It appears that in the PGA-deficient rat the leucopenia can be effectively treated with ascorbic acid. It is also possible that ascorbic acid functions in the formation of normoblasts but PGA is required for red cell maturation. The application of these findings to the treatment of pernicious anemia and sprue has yet to be investigated.

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The Reducing Properties of Serum From Subjects With Malignant Disease

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In 1944 Savignac (*2*) published experiments on the reducing properties of serum. He heated serum at pH 11 for 10 min and, at room temperature, measured under partially anaerobic conditions the time required to reduce a given amount of methylene blue. Savignac concluded that sera from patients with malignant diseases showed a significantly longer reduction time than sera from subjects without malignancy. Recently Black (*1*) reported similar experiments, using a modification of the Savignac method. He concluded that "plasma of patients with malignant diseases tended to have a lowered reducing power and could be differentiated with a high degree of accuracy."

A considerable amount of work on the biochemistry of this phenomenon has been done in this laboratory, and the following conclusions can be made:

(1) Methylene blue is reduced slowly by unheated serum. Heated alkaline serum rapidly reduces methylene blue. It can be easily shown that heating converts a large part of the bound sulfur of protein into sulfide ions (the so-called "labile sulfur"); sulfide ion is insignificant in unheated serum. The reduction of methylene blue is due *solely* to the presence of newly formed S^{--}. When the sulfide ion is removed by appropriate methods, reduction of methylene blue is insignificant (see Table 1). It is therefore more correct to state that the phenomenon under discussion is the anaerobic oxidation of sulfide ion by a suitable hydrogen acceptor such as methylene blue.

(2) Sulfide ion in aqueous solution is oxidized very slowly by methylene blue. The addition of heated or unheated serum, however, greatly accelerates the rate of oxidation. In other words, by some mechanism not yet established, serum catalyzes the oxidation of S^{--}. This peculiar catalytic activity is not specific for any one protein constituent of serum, but is observed with all purified protein components of serum as well as with other proteins, viz.: serum albumin, alpha globulin, beta globulin, fibrinogen, insulin, edestin, urease. It is not observed with starch. The catalysis can be demonstrated by aerobic as well as anaerobic methods.

(3) When known and varying amounts of sulfide ion are added to unheated or heated serum, the reduction time of methylene blue can be expressed with considerable accuracy by the equation $t = k/\sqrt{S^{--}}$. In other words, the reduction time is an indirect but crude method for the estimation of S^{--}, *i.e.* the "labile sulfur" of the serum. This is further established by the fact that the sulfide ion in heated serum (determined by iodometric titration) is significantly correlated with the reduction time.

(4) The catalytic activity of serum in bringing about the anaerobic oxidation of known amounts of added sulfide ion by methylene blue is greatest with unheated serum. Heated serum with the same amount of sulfide ion requires 3–10 times longer, depending upon the concentration of sulfide.

TABLE 1

Serum RS-12 (2/16/45): Add 0.20 ml of 1.25 N NaOH/ml of serum. Heat (100° C) for 10 min. Place three portions of 1 ml each into Thunberg tubes with 1 ml of 0.1% methylene blue in side sacs. Treat the samples as follows:

(A) *Control*: Add 0.1 ml of saline.

(B) *Secondary control:* Add 0.10 ml of 5 N HCl,* let stand without evacuation for same time as C, then add 0.12 ml of NaOH exactly equivalent to added acid to restore original pH.

(C) *Experiment*: Add 0.10 ml of 5 N HCl,* evacuate 3 min, and add 0.12 ml of NaOH exactly equivalent to added acid.

After restoration of alkaline pH, evacuate all tubes, admix methylene blue, and determine time of reduction by the conventional Thunberg Method. The results are given below.

Tube No.	Reduction time (sec)	Final sulfide ion (iodometric titration) (micromole/ml of serum)
A	90	29
B	70	29
C	> 1,800	7
Original unheated alkaline serum		0

* Since pK of H_2S is 6.5, it is necessary to bring to pH ca. 5.5 to remove sulfide by evacuation. The amount of acid needed to bring the alkaline serum to pH 5.5 was determined independently. S^{--} cannot be removed from alkaline solutions by evacuation.

(5) In 315 experiments on sera from miscellaneous patients there was no significant relation between the reduction time of methylene blue, as measured in a variety of ways, with the presence or absence of malignancy. Quantitative measurements of "labile sulfur" in these sera also failed to show any such relationship. These data lead to conclusions contrary to those of Savignac and Black cited above.

References

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