pH Studies of Malignant Tissues in Human Beings

Karl A. Meyer, M.D., Erwin M. Kammerling, M.D., Leo Amtman, M.D., Margaret Koller, B.S., M.S., and Samuel J. Hoffman, M.D.

(From the Hektoen Institute for Medical Research of Cook County Hospital, the Jerome D. Solomon Memorial Research Foundation and Department of Medicine, University of Illinois College of Medicine, Chicago 2, Illinois)

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INTRODUCTION

On the basis of extensive studies, it has long been contended by many investigators that the hydrogen ion concentration of malignant tissues differs from that of normal tissues in a characteristic way. The results obtained, however, are highly controversial. Many workers have found an alteration of pH to the alkaline side (1, 4, 5, 9, 14, 16). Others have found an alteration to the acid side (7, 8, 13, 15, 20). Still others (2, 11, 23) have found no specific pH change in neoplastic tissues. These wide differences in results are explained by Stern and Willheim in several ways:

1. The hydrogen ion concentration of living tissue undergoes constant variations as a result of continuous metabolic processes. 2. Comparisons with values of normal tissues are few, in experimental animal work as well as with human tumors. 3. The technic used, the changes introduced by the method itself and the individual interpretation of results all contribute to the varying conclusions drawn as to pH alteration in neoplastic tissues.

Some workers have tested freshly prepared extracts of tumors by a colorimetric method while others have injected indicator substances into tumor-bearing animals. Both quinhydrone and glass electrodes have been used in tissue cultures, in material surgically-removed in vitro and in living, lightly anaesthetized animals in vivo.

Many, including Voegtlin, Kahler and Fitch (22) agree that the glass electrode is best suited for the purpose of study of this problem. It has long been demonstrated that the presence of oxidizing and reducing substances derived from tissues did not introduce any error in the pH determinations made by the bulb type of glass electrode (22). Voegtlin, DeEds, Kahler and Rosenthal (19) in 1929 demonstrated that the insertion of bulb type of electrodes into skeletal muscle would give rough estimations of pH changes under varying physiological conditions. Snell and Biffen (17), in discussing the advantages of the glass electrode, state that “the glass electrode is not affected by oxidation-reduction potentials in the solution. It has no salt or protein error and usually comes to equilibrium immediately. Most instruments have incorporated temperature compensators. Many types of glass electrodes are available and may frequently be used interchangeably with those in the original apparatus.”

Our study was carried out at the Cook County Hospital in Chicago where pH determinations were made by means of the Beckman pH meter, Model G, using both glass bulb type and capillary-type electrodes (No. 1190 and No. 1190 X 5) on tissues freshly removed at surgery (Figs. 1 and 2). The Beckman 1190 X 5 glass electrode is built with a special pH sensitive glass bulb blown on the end of standard lead glass tubing. The bulb is subsequently shaped into a taper for ease of inserting into soft materials. The buffer solution used in the tip of the glass electrode is such that when this electrode is employed in combination with the standard 1170 Reference Calomel Electrode it provides the proper emf-pH characteristics to the Model G circuit so that the resulting measurements made with the instrument read directly in pH units. The solution used in the glass electrode does not deteriorate with age. In the pH range from 0 to approximately 9.5-10.0 pH, glass electrodes act quite accurately as a hydrogen electrode. The calomel electrode consists of a permanently filled, calomel-mercury internal, and an external body around it which is kept filled with saturated KCL solution. The liquid junction potentials between the solution under measurement and the KCL solution is achieved by adjustment of the concentrations of the components of the special filling solution used in the glass electrode. Because the composition of all of the solutions used in the two electrodes is always the same and the
glass used in the glass bulb is the same for each electrode, the EMF temperature relationship for all electrodes is the same. Therefore, a temperature-compensating electrical circuit is constructed within the instrument. This temperature compensator does not correct pH readings to a base temperature value but merely adjusts the circuit to read the proper pH at the temperature of measurement. For example, a pH measurement taken at 40°C with the temperature compensator at 40°C gives the actual pH of the solution at 40°C.

Although it was our original intention to test all specimens at a given time interval following surgical removal, local conditions in the operating rooms prevented this to a certain extent. The pH of neoplastic and other tissues was determined at periods ranging from 5 to 90 minutes after surgical removal. In all cases, multiple determinations were made on each sample and at several sites of the tissue. Many specimens were examined at repeated intervals in an attempt to evaluate the influence of time following removal on the pH of the specific tissue. Other factors considered and noted were the type of preoperative treatment (medication, x-ray and radium treatment of the tumor), type of anaesthetic and the administration of glucose, saline, plasma and blood during surgery. The Beckman apparatus was kept in a stationary place in a room on the surgical floor and all specimens were carried to the apparatus. The electrodes were standardized against phosphate buffer (specifically prepared for use with Beckman pH meter) at the specific room temperature before and after examination of each surgical specimen. The outer surface of the tissue to be examined was carefully dried, using tissue paper with neutral pH and a small incision into an avascular area was made with a clean scalpel. The electrode was then carefully inserted, making contact with the desired area with as little trauma as possible. Care was taken to avoid contamination with blood. The tissue was examined after the determination to detect evidence of any hemorrhage; results were discarded when this occurred. Readings were taken of the deeper, more central portions of the tumor as well as of the superficial portions. Since it has been shown that necrosis of tumor tissue alters tissue pH (21), the degree of macroscopic degeneration was noted and recorded. Several of the tumors or portions thereof were noticeably degenerated. All specimens were examined histologically by the hospital surgical pathologist, Dr. Alex Ragins.

To date, specimens from 110 surgical patients have been examined. Many of the specimens, for example, benign tumors of the uterus and breast, afforded the opportunity to make determinations of more than one distinct lesion in the same specimen. pH determinations of malignant tumors, benign tumors, normal tissues and a few inflammatory tissues were included in this study.

Included in the series of malignant tumors are: 20 carcinomas of the gastrointestinal tract, 10, 13 carcinomas of the breast, 1 carcinoma of the penis, 1 fibrosarcoma of the hip and 1 dermoid cyst of the ovary. Among the benign tumors were: uterine fibroids of 50 patients, 2 lipomas and 7 fibroadenomas and adenofibrosis of the breast. The small number of inflammatory tissues included appendix, gall bladder, cervix and sigmoid colon. The pH of normal tissues in specimens containing both neoplastic and normal areas were determined whenever possible. Incision into many areas of normal tissue caused bleeding from the tissue surface; these determinations were not recorded.

RESULTS

The pH of specimens of malignant tissue varied from 5.44 (carcinoma of the stomach) to 7.96 (carcinoma of the breast). Excluding those specimens which had undergone grossly marked degeneration and those in which preoperative irradiation had occurred, the pH range of these malignant tissues varied from 5.44 to 6.75.

A. MALIGNANT TISSUES.

Included in the 36 cases of malignant tumors studied were 14 cases in which we were able to determine the pH of normal areas as well as of malignant areas in the same specimen. In 12 of these cases, there was a difference in pH ranging from 0.17 to 1.15 with an average pH difference of 0.49, the lower pH representing that of the malignant tissue. In the remaining two cases, the pH of the uninvolved normal pectoral muscle was lower than that of the malignant area while the pH of the normal fatty and connective tissue was higher. These results are recorded below in Table I.

Specimen No. 94, representative of this group presented (Table I) is listed below in some detail in Table II.

Thirteen cases of carcinoma of the breast were examined. These included 6 cases which had not received preoperative irradiation and in which there was no gross evidence of necrosis at examination, 2 cases that had received preoperative irradiation and 5 cases in which there was gross evidence of necrosis. The following histological types were represented: medullary carcinoma, papillary ade

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TABLE I: pH OF MALIGNANT TISSUES AS COMPARED TO pH OF NORMAL TISSUE IN THE SAME SPECIMEN (14 CASES)

<table>
<thead>
<tr>
<th>Specimen and organ</th>
<th>Microscopic diagnosis</th>
<th>Number of readings</th>
<th>Mean pH</th>
<th>Normal tissue</th>
<th>Number of readings</th>
<th>Mean pH</th>
<th>Difference pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 59, carcinoma, esophagus</td>
<td>Infiltrating squamous cell carcinoma</td>
<td>4</td>
<td>6.54–6.56</td>
<td>6.55 Muscularis</td>
<td>2</td>
<td>7.06–7.07</td>
<td>-.51</td>
</tr>
<tr>
<td>No. 73, carcinoma, esophagus</td>
<td>Anaplastic squamous cell carcinoma</td>
<td>6</td>
<td>6.32–6.43</td>
<td>6.39 “</td>
<td>2</td>
<td>6.77–6.78</td>
<td>-.48</td>
</tr>
<tr>
<td>No. 34, carcinoma, stomach</td>
<td>Adenocarcinoma antrum</td>
<td>8</td>
<td>6.37–6.72</td>
<td>6.55 “</td>
<td>2</td>
<td>7.19–7.20</td>
<td>-.64</td>
</tr>
<tr>
<td>No. 92, carcinoma, stomach</td>
<td>Papillary adenocarcinoma</td>
<td>6</td>
<td>5.94–6.24</td>
<td>6.10 “</td>
<td>4</td>
<td>6.59–6.72</td>
<td>-.55</td>
</tr>
<tr>
<td>No. 94, carcinoma, stomach</td>
<td>Infiltrating adenocarcinoma</td>
<td>8</td>
<td>6.19–6.32</td>
<td>6.24 “</td>
<td>6</td>
<td>6.52–6.70</td>
<td>-.38</td>
</tr>
<tr>
<td>No. 107, carcinoma, stomach</td>
<td>Scirrhous adenocarcinoma</td>
<td>6</td>
<td>5.93–6.08</td>
<td>6.01 “</td>
<td>4</td>
<td>6.39–6.42</td>
<td>-.40</td>
</tr>
<tr>
<td>No. 109, carcinoma, rectum</td>
<td>Infiltrating adenocarcinoma</td>
<td>8</td>
<td>5.61–5.82</td>
<td>5.72 “</td>
<td>4</td>
<td>6.06–6.10</td>
<td>-.37</td>
</tr>
<tr>
<td>No. 109, carcinoma, rectum</td>
<td>Infiltrating mucus-producing adenocarcinoma</td>
<td>6</td>
<td>6.72–6.75</td>
<td>6.73 Fatty tissue</td>
<td>2</td>
<td>6.90</td>
<td>-.17</td>
</tr>
<tr>
<td>No. 63, carcinoma, breast</td>
<td>Infiltrating papillary cystadenocarcinoma</td>
<td>6</td>
<td>6.27–6.38</td>
<td>6.33 “</td>
<td>2</td>
<td>6.80</td>
<td>+.47</td>
</tr>
<tr>
<td>No. 66, carcinoma, breast</td>
<td>Adenocarcinoma</td>
<td>6</td>
<td>5.86–6.11</td>
<td>5.95 Pectoral muscle</td>
<td>2</td>
<td>6.62</td>
<td>-.67</td>
</tr>
<tr>
<td>No. 103, carcinoma, breast</td>
<td>Infiltrating medullary carcinoma</td>
<td>10</td>
<td>6.01–6.17</td>
<td>6.09 Pectoral muscle</td>
<td>6</td>
<td>5.66–5.82</td>
<td>+.37</td>
</tr>
<tr>
<td>No. 106, carcinoma, breast</td>
<td>Anaplastic medullary carcinoma</td>
<td>6</td>
<td>5.76–5.85</td>
<td>5.81 Pectoral muscle</td>
<td>4</td>
<td>5.24–5.30</td>
<td>+.54</td>
</tr>
</tbody>
</table>

carcinoma, anaplastic carcinoma, carcinoma simplex, scirrhous carcinoma and adenocarcinoma. No correlation between histological type and tissue pH was determined. The influence of irradiation and that of degeneration on the pH of carcinoma of the breast is illustrated in Table III.

Single cases of carcinoma of the penis, teratodermoid of the ovary and fibrosarcomas were examined. The results of this miscellaneous group comprise Table IV below.

B. BENIGN TUMORS

Fifty cases of uterine fibromyomas were examined. Many of the uteri contained multiple fibroids and in approximately one-half of the cases, pH determinations were made of non-tumor bearing portions of the uterus as well as of the tumors. Two or more pH readings were taken in various
TABLE III: THIRTEEN CASES OF BREAST CARCINOMA

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No. of cases</th>
<th>No. of areas examined</th>
<th>pH range</th>
<th>Mean pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>No preoperative irradiation. No degeneration</td>
<td>6</td>
<td>20</td>
<td>5.76-6.75</td>
<td>6.18</td>
</tr>
<tr>
<td>Preoperatively irradiated tumors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grossly degenerated tumors</td>
<td>2</td>
<td>7</td>
<td>6.90-7.36</td>
<td>7.16</td>
</tr>
</tbody>
</table>

TABLE IV: MISCELLANEOUS GROUP OF MALIGNANT TUMORS

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No. of areas examined</th>
<th>pH range</th>
<th>Mean pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hornifying anaplastic squamous cell carcinoma of penis</td>
<td>3</td>
<td>6.57-6.62</td>
<td>6.59</td>
</tr>
<tr>
<td>Necrotic portion of above tumor</td>
<td>2</td>
<td>7.23-7.29</td>
<td>7.26</td>
</tr>
<tr>
<td>Teratodermoid ovary</td>
<td>2</td>
<td>6.82-6.86</td>
<td>6.84</td>
</tr>
<tr>
<td>Pleomorphic giant cell fibrosarcoma</td>
<td>3</td>
<td>6.17-6.39</td>
<td>6.25</td>
</tr>
</tbody>
</table>

TABLE V: FIFTY CASES, 151 DETERMINATIONS, OF UTERINE FIBROMYOMAS

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No. of areas examined</th>
<th>pH range</th>
<th>Mean pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal uterus</td>
<td>21</td>
<td>6.57-7.17</td>
<td>6.94</td>
</tr>
<tr>
<td>Fibromyomas. No visible degeneration</td>
<td>112</td>
<td>6.11-7.31</td>
<td>6.76</td>
</tr>
<tr>
<td>Fibromyomas. Visibly degenerated</td>
<td>18</td>
<td>6.32-7.91</td>
<td>6.91</td>
</tr>
</tbody>
</table>

Seven cases of histologically benign breast tumors were examined, among which were 4 cases of adenoma and 3 of adenofibrosis. Table VI reveals that the mean pH of these tumors was higher than that of several normal areas of breast tissue in the same specimens.

Two cases of lipomas which were examined had a pH range of 6.77-7.21 with a mean pH of 6.96.

Experiments were carried out to determine the change in tissue pH in tumor specimens over prolonged periods following surgical removal. pH determinations were repeated in the same area at varying intervals. Representative of this group are 3 cases listed in Table VII.

### DISCUSSION

The interpretation of pH determinations of tissues, especially those examined at varying intervals following removal from the body is at best somewhat difficult. First, we cannot evaluate with accuracy the rapidity and extent of change of tissue pH immediately after its blood supply has been severed and the tissue subsequently removed from the animal body. The formation of lactic acid and other acid metabolites in the tissues, the buffering capacity inherent in neoplastic tissue, the effect of the anaesthetic employed and the depth of anesthesia, the effect of parenteral glucose given during surgery, are important factors known to influence the pH of tumors. Further it is known that the pH of living tissue undergoes continuous variations and that the data obtained by various physicochemical measurements represent at best only a transient time differential. Another important factor, difficult to evaluate grossly and recognized by all workers as influencing tissue pH is the presence and variable degree of necrosis in different parts of the tumor. We know also that the electrode makes contact with many cells and with the intercellular fluid and not with one single cell. Obviously, we do not measure intracellular pH. Finally, the possibility that insertion of the electrode into the tissue may injure the smaller blood vessels and thus contaminate the electrode must be considered.

However, while it is impossible to eliminate certain of these potential sources of error, we feel that the careful technics employed, the accuracy of the apparatus used, as well as critical analysis of the results, enable us to draw some valid conclusions. Careful handling of the specimens following their surgical removal and careful insertion of a very small caliber electrode into the tissue avoids injury to small blood vessels and subsequent contamination of the electrode. When careful examination of the tissue and electrode following the determination revealed evidence of hemorrhage, the results were discarded. pH determinations of bloody fluid in other areas for comparison support this assumption. Loss of CO₂ from the tissue and consequent change of tissue pH is avoided as the capillary...
trode is deeply inserted into the tissue and the insulating electrode coating covers the upper portion of the tissue.

Using the fine capillary type of electrode, we noted an initial fall in pH of about 0.05 to 0.10 units during the first 1 to 2 minutes, this being followed with a gradual rise until the equilibrium value was reached. This primary fall is interpreted as being caused by cell injury and is of short duration.

Repeated experiments revealed that by withdrawing the electrode and carefully reinserting it into the tissue, pH readings could be duplicated. Therefore, the results are reproducible.

pH readings carried out at intervals in the same area of the tissue revealed that the voltage maintained a constant value for 90 minutes or longer, deviating at most 0.02 to 0.03 pH units. Confirming results of other investigators, we noted that necrotic portions of tumors gave consistently higher pH values than tumors which had not undergone degeneration. Of greatest significance in these experiments was the consistent pH difference between normal areas and malignant areas in the same specimen (in which cases almost all other factors are the same).

SUMMARY

Despite the many possible sources of error inherent in this approach to the study, we feel that they have been minimized by the careful technics and the accuracy of the apparatus employed. We have concluded that:

1. The pH of malignant tumors is lower than that of normal tissue in the same specimen.
2. The pH change of benign tumors varies. Some have a higher pH while others have a lower pH than that of the normal tissue in the same specimen.
3. Degenerative changes in malignant tissues increase the tissue pH.
4. Following irradiation, the pH of malignant tissue is increased.
5. The initial drift in pH to the acid side following insertion of the electrode appears to be less and its duration less in these specimens studied in vitro than in living tissue studied in vivo.

REFERENCES


pH Studies of Malignant Tissues in Human Beings

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