following chemotherapy, 25 days later</td>
<td>600</td>
<td>722</td>
</tr>
<tr>
<td>2a</td>
<td>Metastatic melanoma</td>
<td>621</td>
<td>887</td>
</tr>
<tr>
<td>2b</td>
<td>Progression 26 days later</td>
<td>466</td>
<td>683</td>
</tr>
<tr>
<td>3</td>
<td>Breast carcinoma</td>
<td>711</td>
<td>956</td>
</tr>
<tr>
<td>4</td>
<td>Squamous carcinoma, primary undetermined</td>
<td>445</td>
<td>518</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean, 76 ± 6</td>
<td></td>
</tr>
</tbody>
</table>

In certain patients the reproducibility of the 50-stroke method was clearly demonstrated (e.g., Chart 3, Patient No. 3; Chart 4, Patient No. 2). This affords greater credibility to alterations in activity that do occur. The data of the study are relevant to dynamic changes in catalase activity during the course of tumor growth. No correction factors have been used. (The patients and specimens in Tables 2 and 3 are not included in other charts, tables, or totals in the text.)

The 50 patients dead with cancer had about 40% of the hepatic catalase activity of the 10 normal male volunteers (Chart 1).

To determine if decreased catalase activity was due to absolute reduction or merely to dilution by new hepatic protein, whole liver catalase activity was also compared between healthy volunteers and 10 additional patients dead with cancer. Catalase activity of the whole liver of control subjects was calculated based on the assumption that the liver weight is approximately 1/14th of body weight (8) and the range of liver protein 11.4–16.6 gm/100 gm of total liver weight (16). For these calculations we have used an average figure of 14 gm/100 gm of liver. The cadaveric livers were weighed and their weights were also estimated from the last clinically recorded body weights for comparison with the observations at autopsy. Ten patients who died with cancer had mean whole liver catalase activity of 10,992 while 10 healthy volunteers had a mean of 32,558. Thus, the total liver catalase activity at death with cancer was reduced to one-third (Table 4).

The 59 patients under study all had metastatic cancer. They were divided into 5 groups: malignant melanoma, adenocarcinoma of the breast, adenocarcinoma of other sites excluding breast, squamous cell carcinoma, and miscellaneous neoplasms. The graph of each group (Charts 2-6) is divided into 2 parts. The upper part represents a series of curves of catalase activity in patients who responded to chemotherapy, surgery, or radiation, while the lower parts of the graph are those in patients who failed to demonstrate the reduction in tumor size of patients in the response category.

Patients who seemed in good clinical condition at the onset of the study despite their metastatic cancer had relatively high liver catalase activities approaching the normal mean value. Progressive fall in liver catalase activity occurred almost invariably in patients who did not sustain recognizable tumor response from the treatment employed. Response was not synonymous with remission, although clinical benefit often did accompany the effect. The brief case histories of all the patients whose tumors responded are presented below.

**Case Reports**

**MELANOMA No. 1 (CHART 2).** This 66-year-old housewife was fully active without stigmata of cancer cachexia at the time of the initial catalase study. She received vinblastine initially and her growing subcutaneous metastases became static and some lesions partially regressed. At the 2nd biopsy, the hepatic catalase had increased. Two weeks after the 2nd biopsy (Day 50), increase in tumor size was noted. On Day 63, two new subcutaneous lesions appeared. The catalase activity at this time had fallen. Definite but minor decrease in tumor size and apparent stasis of growth in others occurred during subsequent vincristine administration.

![Chart 1](chart1.png)

*Chart 1. Hepatic catalase activity and mean ± 1 S.D. in 50 patients dead with cancer and in 10 male volunteers.*
Hepatic Catalase Activity

TABLE 4
Catalase Activity* in Whole Liver of Normal Subjects and Patients Dead with Cancer

<table>
<thead>
<tr>
<th></th>
<th>Healthy male volunteers</th>
<th>Patients with cancer at autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Liver catalase units/gm of liver nitrogen</td>
<td>704 ± 255</td>
<td>308 ± 92</td>
</tr>
<tr>
<td>Actual liver wt./body wt.</td>
<td>1/35.6 ± 10.6</td>
<td>10,962 ± 3316</td>
</tr>
<tr>
<td>Whole liver catalase calculated from liver wt.</td>
<td>32,558 ± 10,343</td>
<td>11,236 ± 4143</td>
</tr>
</tbody>
</table>

* Sample means ± population S.D.'s.

Vincristine was abandoned on Day 38 because of moderate neurologic toxicity and alopecia. The 4th catalase activity measured after clearing of toxicity, however, was again increased. Thereafter, slow growth of metastases was observed despite methylglyoxal-bis-guanylhydrazone, and catalase activity declined.

MELANOMA No. 2 (CHART 2). This 41-year-old housewife had melanoma of 1 year's duration with massive tumor involvement of the left lower extremity. During treatment with vinblastine, transitory regression of leg tumor was noted by Day 40, accompanied by an increase of hepatic catalase activity at the 2nd liver biopsy. Three weeks later, pulmonary metastases first became apparent. The catalase fell. During vincristine treatment, her pulmonary metastases and the leg lesions remained constant in size and number. The catalase activity showed no change from the previous level.

MELANOMA No. 3 (CHART 2). This 72-year-old man had pulmonary metastases 1 year after amputation for melanoma of the leg. He received orthophenylenediamine (OPDA) between the 1st and 2nd liver catalase study (15). He experienced frequent vomiting, his pulmonary metastases increased in size, and weight loss and general malaise progressed during this drug trial. Thirteen days after the 2nd catalase study, vinblastine was started. Nine days later, the pulmonary lesions had not grown but the catalase was increased. On the 15th day, cavitation with decrease in size occurred. There was no appreciable subsequent change in the pulmonary metastases. Death occurred on the 74th drug day from bilateral bronchopneumonia.

Progression of melanoma and the lack of response to chemotherapy of the remaining patients was associated with stable or falling liver catalase activity.

The 2nd group consists of patients with metastatic adenocarcinoma of the breast (Chart 3).
Case Reports

**Breast Cancer** No. 1. This 56-year-old postmenopausal woman had an adrenalectomy performed for inflammatory breast cancer 3 days after an initial liver biopsy. She was studied again 1 week later. An objective regression and subjective remission of her cancer was first noticed on her 13th postoperative day and the response lasted for 6 months. Her catalase increase anticipated the clinical response.

**Breast Cancer** No. 2. This 75-year-old lady had metastatic visceral cancer 2 years after radical mastectomy. She did not respond to methotrexate but had an excellent response to triethylenethiophosphoramide (Charts 3 and 7a). During methotrexate treatment, her cancer was progressive, and ascites and pleural effusion precluded repeat liver biopsies. After triethylenethiophosphoramide was started, all evidence of tumor disappeared. The 2nd biopsy was done when objective regression was well in evidence, and disclosed an increase in hepatic catalase activity. The administration of triethylenethiophosphoramide was interrupted after 10 months because of thrombocytopenia. Relapse was noted 11 months later, and she died 25 months after the start of triethylenethiophosphoramide.

**Breast Cancer** No. 3. This 79-year-old lady had extensive exophytic breast cancer of 15 years' duration. It was nonprogressive during cyclophosphamide treatment and accompanied by a rather flat catalase curve. Decrease of the catalase activity in the late part of the study may be related to severe toxicity from vincristine when she lost 7 kg of body weight in 40 days. She died 15 months later.

Absence of favorable response to adrenalectomy in patient Breast Cancer No. 5 was characterized by a fall in liver catalase activity. Lack of response to hypophysectomy in patient Breast Cancer No. 6 was followed by progressive weight loss, fall of liver catalase, and death.

A group of patients with adenocarcinomas other than breast cancer was studied (Chart 4).

**Case Reports**

**Adenocarcinoma** No. 1 (Charts 4 and 7b). This 51-year-old farmer had a large carcinoma of the prostate ulcerating into the rectum. Metastases were present in the left clavicle. After an initial biopsy for catalase assay, bilateral orchiectomy and stilbestrol administration were followed by marked regression of primary and metastatic tumor, decrease of prostatic acid phosphatase, and weight gain. His liver catalase activity rose sharply.

**Adenocarcinoma** No. 2. This 47-year-old housewife had a carcinoma of the colon with metastases to the left lobe of the liver. She responded well to intermittent courses of methotrexate for 8 months. The hepatic mass regressed and she gained weight. Her catalase activity remained approximately stable. Two months after the last liver biopsy, she was noted to be in relapse with rapidly growing hepatomegaly and jaundice.

Two patients showed a rise in liver catalase activity, Adenocarcinoma 6, two months prior to death, and Adenocarcinoma 10 at autopsy, without evidence of tumor regression. These 2 patients both had clear-cell carcinoma of the kidney, and were the only patients under study with this type of neoplasm.

Seven patients with squamous cell carcinoma were studied (Chart 5).

**Case Reports**

**Squamous Cancer** No. 1. This 66-year-old housewife had squamous cell carcinoma of the anus of 5 months' duration.
During the 1st and 2nd catalase studies, she received vinblastine without response. Radiation was given to the perineum starting at 0 month, and the anorectal mass became soft and later disappeared. Catalase activity substantially increased despite the appearance of severe monarticular tuberculosis of the knee. She died elsewhere from volvulus and gangrene of the small bowel, 1 year after the radiation therapy. No tumor was found at autopsy.
Patients with Miscellaneous Tumors

Chart 6. Hepatic catalase activity/gm of liver nitrogen in patients with miscellaneous malignant neoplasms. See legend, Chart 2, for symbols. See Chart 7c for a more detailed presentation of Patient No. 5.

Squamous cancer No. 2. This 70-year-old man had extensive carcinoma of the larynx and a small pulmonary metastasis. A large part of the total tumor was removed by laryngopharyngectomy. He improved clinically. An increase of catalase activity was seen 5 weeks postoperatively.

The remaining 5 patients had progressive disease.

The group of miscellaneous neoplasms (Chart 6) consists of embryonal carcinoma, teratocarcinoma, lymphoma, multiple myeloma, neuroblastoma, and carcinoid.

Case Reports

Miscellaneous cancer No. 1 (Chart 6). This 52-year-old housewife had teratocarcinoma of the abdominal wall for 2 years. She received 3 courses of methotrexate between the 2 catalase studies. In this patient no objective tumor regression was confirmed because of difficulty in measurement, although pain disappeared and she gained 3 kg of body weight after the 3rd course of drug. The 2nd liver biopsy was done 11 months after the initial study during which time the patient had shown no progression of her disease. A rise in catalase activity of 95 units/gm of liver nitrogen was noted. Progression was found 5 months later as an ulcerating tumor mass at the old scar.

Miscellaneous cancer No. 2. The clinical course of multiple myeloma in this 59-year-old woman was relatively benign. Protruberant lesions from bone failed to respond to vinblastine but responded unequivocally though evanescently to methylglyoxal-bis-guanhydrazone. The fall in her catalase activity despite substantial tumor regression occurred when severe systemic and gastrointestinal toxicity from the drug was present.

Miscellaneous cancer No. 3. This 53-year-old man had generalized lymphosarcoma characterized by hepatosplenomegaly and axillary and inguinal adenopathy. He was given weekly nitrogen mustard treatment after an initial liver biopsy. He responded well to the drug with disappearance of hepatomegaly and decrease of axillary and inguinal lymph nodes and splenomegaly. Clinical improvement had begun by the 3rd week, but increase in catalase activity was delayed.

Miscellaneous cancer No. 4. This 44-year-old housewife with generalized reticulum cell sarcoma responded well to vincristine with disappearance of palpable lesions. There was an associated sharp increase in catalase activity and weight gain. Her remission lasted 22 months.

Miscellaneous cancer No. 5 (Chart 7c). This 32-year-old clerk had recurrent testicular embryonal carcinoma presenting as a large retroperitoneal mass. A liver biopsy for catalase assay was done immediately before placing him on vincristine. Two-thirds decrease of tumor in 4 months was accompanied by clinical well-being and rise in catalase activity, despite loss of 10 kg of body weight ascribed to vincristine. Thereafter, progression of pulmonary metastases was noted.

The remaining patients in each of the 5 groups did not respond to treatment. Their course was associated with continuing cachexia, often with measurably advancing cancer, fall in liver catalase activity, and subsequent death.

Thus 11 of 14 responders had rise in catalase activity temporally related to their tumor response. In 1 patient (Miscellaneous No. 1) response was defined by subjective improvement and apparent host benefit only. In 1 patient (Adenocarcinoma other than Breast No. 2) the catalase activity remained on a plateau.
Hepatic Catalase Activity

Chart 7a. Patient Breast No. 2. Change in hepatic catalase activity/gm of liver nitrogen with tumor regression caused by triethylene-thiophosphoramide (TSPA). Schematic representation of abdominal mass with ascites; transverse diameter is given in centimeters (cm). MTX indicates methotrexate.

Chart 7b. Patient Adenocarcinoma No. 1. Change in hepatic catalase activity/gm of liver nitrogen, weight, and prostatic acid phosphatase activity in a 53-year-old male with adenocarcinoma of the prostate following castration and stilbestrol administration.

during the period of tumor responsiveness. The 14th patient (Miscellaneous No. 2) had unequivocal regression of myeloma with marked systemic toxicity and fall in catalase activity.

Of 25 patients with more than 1 catalase assay who had progression of tumor, only 2 had rises in catalase activity, 18 had unequivocal decrease in catalase, and 5 had essentially plateau values.

A total of 201 observations of hepatic catalase activity, including 50 from necropsy specimens, were divided into 3 groups according to survival status; catalase activity at death, catalase
activity in the period less than 3 months prior to death, and catalase activity determined on liver specimens more than 3 months before demise. The cumulative percentage frequency distribution for each group is plotted in Chart 8. It can be noted that the graph shows good separation: the distributions were found to be significantly different from each other (A vs. B at the 1% level, and B vs. C at the 5% level). Thus, one can recognize groups of patients retrospectively in which catalase activity rather than clinical impression correlated with survival. Although imperfect, such indices based on measurable parameters are rare in neoplastic disease.

The survival of patients who responded or failed to respond to chemotherapy were also studied. Among 59 patients observed, 5 were omitted from the analysis. Three of these were lost to follow-up. The other 2 patients are alive and well 4 years later; biopsy proof of intraabdominal tumor recurrence had not been obtained. Another 3 patients who failed to respond to chemotherapy during the time under observation in the present study subsequently responded to other chemotherapy. They were included in the group of responders in the survival study, after finding that their exclusion did not change the shape of the curves in any way. These 54 patients were divided into 4 groups according to their initial catalase level and the survival from the time of biopsy was plotted in each group as shown in Chart 9. It is noted that the responders in all groups live longer than nonresponders without regard to the initial catalase activity. Because of the small size of the groups, this difference is significant at the 5% level only for the 2 groups with lowest initial catalase activity. The difference in survival for responders in all groups in contrast to all patients who failed to respond, however, is significant at the 1% level. This is a clear demonstration that prolonged survival occurs in patients with chemotherapeutic tumor regression judged on an objective basis. The data also demonstrate that initial catalase level per se is not as influential a determinant of prospective survival as response to chemotherapy.

In Chart 10, the % change in catalase from the initial value is plotted against time for those who responded and those who failed to respond in all tumor categories. It can be seen that 2 populations of points result, with little overlap. Those who respond have a rise in activity above the initial value significant at the 1% level. Those who fail to respond fall below the initial value with significance at the 1% level.

The relationship between changes of hepatic catalase activity/gm of liver nitrogen and body weight is shown in Chart 11. Although in some patients, decrease of body weight, progression of the disease, and fall in the catalase activity correlate well, and in other patients, response to medical management is accompanied by increase in body weight and hepatic catalase, an overall statistical analysis failed to show a correlation between changes of hepatic catalase activity and body weight. The importance of this finding is clouded, however, by the possibility that the water accumulation commonly present in advanced cancer might have spuriously increased weight in a systematic fashion.

Discussion

The present communication describes the measurement of hepatic catalase activity in man using a simple and rapid technie,
Hepatic Catalase Activity

Chart 9. Survival of responders (top line in each graph) and nonresponders in 4 groups of patients grouped by initial hepatic catalase activities/gm of liver nitrogen.

Chart 10. Changes in hepatic catalase activity/gm of liver nitrogen from the initial activity in responders and nonresponders. All patients are plotted as 100% on their initial biopsy.
The spectrophotometric measurement of hydrogen peroxide disappearance (1) upon incubation with liver homogenate obtained by needle biopsy permitted us to make sequential studies on unanesthetized patients during advancing cancer, and on normal subjects.

The technic employed recovered approximately 90% of the activity in cadavers, and 75% of that found in patients when nonionic detergent and vigorous mechanical homogenization were used. Dynamic changes were concordant and similar when observed: 24% and 11% rise during tumor regression by 50-stroke homogenization or detergent omnimixing, respectively, and 25% and 23% fall during melanoma progression (Table 3).

Changes in catalase activity were not influenced by alteration in hepatic water or fat content, since the data are expressed in terms of hepatic nitrogen. Systematic alteration in the proportion of hepatic and reticuloendothelial cells might produce alterations in catalase activity/gm of liver nitrogen, but histologic study of biopsy specimens failed to support such a possibility.

The catalase level at death was measured in 60 cadavers (Chart 1, Table 2), and was found to be 294 ± 111 units/gm of liver nitrogen. Furthermore, catalase activity in refrigerated cadavers was demonstrated to be stable, therefore permitting these and other postmortem studies. The decrease of catalase activity is a decrease in total activity (Table 2), and not merely a dilution phenomenon as previously claimed (7).

Catalase studies on groups of patients with morphologically related types of neoplasms are presented in Charts 2-6. Clearly, the majority of patients under study had no improvement from chemotherapeutic or other treatment procedures, and are shown in the bottom halves of the charts. Response to chemotherapy or other treatment was objectively determined as described, independently from considerations of behavior of catalase activity. There is a great preponderance of negative slopes of catalase activity in the 25 patients who failed to respond, and contrariwise, of 14 patients who sustained objective improvement among all 5 groups, 11 had positive slopes of catalase activity. There is no
appreciable difference among tumor types nor sites of metastasis that influenced initial catalase levels or subsequent behavior. Steep negative slopes were associated with rapid progression of tumors, and regressing tumors were accompanied by positive slopes. Individual case presentations (Chart 7) demonstrate the clear correlation of therapeutic effect and change in catalase activity. Catalase increase has provided objective measurement in the host which accompanies objective regression of neoplasm.

Two endocrine ablation procedures were used as treatment which caused tumor response and rise in hepatic catalase activity. Adrenalectomy (with corticosteroid replacement) in Breast Cancer Patient No. 1 and orchiectomy and estrogen administration in Adenocarcinoma Patient No. 1 both were followed by tumor regression and rise in catalase. These same procedures in nontumor-bearing mice have been shown to result in fall in catalase activity (5). Breast Cancer Patient No. 5, who also underwent adrenalectomy but failed to demonstrate objective regression of tumor, showed a further decline in hepatic catalase.

Lack of therapeutic response to hypophysectomy in Breast Cancer Patient No. 6 was associated with progressive cachexia, and fall in catalase. This is consistent with the fall in catalase activity of tumor-bearing rats despite hypophysectomy (14).

The effect of these hormonal manipulations on liver catalase in man without cancer is unknown. In these 4 cancer patients, however, it appears that behavior of the tumor was of greater influence than whatever hormonal influence may have been exerted directly on the liver.

Severe drug toxicity can apparently affect hepatic catalase activity more than tumor behavior. Thus impressive regression of myelomatous masses in Miscellaneous Patient No. 2 was accompanied by fall in hepatic catalase activity in the presence of formidable toxicity.

When the catalase activities of all cancer patients as a group are plotted according to survival rank of 3 months or more, less than 3 months, or dead already, significantly different curves are found (Chart 8). They show, when considering the entire group, that as death approaches, the catalase activity declines.

When the survival of patients who responded to treatment and those who did not are plotted (Chart 9) there is evidence of an effect of chemotherapy which is more than mere shrinkage of tumor size. It is noteworthy that the catalase activity usually rose in the responders but fell in those who did not respond.

It has been speculated in the past that depletion of total body protein, measured as body weight, was the responsible mechanism for catalase depression (12). When changes in catalase activity/gm of liver nitrogen and changes in body weight were measured for all patients as a single large group (Chart 11), no correlation was found. Indeed, our experience encompasses substantial decrements in body weight (conceivably due to loss of fluid) with concurrent lowering of catalase (11), but consistent increase in catalase activity also has occurred despite weight loss (Charts 7a, c). Since total body weight incorporates the divergent influences of fat loss, protein loss, and fluid gain in advanced cancer, and fat, protein, and fluid change in the opposite direction after effective therapy, we see no basis to believe that catalase activity should be directly correlated with this composite. Nonetheless, it may indeed be true that catalase is a representative protein whose synthesis is diminished in advanced cancer, in part related to nutritional deficiency. The experiments of Hozumi and Sugimura (4) demonstrate that catalase depression caused by toxohormone administration to mice is due to inhibition of catalase synthesis. It is likely that the depression in catalase activity caused by tumor growth in vivo is also explained by deficient catalase synthesis, although unequivocal proof is not available.

It is certainly possible that whatever mechanism diminishes catalase activity may also interfere with the synthesis of other proteins. Hemoglobin is another heme containing protein often lowered in advanced cancer. Although correlative analysis with fall in catalase would be of interest in man, appropriate studies were not obtained in our patients, and transfusions were used electively.

Studies of bacterial contamination of the patients' tumors were not made routinely. Some patients had ulcerated neoplasms with gross surface contamination by a wide spectrum of microorganisms. Others did not. Urinary and pulmonary infections punctuated the course of many of these patients with advanced cancer. Antibacterial treatment was used according to the same therapeutic criteria in the group of responders and nonresponders. The patients with objective regressions of tumors contemporaneous with the administration of cancer chemotherapy include nearly all those whose catalase activity rose. There is no appreciable effect of the antitumor drugs in the doses used on microbial flora or infections, nor were these patients receiving exceptional amounts or types of other drugs. We doubt, therefore, that a causal relationship between bacterial control and rising catalase activity explains our observation. Furthermore, we find no evidence to suspect that bacterial contamination explained the fall in catalase at the termination of a therapeutic response when tumor began to grow, or in the entire population of patients who failed to respond. Augmentation of catalase depression has, however, been ascribed to bacterial contamination of 1 transplantable rat tumor line (6) and inferentially implicated in the explanation and interpretation of many experiments on transplantable animal tumors and toxohormone preparations (5). Bacteriologically sterile tumor growth in axenic mice has recently been shown to depress catalase activity (E. A. Mirand, personal communication, 1965).

Falkson and deJager (3) have recently described catalase determinations made on epidermal biopsies from the back of patients undergoing radiotherapy or chemotherapy for advanced cancer. In 18 of 25 instances concordant changes occurred: a rise in catalase with regressing tumor or a fall with tumor progression. In several instances the catalase change was apparent before the clinical phenomenon was established. In 14 of the 25, weight changes discordant with catalase behavior were observed: weight loss was not a constant accompaniment of lower catalase.

The normal subjects in this study were prisoner volunteers who had received a nutritious diet, and had voluntarily abstained from alcohol for at least 3 months. Regrettably, the normal assays are limited in number. The wide range of catalase activity from 259 to 1061 units/gm of liver nitrogen is of interest. This range complicates the assumption of a patient's precancer catalase level. Genetic variations of erythrocyte catalase activity have been recognized in man (17). Although genetic variation of hepatic catalase has so far been described only in mouse liver (13), it is likely that genetic factors in man, in addition to environmental and metabolic effects, are operative.
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Appendix

During the early phase of the study, the absorbance of reaction mixtures was read against a blank of 0.05 M phosphate buffer only, rather than buffer containing liver homogenate. Absorbancies therefore were due not only to hydrogen peroxide but to non-specific absorption as well. A technique to use absorbancy changes, rather than absolute absorbancy, for the calculation of rate constants was therefore devised. The following formula for a first order reaction process was introduced.

\[
\frac{\Delta A}{A_0} = \frac{A_1 - A_2}{A_0} = e^{-t_1k} - e^{-t_2k}
\]

where \(A_0\) = calculated absorbance at 0 sec due to the known amount of \(\text{H}_2\text{O}_2\) added (for a few experiments, \(A_0\) was not experimentally determined; in these instances the mean \(A_0\) of all other experiments was used); \(A_1\) = absorbance of reaction mixture at \(t_1\) sec; \(A_2\) = absorbance of reaction mixture at \(t_2\) sec. \(\Delta A\) therefore is the decrement in absorbancy occurring during the interval between \(t_1\) and \(t_2\) and is due solely to disappearance of \(\text{H}_2\text{O}_2\).

A series of graphs was prepared displaying \(\Delta A/A_0\) as a function of \(k\) for all 21 intervals where \(t_1 = 10, 20, \ldots, 60\) sec and \(t_2 = 20, 30, \ldots, 70\) sec. These graphs were then used to obtain estimates of \(k\) for each of the 21 observed values of \(\Delta A/A_0\). The mean of the values so obtained was accepted as the best estimate of the rate constant. The validity of this method was experimentally proven.

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Hepatic Catalase Activity in Advanced Human Cancer

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