Sulfhydryl Reduction of Methylene Blue
With Reference to Alterations in Malignant Neoplastic Disease

Maurice M. Black, M. D.
(From the Department of Biochemistry, New York Medical College, New York 29, N. Y., and the Brooklyn Cancer Institute, Brooklyn 9, N. Y.)
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A significant decrease in methylene blue reducing power of plasma from patients with malignant neoplastic disease was previously reported (1). At that time it was suggested that change in a reducing group of the albumin molecule was a likely source of this alteration. Similar conclusions were reported also by Savignac and associates (7) as the result of analogous studies.

In an attempt to evaluate the effect of the sulfhydryl group on the reduction of methylene blue, a study was undertaken with various compounds of known -SH and S-S structures. In addition, an attempt was made to establish a standard method of calibration of various lots of methylene blue, so that more uniform results would be possible in the plasma reducing test.

Glutathione, cysteine hydrochloride and methionine were made up in equimolar solutions (0.0325 M) in distilled water. One cc. of glutathione was added to 0.2 cc. of 0.13 per cent methylene blue in a Wasserman tube. Similarly, 0.2 cc. of methylene blue (0.13 per cent) was added to cysteine and to methionine. The tubes were immersed in a boiling water bath and observed for time of complete decolorization. The tube containing methionine and methylene blue failed to show any change in color in spite of continued boiling for an hour and a half. On the other hand, complete decolorization was noted in the tubes containing cysteine HCl and glutathione in 6.0 and 15 minutes, respectively.

An attempt was then made to evaluate changes in the reducing time with varying concentrations of cysteine and glutathione. Thus 1 cc. of varying concentrations of cysteine HCl was mixed with 0.2 cc. of methylene blue (0.13 per cent) and the time noted for complete decolorization. Equimolar solutions of glutathione were treated in a similar fashion. The values obtained are indicated in Fig. 1. The results indicate a linear relationship between the cysteine concentration and the reducing power, and a definite limiting value of cysteine concentration for reduction of the methylene blue. The reactions with glutathione are similar, but the reactivity is less than half that of the cysteine. It is noteworthy also that the resultant leuco mixture did not revert back to colored methylene blue on cooling, as was the case with methylene blue reduction by plasma.

Similar relationships were investigated between cysteine and different concentrations of methylene blue. As seen in Fig. 2, similar curves are obtained, but the position of the curve on the graph varies with the concentration of the methylene blue used. It should be noted that there is no appreciable difference in the reducing time of methylene blue on varying the concentrations between 0.10 per cent and 0.2 per cent, although 0.08 per cent shows a decided difference.

Analogous findings were obtained on mixing similar concentrations of methylene blue with a plasma sample. The following reducing times were obtained when a plasma sample was used to reduce the methylene blue solutions:

<table>
<thead>
<tr>
<th>Methylene blue, per cent</th>
<th>Reducing time, minutes</th>
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<tbody>
<tr>
<td>0.08</td>
<td>5.5</td>
</tr>
<tr>
<td>0.13</td>
<td>8</td>
</tr>
<tr>
<td>0.20</td>
<td>8</td>
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The curves depicted in Fig. 2 possess an additional feature which merits attention in relation to the results obtained with plasma, namely, the sharp break in the curves occurring after the methylene blue reducing time of 15 minutes. A similar break is found at 15 minutes when the occurrence of various reducing times of numerous plasma samples from cancer patients is plotted. These figures are based on 109 plasma samples whose reducing times fell between 13 and 25 minutes. As with the cysteine, apparently, a point is reached beyond which decrease in reducing activity is attended by marked change in the reducing time; in short, a steeper rate of change.

In view of the parallel results obtained with cysteine HCl and plasma, the cysteine solutions may be used to test the reactivity of different lots of methylene blue. Concentrations between 0.1
per cent and 0.2 per cent would seem to be most efficacious, since variations in this zone produce minimal changes in reducing time. Thus, a concentration of 0.13 per cent methylene blue has been adopted in evaluating the reducing power of plasma.

The apparent decrease in sulphydryl activity in plasma of patients with malignant neoplastic disease suggested therapeutic trial of sulphydryl compounds on such patients. Glutathione in doses of 50 to 100 mgm. was injected intravenously in several patients with varying types of malignant neoplastic diseases. A reaction of chills and fever occurred in most cases in about 30 minutes and lasted from 20 minutes to an hour. This was usually replaced by a sense of well being and relief from previous symptomatic complaints, viz. pain, asthenia etc. Beneficial effects usually lasted several days and could be regained by repeated injections. Similar results were obtained by injection of 25 to 50 mgm. of cysteine HCl although the initial reaction did not occur. It should be mentioned that although the symptomatic improvement might be great, there was no apparent effect on the growth of the neoplasm.

**DISCUSSION**

The reduction of methylene blue by cysteine and glutathione and not by methionine is an indication that, in these compounds at least, a free -SH bond is required. The more prolonged time found for glutathione (15 minutes) as compared with cysteine HCl (6 minutes) would seem to indicate that the availability or reactivity of the -SH bond may be altered by its location in the molecule. Thus in cysteine the -SH is terminal and presumably unhindered in its reactivity. In
the case of the glutathione, the internal location of the -SH bond seems to decrease its reactivity.

The reversible decrease in reducing power of plasma associated with malignant neoplastic disease might be explained on the basis of changes in the spatial configuration of the albumin molecule. Such changes would be readily reversible and would not necessitate changes in amount of total protein of -SH bonds. This is of importance since the observed decrease in reducing power is not correlated with changes in concentration of plasma proteins.

The observation that glutathione and cysteine were often efficacious in relieving the symptomatic complaints of patients with malignant neoplastic disease finds some parallel in the literature. Thus, Stern and Wilheim (8), in reviewing the relation of sulfhydryl compounds to life processes, point out that the growth inhibition of normal animals treated with various organic carcinogens could be cancelled by increased quantities of sulfhydryl compounds in the diet. This is interpreted as being due to utilization of sulfhydryl compounds in detoxification of the carcinogens and also the competitive utilization of -SH groups by neoplastic tissue itself, leaving relative deficiencies of these indispensable requisites for growth, enzyme activity and metabolism. Therapeutic use of cysteine and glutathione also has been suggested by the observation of tumor growth inhibition in some neoplasms (2, 3, 5), although no such effect was obtained in others (4).

The data presented in this and our previous study would seem to point to the importance of the sulfhydryl group as a factor in the over-all redox substrate of the body rather than as a specific tumor-inhibiting agent. One might consider this group analogous to a buffer in acid-base systems. Loss of such control would lead to suboptimal sulfhydryl potentials with attendant alterations in function of various enzyme systems. The report by Hirshfeld, Duboff, and West (6) of an inhibitory action of serum of cancer patients on the tyrosine-tyrosinase system might be explained on the basis of such alteration in optimal -SH potential.

**SUMMARY**

1. Methylene blue is reduced by boiling with cysteine HCl and with glutathione but not with methionine.

2. The reaction with cysteine HCl is more rapid (6.0 minutes) than the reaction with equimolar glutathione (15 minutes).

3. The reaction between cysteine and methylene blue shows a linear relationship, which may be used for calibration of different lots of methylene blue.

4. A possible relationship between the spatial configuration of the albumin molecule and the decrease in reducing power of plasma in the presence of malignant neoplastic disease is discussed.

5. Effects of the administration of cysteine and glutathione on patients with malignant neoplastic diseases are reported.

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**REFERENCES**


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Maurice M. Black


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