INTERRELATIONS BETWEEN HISTOLOGIC STRUCTURE AND BLOOD CHEMICAL FINDINGS IN CANCER

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The vital activities and the morphology of cells seem to depend to a large extent upon the chemical composition and reaction of the surrounding liquid medium in which the cells are normally found or into which they may be placed for experimental purposes (Haagen, 1). This observation has furnished the basis for the investigations which are herein presented.

Taking the cell and its environment as a unit, this study attempts to correlate certain chemical findings in the blood of cancer patients with the morphologic findings in the tumors from the same patient. Changes in the environment, the blood, might possibly be reflected in the histologic structure of the tumor, and, on the other hand, the vital activity of the tumor cells as expressed by the degree of maturity might affect the chemical reaction and composition of the environment.

Hueper's malignancy index (2) was used for the evaluation of the histologic structure of the carcinomata. The pH of the blood was determined after the method of Hastings and Sendroy (3), modified by Schoonover and Woodward (4). For the determination of the blood sugar the method of Hagedorn-Jensen (5) was used.

The colorimetric method for the determination of blood pH as employed in our laboratory has been checked by Fos binder and Schoonover (6) with the glass electrode and a vacuum tube potentiometer devised by Fos binder (7). An average deviation of 0.002 was found between the two methods, a figure so small as to be negligible. Thus it has been shown that the colorimetric method as modified and used in these studies gives exact and reproducible pH results. A series of normal cases gave an average blood pH of 7.38, which we consider approximately normal.

The Hagedorn-Jensen method expresses very nearly the true blood sugar value. Until recently the blood sugar as generally
reported has been too high, to the average extent of 15 mg. per 100 c.c. of blood. It is known that other reducing substances present in the usual blood filtrates react on the reagents as does glucose, thus producing an additive error. In our experience with the Hagedorn-Jensen filtrate, glutathione has been entirely absent. Herbert and Bourne (8), it is true, have reported that only a part of the glutathione is eliminated by the Hagedorn-Jensen procedure. This is the case when Hagedorn-Jensen filtrates are made from pure aqueous solutions of glutathione. However, in the presence of protein we have demonstrated the removal of glutathione to be complete. We have likewise checked the Hagedorn-Jensen filtrate with the Somogyi zinc hydroxide filtrate (17), the latter having been shown by this author to contain no appreciable amount of glutathione. The effect of this substance is thereby ruled out. Unless, then, there is a hitherto unknown reducing substance in cancer blood, the Hagedorn-Jensen blood sugar value is very close to the true one. By this method we have found the normal figure to be 70 to 89 mg. per 100 c.c.

From a total number of 102 human cases of cancer of various organs in which pH and blood sugar determinations and histologic malignancy indices were made, 34 untreated cases were selected for our study. Basal-cell cancers of the skin were excluded as unsuitable, as they differ biologically from the usual cancer. Cases treated with x-rays or radium before the histologic and blood chemistry examinations were done were also eliminated because such treatment affects the pH and the sugar content of the blood (Held, 9) and produces disturbances in histologic structure which are apt to make a correct and reliable correlation of these factors impossible. Finally, also, those untreated cases were not considered in which the interval between the histologic and blood chemistry examinations was unduly long, exceeding in general more than a week.

**INTERRELATIONS BETWEEN THE MALIGNANCY INDEX AND THE pH OF THE BLOOD**

In previous publications of Hueper (2) it has been shown that there exist definite numerical relations between the histologic structure of a carcinoma, as expressed by the histologic malignancy index, and its prognosis. With the increase of the malignancy index, which represents a numerical evaluation of 20 different
histologic factors pertaining to the parenchyma and stroma of a cancer, the curative results decreased proportionally. The numerical range of the histologic malignancy index is from 20 to 80. As it was found that the average malignancy index of 100 carcinoma of the uterine cervix and 71 carcinoma of the breast was 56, four malignancy groups were formed: Group I ranging from 32 to 44, Group II from 45 to 56, Group III from 57 to 68 and Group IV from 69 to 80. (For the purpose of this paper a blood pH above 7.39 is spoken of as alkalosis; and, correspondingly, a pH below 7.36 as acidosis.)

In a biochemical study by Woodward, Schoonover, Fry, Torrance and McDonald (10) a remarkable interrelation between the longevity of the patient and the pH of the blood was noted. The length of life decreased in direct proportion to the degree of alkalinity of the blood. The degree of alkalinity of the blood in untreated cases is, therefore, closely related to the prognosis of the disease.

This parallelism of the prognostic significance of the two factors, alkalinity of the blood and malignancy index, was made the subject of a comparative study (Table I).

<table>
<thead>
<tr>
<th>Malignancy Index</th>
<th>32 to 44</th>
<th>44 to 56</th>
<th>57 to 68</th>
<th>69 to 80</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH above 7.39</td>
<td>3</td>
<td>12</td>
<td>1</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>pH 7.36 to 7.39</td>
<td>2</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>pH below 7.36</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>12</td>
<td>18</td>
<td>2</td>
<td>34</td>
</tr>
</tbody>
</table>

These figures demonstrate a correlation between the histologic malignancy index and the pH of the blood. With a higher malignancy index, an alkaline pH appears with increasing frequency. A discrepancy in the results obtained is, however, apparent in the acidosis group. Here, while the pH values indicate a relatively good prognosis, the histologic malignancy indices contradict this conclusion. No satisfactory explanation for this discrepancy can be offered at the present time, but it may be suggested that the high malignancy indices in this group are due to the morphologic distortions resulting from a pathologic pH of the environmental fluid. Cellular irregularities associated with an acid blood pH may, however, possess a prognostic signifi-
cancer differing from that of similar irregularities associated with an alkaline pH. If this assumption should be correct, the pH determinations of the blood would represent an important additional factor in the laboratory evaluation of malignancy. The unexpectedly good curative results in certain histologically highly malignant tumors might thus find a satisfactory explanation.

**Special Cell Type of Carcinoma and pH of the Blood**

In the histologic malignancy index, the factor—special cell type—designates the degree of differentiation or maturity of the tumor. The following scale is used:

I. Primary solid carcinomas
   1. Spinous-cell carcinoma with cornifications ........ 1 point
   2. Spinous-cell carcinoma without cornifications ...... 2 points
   3. Round-cell or transitional-cell carcinoma .......... 3 points
   4. Spindle-cell carcinoma .......................... 4 points

II. Glandular carcinoma
   1. Malignant adenoma .................................. 1 point
   2. Gelatinous and papillary adenocarcinoma .......... 2 points
   3. Simple adenocarcinoma ............................. 3 points
   4. Solid adenocarcinoma .............................. 4 points

Woodward, Schoonover, Fry, Torrance and McDonald (10) believe that the alkalinity of the blood is rather a consequence than a cause or a predisposing factor in cancer (Reding, 11). This conception is supported by the findings of Chambers (12), Roffo and Corrêa (13), and Guthmann and Wirz (14). These latter authors believe that there is an acidosis in early cancers, but an alkalosis in advanced cancers, increasing with the extent of the disease as the result of an overcompensated acidosis. As there is a possibility that the alkalosis of the blood might affect and possibly prevent the maturation of the tumor cells, even if the tumor cells should be responsible for the production of the alkalosis, the coincidence of these two factors was determined. If this explanation is correct, there would exist a sort of vicious circle in regard to the interrelation and interaction of the degree of maturity and the alkalosis of the blood. The correlations set forth in Table II were observed:

A more alkaline blood pH is, according to these figures, more frequently associated with a cancer of an immature type than with one of mature type. This observation is more strikingly demonstrated, if special cell types 1 and 2 and special cell types 3 and 4 are grouped together (Table III).
The more alkaline pH of the blood of cancer patients seems to be not only a function of the extent of the disease, but also of the vital activities of the tumor cells as represented in the degree of morphologic maturity and histologic malignancy of the tumor.

**Table II**

<table>
<thead>
<tr>
<th>pH above 7.39</th>
<th>pH 7.36 to 7.39</th>
<th>pH below 7.36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Special Cell Type</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>pH above 7.39</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>pH 7.36 to 7.39</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>pH below 7.36</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>12</td>
</tr>
</tbody>
</table>

**Table III**

<table>
<thead>
<tr>
<th>pH above 7.39</th>
<th>pH 7.36 to 7.39</th>
<th>pH below 7.39</th>
</tr>
</thead>
<tbody>
<tr>
<td>Special Cell Type</td>
<td>1 and 2</td>
<td>3 and 4</td>
</tr>
<tr>
<td>pH above 7.39</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>pH 7.36 to 7.39</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>pH below 7.39</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>18</td>
</tr>
</tbody>
</table>

**CELLULAR IRREGULARITY AND pH OF THE BLOOD**

The morphology of a cell depends upon four factors: (1) inherited qualities; (2) developmental stage; (3) physico-chemical condition of the cell (functional activity); (4) physico-chemical condition of the environment. Any disturbance in the normal equilibrium of these factors may produce changes in the cellular morphology. As morphologic cellular irregularity is one of the main histologic characteristics of malignant growth, an investigation into the possible relation between the degree of cellular irregularity in cancers and the pH of the blood seemed to be indicated. The morphologic factor was determined on a numerical basis by an evaluation of five factors pertaining to the size and shape of the cells and their nuclei and the relative frequency of atypical mitoses, using the principles embodied in the method of the histologic malignancy index. The sum of the values thus obtained ranges from 4 to 20 points. As none of the carcinomata
evaluated had an irregularity index below 7 the range from 7 to 20 was divided into two equal groups: Group I ranging from 7 to 13 and Group II from 14 to 20. The comparison with the blood pH values gave the following results (Table IV).

**Table IV**

<table>
<thead>
<tr>
<th>Cellularity</th>
<th>Group I: 7-13</th>
<th>Group II: 14-20</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH above 7.39</td>
<td>5 (31.25%)</td>
<td>11 (68.75%)</td>
<td>16</td>
</tr>
<tr>
<td>pH 7.36 to 7.39</td>
<td>6 (61.50%)</td>
<td>3 (38.50%)</td>
<td>9</td>
</tr>
<tr>
<td>pH below 7.36</td>
<td>1 (20.00%)</td>
<td>4 (80.00%)</td>
<td>5</td>
</tr>
</tbody>
</table>

Group I: 57.20 per cent of cases in normal range of pH; 36.72 per cent in alkaline range; 7.08 per cent in acid range.

Group II: 25 per cent of cases in normal range of pH; 55 per cent in alkaline range; 20 per cent in acid range.

High cellular irregularity of the cancer cells coincides frequently with a pathologic pH of the blood, as is evident from this compilation.

**CELLULARITY AND pH OF THE BLOOD**

Considering the fact that proliferation is increased in tissue culture in an alkaline medium (optimum between pH 7.4 and 7.8), the tumors were grouped into four groups according to their degree of cellularity, using the method employed in the malignancy index. A tumor consisting, in its actively proliferating peripheral portions, of more than 75 per cent of parenchyma and less than 25 per cent of stroma received 4 points, while a tumor composed of more than 75 per cent of stroma and less than 25 per cent of
parenchyma received 1 point. The intermediary grades were determined accordingly. Plotting these evaluations against the pH figures of the blood, the results set forth in Table V were obtained:

There exists apparently no definite relation between the pH of the blood and the degree of cellularity of the tumor.

**Fibrosis and pH of the Blood**

The character of the stroma was evaluated according to the method used in the determination of the histologic malignancy index, but the numerical values were reversed. A very loose, edematous stroma received 1 point, a moderately loose one 2 points, a fibrous one 3 points, and a stroma composed of hyalinized connective tissue 4 points. The values thus obtained were plotted against the pH values of the blood (Table VI).

<table>
<thead>
<tr>
<th>pH above 7.39</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.36 to 7.39</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>pH below 7.36</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>10</td>
<td>6</td>
<td></td>
<td>34</td>
</tr>
</tbody>
</table>

From this study it appears that the factor of fibrosis, also, is without definite relation to the pH of the blood. There is, however, a higher tendency to cancers with a fibrous stroma in the alkaline group than in either of the other two groups.

**Number of Mitoses and Blood Sugar**

The dependence of proliferation in tissue cultures on the amount of glucose present in the culture medium is a well known fact. It appeared, therefore, to be of interest to examine the relation of the number of mitoses in the tumor to the amount of blood sugar. The number of mitoses was determined and evaluated according to the method of Hueper employed in the determination of the histologic malignancy index. The presence of 0 to 10 mitotic figures in 10 oil immersion fields was evaluated with 1 point, 11 to 15 mitoses received 2 points, 16 to 20 mitoses 3 points, and any number above 20 mitoses 4 points. The values thus
obtained were plotted against the blood sugar figures, as shown in Table VII.

**Table VII**

<table>
<thead>
<tr>
<th>Blood Sugar</th>
<th>Mitoses</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>Below 70 mgr.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>70 mgr. to 89 mgr.</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Above 90 mgr.</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>3</td>
</tr>
</tbody>
</table>

Two important observations can be made from this compilation:

1. The blood sugar level is increased in a high percentage of cancer patients. In our series of 29 cases, 22 (73.3 per cent) have a blood sugar over our normal range.

2. In 36.36 per cent of the cases with hyperglycemia, a high number of mitoses was found in the cancer tissue.

The first mentioned observation supports that made by Jackson (15), who claims that cancer patients have, also, a lowered sugar tolerance. He notes, moreover, that patients with a high blood sugar level and a lowered sugar tolerance have a poor prognosis on account of frequent recurrences and metastases, while patients with a low blood sugar and a normal sugar tolerance react well upon treatment.

The increased number of mitoses found in about one third of our hyperglycemic cases seems to support this contention. It throws, moreover, an interesting light upon the attempt of Mayer (16) to increase the radiosensitivity of cancers by intravenous glucose injections preceding the irradiation. Mayer believes that the additional glucose available in the blood stimulates the mitotic processes in the tumor and makes thereby for a larger number of radiosensitive cells. The therapeutic effect of x-ray treatments may also be partially due to the hypoglycemia produced by the rays (Held, 9).

**Summary**

1. The malignancy indices and the blood pH values of 34 cases of carcinoma were correlated and a preponderance of histologically highly malignant tumors was found in patients with an alkalosis, while the tumors of histologically low malignancy predominated in patients with a normal blood pH.
2. The alkalosis of the blood in cancer patients varies not only with the extent of the disease, but also with the degree of malignancy and maturity of the tumor.

3. The degree of cellular irregularity of cancerous tissue is increased in patients with an alkaline pH of the blood as compared with patients with a blood pH in the normal range.

4. In about one-third of the cancer patients with a hyperglycemia (which was present in 78.3 per cent of our cases) a high number of mitoses was found, while this phenomenon was absent in cancers with a normal blood sugar.

BIBLIOGRAPHY

   Arch. Path. 6: 1064, 1928.
   Am. J. Obst. & Gynec. 17: 733, 1929.