Changes in the Reducing Power of Serum or Plasma of Patients with Malignant Neoplastic Disease

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Many attempts have been made to demonstrate differential reactions in the serum of patients suffering from neoplastic diseases. In view of the relatively distinct alterations found in cellular metabolism with the advent of malignancy, particularly in regard to the oxidation-reduction reactions, it was felt that review of the properties of the blood from that point of view might prove fruitful. Further, there had been previous investigations along these lines which seemed to justify further study. Thus, Schütt in 1929 (2) performed estimations of the oxidase activity of the blood using the Nadi reaction and reported an increased oxidase activity in the blood of 90 per cent of the cancer patients tested. Savignac and associates (1) reported an interesting correlation between the presence of malignancy and the reducing power of serum as tested by reaction with methylene blue.

Alterations in redox potential and reducing substances as found in tumor tissue and in the serum of tumor hosts are reviewed by Stern and Wilhelm (3). The authors point out that “evidence derived from work undertaken from various points of view appears to point clearly to an increased presence of reducing systems in malignant tumors.” The papers reviewed by these authors failed to show any clear-cut differentiation in serum reducing power, although contradictory reports have been put forth from time to time.

It was the purpose of this investigation to evaluate alterations in the reducing power and throw some light on the significance of such variations.

METHOD

The evaluation of the reducing power was made by studying the interaction of plasma (or serum) with redox dyes. The following dyes were studied: methylene blue, brilliant cresyl blue, 2,6-dichlorobenzenoneindophenol, and cresyl violet. The potentials of these dyes as measured by the Weston electrometer were as follows: brilliant cresyl blue, 190 mv.; methylene blue, 220 mv.; cresyl violet, 295 mv.; 2,6-dichlorobenzenoneindophenol, 355 mv. It is to be noted that these are not E0 values, but are potentials as measured in the course of these experiments. These values are obtained by direct reading from the scale after the electrodes have been in contact with the solution tested for 7 minutes. The pH values of the mixtures of the dye and plasma were about 7.4 to 7.6. Of these, it was found that brilliant cresyl blue and methylene blue gave significantly different reactions with plasma or serum from patients with and without neoplastic disease.

The technic consists of adding 0.2 cc. of a 0.1 per cent solution of methylene blue to 1 cc. of serum or plasma in a Kahn tube. A like amount (0.2 cc.) of a 0.1 per cent solution of brilliant cresyl blue is added to 1 cc. of serum or plasma in a second tube. After mixing, the tubes are immersed in a boiling water bath. A beaker served as a water bath so that changes in color could be seen with ease. The methylene blue used in this study was made by Mallinckrodt and differences in reaction were found with several other samples tested.

The tubes are watched for decolorization of the dyes. Thus, the tube containing the plasma and methylene blue is watched for complete decolorization and the time noted. In the case of the tube containing the brilliant cresyl blue, a slightly different technic is employed. Here the reaction is read at 10 minutes. Complete reduction of the brilliant cresyl blue results in a white or greyish-white color, while incomplete reduction leaves a lavender shade of varying intensity. The color is noted at 10 minutes and the tube then removed from the water bath and cooled under tap water. With cooling, characteristic alterations are seen to occur at the surface and in the mass of the coagulated plasma. These changes are of two main types: 1. surface—bluish-
green, mass—white or grey; 2. surface—blue-violet, mass—lavender. While a little experience is needed to familiarize the eye with these colors, they prove to be highly specific. Thus, plasma from a patient with malignant neoplastic disease is seen to be lavender after 10 minutes in the boiling water bath and to react on cooling with a violet surface and a lavender mass. On the other hand, complete reduction to a grey-white color is found in the non-malignant groups and on cooling, the surface becomes blue-green and the mass grey-white.

The time values found for the reduction of methylene blue by the plasma of patients with malignant neoplastic disease differed from the time values of patients with non-malignant neoplastic disease. In the majority of cases, the blood of patients suffering from malignant neoplastic diseases gave reducing times of 10 minutes or more as tested by this method. The blood of normal individuals showed the greatest concentration of values below 9 minutes, while the plasma of patients with non-malignant disease tended to give values below 10 minutes.

As will be shown below, various types of malignant growths tended to be associated with somewhat characteristic alterations in the reducing power of the plasma.

Between the range of 0.50 to 0.120 per cent, the potential of the methylene blue solution varies inversely with the concentration. On further dilution the slope flattens considerably. Thus, the following readings are obtained: 0.5 per cent, 150 my.; 0.250 per cent, 187 mv.; 0.12 per cent, 220 mv.; 0.10 per cent, 225 mv.; 0.03 per cent, 220 mv. The reducing times of the 3 latter solutions measured with 1 plasma sample as a control vary greatly in spite of the proximity of the potential values; viz., 0.03 per cent, 6.5 minutes; 0.10 per cent, 9 minutes; 0.12 per cent, 15 minutes.

The calibration of a new lot of 0.1 per cent methylene blue is accomplished by comparing the time needed for complete decolorization of a control plasma sample with the time needed with the standard solution. If the time value of the new lot is prolonged, the lot should be diluted with distilled water. If the time value is low, the solution should be concentrated by evaporation in an open vessel. It follows that solutions should be carefully stoppered when not in use.

The standardization of the brilliant cresyl blue solution is done in the same manner with one exception. This exception is the end point. With brilliant cresyl blue, the end point is not a complete decolorization phenomenon but a definite color change, and as a result, this solution must be standardized with two plasma samples—one from a patient with malignant disease and another from a normal individual. The end points of the control samples are those described above.

RESULTS

Analysis of the results obtained with methylene blue may be made along several lines: 1. the spread of values with (a) normal controls, (b) non-malignant disease, and (c) malignant disease; 2. study of the alterations with the various types of malignant tumors; 3. the effect of therapy on the reducing power of the plasma.

In the studies with brilliant cresyl blue, the reactions as studied here are of the all-or-none type, plasma from patients with malignant tumors showing incomplete reduction and that from patients with non-malignant processes resulting in complete reduction. Thus, the final evaluation of the serodiagnostic value of the reducing power of the blood rests on the reaction with both dyes.

### Table I: Methylen Blue Reduction Time for Normal Blood Plasma

<table>
<thead>
<tr>
<th>Time min.</th>
<th>No. of cases</th>
<th>Per cent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.7</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>7.8</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>8.9</td>
<td>32</td>
<td>41</td>
</tr>
<tr>
<td>9.10</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>10-10.5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>10.5-11</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>76</td>
<td>100</td>
</tr>
</tbody>
</table>

*The interaction with brilliant cresyl blue gave complete reduction in all these cases; viz., surface—blue-green, mass—gray-white.

When the plasma of presumably healthy individuals is tested by this method, it is found that the great majority shows complete reduction of the methylene blue before 10 minutes (Table I). In a few cases, the time values are longer, but even in these cases complete reduction of brilliant cresyl blue has been found, so that differentiation is possible. It might be mentioned that it is not necessary to limit tests to individuals who have fasted, although in some cases a fatty plasma makes it more difficult to evaluate color changes with the brilliant cresyl blue. The reactions obtained with the plasma of patients suffering from a variety of non-neoplastic diseases were for the most part similar to those obtained in the control group (Table II).
TABLE II: NON-NEOPLASTIC DISEASE, METHYLENE BLUE REDUCTION

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>False interpret.*</th>
<th>Av. Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenopathy, infectious</td>
<td>2</td>
<td></td>
<td>6.5</td>
</tr>
<tr>
<td>Anorexia</td>
<td>1</td>
<td></td>
<td>8.5</td>
</tr>
<tr>
<td>Appendicitis</td>
<td>1</td>
<td></td>
<td>8.5</td>
</tr>
<tr>
<td>Arthritis</td>
<td>1</td>
<td></td>
<td>8.3</td>
</tr>
<tr>
<td>Arteriosclerotic heart disease</td>
<td>5</td>
<td></td>
<td>8.2</td>
</tr>
<tr>
<td>Asthma, bronchial</td>
<td>3</td>
<td></td>
<td>8.6</td>
</tr>
<tr>
<td>Bronchietasis</td>
<td>1</td>
<td></td>
<td>9.5</td>
</tr>
<tr>
<td>Beck sarcoid</td>
<td>1</td>
<td></td>
<td>8.5</td>
</tr>
<tr>
<td>Bronchial cyst</td>
<td>1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Cardiac decompensation</td>
<td>1</td>
<td></td>
<td>7.0</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>1</td>
<td></td>
<td>7.0</td>
</tr>
<tr>
<td>Cardiopasm</td>
<td>1</td>
<td></td>
<td>10.0</td>
</tr>
<tr>
<td>Cirrhosis, hepatic</td>
<td>6</td>
<td>3</td>
<td>10.9</td>
</tr>
<tr>
<td>Cholecystitis</td>
<td>1</td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>Coitus</td>
<td>2</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Coronary insufficiency</td>
<td>2</td>
<td></td>
<td>8.5</td>
</tr>
<tr>
<td>Cystitis</td>
<td>2</td>
<td></td>
<td>8.5</td>
</tr>
<tr>
<td>Dermatitis, subacute and chronic</td>
<td>6</td>
<td></td>
<td>8.3</td>
</tr>
<tr>
<td>Diabetes, HCVD</td>
<td>1</td>
<td></td>
<td>8.5</td>
</tr>
<tr>
<td>Endocarditis, subacute bacterial</td>
<td>1</td>
<td></td>
<td>8.5</td>
</tr>
<tr>
<td>Endomicrotis</td>
<td>2</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Fever, undetermined origin</td>
<td>1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Gastritis</td>
<td>3</td>
<td></td>
<td>9.6</td>
</tr>
<tr>
<td>Granuloma, fungus</td>
<td>1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Hemorrhoids</td>
<td>1</td>
<td></td>
<td>6.0</td>
</tr>
<tr>
<td>Hepatoplenomocely</td>
<td>2</td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>Hypertensive cardiovascular disease</td>
<td>9</td>
<td>1</td>
<td>9.1</td>
</tr>
<tr>
<td>Hypertension, essential</td>
<td>1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Infectious mononucleosis</td>
<td>1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Lues</td>
<td>1</td>
<td></td>
<td>7.0</td>
</tr>
<tr>
<td>Lymphedema</td>
<td>1</td>
<td></td>
<td>10.0</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Menopausal syndrome</td>
<td>2</td>
<td></td>
<td>7.7</td>
</tr>
<tr>
<td>Muscular dystrophy</td>
<td>1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Nephritis</td>
<td>1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Nevis, pigmented</td>
<td>1</td>
<td></td>
<td>8.7</td>
</tr>
<tr>
<td>Nucleus pulposus</td>
<td>2</td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>Oophorctomy</td>
<td>1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Paget's disease</td>
<td>1</td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>Parkinsonism</td>
<td>1</td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>Pernicious anemia</td>
<td>5</td>
<td>1</td>
<td>9.2</td>
</tr>
<tr>
<td>Pharyngitis, streptococcus</td>
<td>1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>1</td>
<td></td>
<td>9.4</td>
</tr>
<tr>
<td>Pregnancy, 5 months</td>
<td>3</td>
<td></td>
<td>10.0</td>
</tr>
<tr>
<td>Pregnancy, 8.9 months</td>
<td>2</td>
<td>2</td>
<td>14.0</td>
</tr>
<tr>
<td>Prostatic hypertrophy</td>
<td>2</td>
<td></td>
<td>9.5</td>
</tr>
<tr>
<td>Prostatitis</td>
<td>1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Psychosis, senile</td>
<td>1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Premilis</td>
<td>2</td>
<td></td>
<td>10.8</td>
</tr>
<tr>
<td>Rheumatic heart disease</td>
<td>2</td>
<td></td>
<td>10.0</td>
</tr>
<tr>
<td>Tuberculosi</td>
<td>7</td>
<td></td>
<td>8.8</td>
</tr>
<tr>
<td>Ulcer, peptic</td>
<td>5</td>
<td>1</td>
<td>10.0</td>
</tr>
<tr>
<td>Ulcer, trophic, leg</td>
<td>1</td>
<td></td>
<td>7.0</td>
</tr>
<tr>
<td>Vaginitis</td>
<td>1</td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>Weber-Christian syndrome</td>
<td>1</td>
<td></td>
<td>10.3</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>112</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

*The reactions with methylene blue and brilliant cresyl blue were indistinguishable from those obtained with blood from patients with neoplastic diseases. These determinations are included in the average time values.

However, it should be noted that some samples of plasma showed prolonged reducing time with the methylene blue and incomplete reduction of the brilliant cresyl blue. These divergencies occurred particularly in cases where there was hypoprothrombocytopenia, viz., cirrhosis. Thus, in the presence of a marked hypoprothrombocytopenia, less than 5 per cent, it is possible to obtain false positive results.

The presence of non-malignant neoplasia fails to induce significant variations in the plasma of patients as measured by this method (Table III). Thus, plasma of patients with benign tumors as well as such diseases as chronic leukemia, multiple myeloma, and polycythemia reacts similarly to the plasma of the controls.

TABLE III: NEOPLASIA, NON-MALIGNANT, METHYLENE BLUE REDUCTION

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>False interpret.*</th>
<th>Av. Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papilloma, bladder</td>
<td>1</td>
<td></td>
<td>9.5</td>
</tr>
<tr>
<td>Breast mass, non-malignant</td>
<td>4</td>
<td></td>
<td>8.8</td>
</tr>
<tr>
<td>Giant Cell Tumor</td>
<td>1</td>
<td></td>
<td>9.5</td>
</tr>
<tr>
<td>Lipoma</td>
<td>2</td>
<td></td>
<td>8.5</td>
</tr>
<tr>
<td>Leukoplakia</td>
<td>2</td>
<td></td>
<td>6.5</td>
</tr>
<tr>
<td>Leukemia, chronic, myelop.</td>
<td>4</td>
<td>1</td>
<td>9.8</td>
</tr>
<tr>
<td>Leukemia, chronic, lymphop.</td>
<td>3</td>
<td></td>
<td>9.3</td>
</tr>
<tr>
<td>Leukemia, chronic, lymphop.</td>
<td>1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>5</td>
<td></td>
<td>9.2</td>
</tr>
<tr>
<td>Polycythemia</td>
<td>2</td>
<td></td>
<td>10.0</td>
</tr>
<tr>
<td>Xanthoma</td>
<td>1</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>26</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

*These cases give "non-malignant type" type of reaction with brilliant cresyl blue.

The plasma of patients suffering from malignant neoplasia showed characteristic increase in methylene blue reduction time as well as incomplete reduction of brilliant cresyl blue (Table IV). In addition, there was a much greater spread of values. There tended to be somewhat characteristic time values for the methylene blue reduction with plasma of patients with the various types of malignant conditions. Thus, plasma from patients with ovarian and cervical carcinomas gave long methylene blue reduction times.

TABLE IV: ACTIVE MALIGNANT NEOPLASIA, METHYLENE BLUE REDUCTION

<table>
<thead>
<tr>
<th>Site of origin</th>
<th>No. of cases</th>
<th>False*</th>
<th>Av. Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder, adenocarcinoma</td>
<td>2</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>Breast, duct cell carcinoma</td>
<td>22</td>
<td>4</td>
<td>12.4</td>
</tr>
<tr>
<td>Carcinomatosis</td>
<td>1</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>Cecum, adenocarcinoma</td>
<td>1</td>
<td>12.5</td>
<td></td>
</tr>
</tbody>
</table>

*The reactions with methylene blue and brilliant cresyl blue were indistinguishable from those obtained with blood from patients with neoplastic diseases. These determinations are included in the average time values.
The therapeutic procedure may alter the reactions under discussion. Vidcs an objective index of the efficacy of a therapeutic procedure. Thus, examination of Table V reveals these reactions is of particular importance for it provides an objective index of the efficacy of a therapeutic procedure. The ability of therapeutic measures to alter previous or coincident therapy, either surgery or radiation, may have caused reversal of reactions (Table V). The ability of therapeutic measures to alter these reactions is of particular importance for it provides an objective index of the efficacy of a therapeutic procedure. Thus, examination of Table V reveals how therapeutic procedure may alter the reactions under discussion.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>No. false</th>
<th>Accuracy ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>76</td>
<td>0</td>
<td>76/76</td>
</tr>
<tr>
<td>Non-neoplastic diseases</td>
<td>112</td>
<td>9</td>
<td>103/112/203/214</td>
</tr>
<tr>
<td>Non-malignant neoplasia</td>
<td>26</td>
<td>2</td>
<td>24/26</td>
</tr>
<tr>
<td>Active malignancy</td>
<td>237</td>
<td>48</td>
<td>189/237</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>451</strong></td>
<td><strong>59</strong></td>
<td><strong>392/451</strong></td>
</tr>
</tbody>
</table>
DISCUSSION

The results obtained in this investigation add additional evidence to the concept that malignant neoplasms induce changes in the host at distant sites. In this case, it would appear that the reaction of reduction of methylene blue and brilliant cresyl blue depends on an alteration in the reducing groups of a protein fraction, probably the albumin fraction.

In the presence of a marked hypoproteinemia (plasma protein of 5 per cent or less) the test is invalid for it will tend to give false positive reactions. Thus, false positive reactions were obtained in some cirrhotic patients and, in a case of chronic leukemia, with marked recurrent ascites. These conclusions are in accord with those of Savignac and associates (1).

SUMMARY

1. Determination of the reducing power of plasma (or serum) was made by the use of the redox dyes, brilliant cresyl blue, and methylene blue.

2. Plasma of patients with malignant diseases tended to have a lowered reducing power and could be differentiated with a high degree of accuracy.

3. Adequate therapy reversed the characteristic alterations in reducing power of the plasma of patients with malignant neoplastic diseases.

4. The various malignant neoplasias gave varied degrees of alteration of the reducing power.

ACKNOWLEDGMENT

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REFERENCES


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