CORRESPONDENCE

Sulphydryl Compounds

To the Editor:

In Blood 32:49, 1968 Kann et al. report that normal red cells treated with a sulphydryl compound, reduced glutathione (GSH), under suitable experimental conditions, become similar in some respects to the red cells of paroxysmal nocturnal hemoglobinuria (PNH).

The reported results are similar to those we obtained in 1965 with two other sulphydryl compounds, i.e., cysteine and 2-aminoethylisothiouronium bromide (AET), under the same experimental conditions. However, the authors claim that "normal RBCs incubated with solutions of GSH are, apparently, the most nearly perfect 'induced' PNH RBCs thus far reported." GSH-treated cells would resemble PNH cells more closely than AET- or cysteine-treated cells, essentially for the following two reasons:
1) GSH-treated normal red cells are said to show, in the acidified-serum test, the same optimum pH of PNH cells, simultaneously tested, i.e., pH 6.0-6.2. AET- and cysteine-treated cells had an optimum pH lower than that of the PNH cells we tested (6.1-6.2 instead of 6.7-7.0).2
2) GSH-treated normal red cells are said to give a positive cold-antibody hemolysis test, while AET- and cysteine-treated cells did not in our original experiment.

We do not agree with Kann et al. on the above arguments.

In fact, it appears from Table No. 3 of the authors' paper that GSH-treated cells show an optimum pH for acid lysis comprised between 6.0 and 6.2, while according to the literature this optimum pH, with rare exceptions, is in the region of 7 for PNH cells.3,5

It is this unusual finding that probably led the authors to conclude that GSH-treated cells are the artificially altered normal cells that more closely resemble PNH red cells. Actually, as far as the optimum pH for acid lysis is concerned, there appears to be no difference between GSH-treated and AET- or cysteine-treated red cells.

Insofar as the second argument is concerned, we wrote in our first paper that "the results (of the test of sensitivity to iso-antibodies) appeared not constant, in spite of the fact that altered red cells showed a certain aptitude to be hemolyzed by anti-A and anti-B agglutinins." This conclusion, drawn from the use of inappropriate commercial anti-A and anti-B antisera, was modified in a subsequent paper,5 where it was shown, by using a suitable cold antibody (anti-i), that AET-treated cells are approximately 20 times as sensitive to the lytic action of antibody and complement as normal cells, closely resembling PNH cells in this respect. Therefore, GSH-cells also appear to be not dissimilar from AET-cells in regards to the high sensitivity to immune lysis.

Anyway, we think that what is important is the finding that some (and probably many) sulphydryl compounds are capable of modifying a normal red cell membrane in such a way as to cause a lesion that may be similar to that occurring spontaneously in PNH.

G. Sirchia, S. Ferrone and F. Mercuriali

References


2. Sirchia, G., Ferrone, S., and Mercuriali,
As discussed in reference four above, the value one obtains for the optimum pH for erythrocyte lysis in acidified serum will vary, depending upon whether the pH is measured before the addition of RBCs, after the addition of RBCs but before the one hour incubation at 37 degrees, or after the one hour incubation. The serum pH will increase by 0.2-0.7 units after the addition of RBCs (again, reference 4 above), due to the inherent buffering capacity of the RBCs. The serum pH may also increase during incubation at 37 degrees for one hour, due to loss of carbon dioxide from the serum. In light of these facts, the pH optimum of 6.0-6.2, reported in our study in which the serum pH was measured prior to the addition of RBCs, was not considered to be unusually low, even though values in the region of 6.8-7.0 have been found in studies in which the pH measurement was performed after addition of RBCs to serum and after 60 minutes' incubation (for example, reference 3 above).

The optimum pH for lysis in acidified serum has now been tested simultaneously in GSH-incubated normal RBCs and RBCs from additional patients with PNH, and the consistent finding has been that the optimum pH for lysis is the same for both kinds of RBCs. We therefore feel that the suggestion offered by Sirchia et al., that our finding of an identical pH optimum for GSH-incubated normal RBCs and PNH RBCs was based on our having compared GSH-incubated normal RBCs to PNH RBCs that exhibited a fortuitously low optimum pH for acid-serum lysis, is most likely incorrect.

We suspect, however, that the alterations produced by GSH in normal RBCs are more or less similar to the alterations produced by cysteine or AET. We know of no reason why the optimum hydrogen ion concentration for acid-serum lysis should be the same when GSH-incubated normal RBCs and PNH RBCs are compared, but different, by an order of magnitude, when cysteine- or AET-incubated normal RBCs and PNH RBCs are compared. We wonder whether the incubation of normal RBCs in concentrated, alkaline solutions of sulfhydryl compounds decreases their buffering capacity to an appreciable degree. If this were the case, the serum pH would increase less than usual when these RBCs were studied in the acid-serum lysis test. Determination of serum pH at the end of the test might then provide information which would be misleading if it were the same, the serum pH would increase less than usual when these RBCs were studied in the acid-serum lysis test. Determination of serum pH at the end of the test might then provide information which would be misleading if one had assumed equal buffering capacities for the altered normal RBCs and PNH RBCs.

If there were differences in the buffering capacities of "artificial" and "true" PNH RBCs, it is also possible that measurement of serum pH prior to the addition of RBCs to serum might not have given an accurate indication of the pH of the RBC-serum mixture during the early phase of the one hour incubation at 37 degrees. Experiments in which serum pH is measured immediately before and after the addition of RBCs, and again at the end of the one hour incubation, using both "artificial" and "true" PNH RBCs may help to clarify these issues.

The second point raised, regarding the results of cold antibody tests in AET-treated normal RBCs, seems to require no comment other than that we had, until now, been unaware of these results.

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To the Editor:

We wish to correct a conclusion attributed to us by Doctors Baker, Pereira and Begum in their recent paper, "Failure of Vitamin E Therapy in the Treatment of Anemia of Protein-Calorie Malnutrition."

They state, "Oski and Barness found that the administration of vitamin E to premature infants with low serum tocopherol levels between six and eleven weeks of age caused a significant rise in hemoglobin. However, in a subsequent study they were unable to duplicate these results." The subsequent study these authors refer to was not designed to duplicate the original findings but was, as is clearly stated in the manuscript, an attempt to determine the minimum requirement for vitamin E in these small infants. This was unsuccessful because the absorption of E proved erratic in the presence of the diet employed—one very low in unsaturated fat and total fat.

At no time did we state or imply that we are unable to duplicate our first results. In fact, we have not yet tried to duplicate them systematically with the first formula though we are planning this shortly. Others have found a similar state in infants fed the same formula, and we have continued to note this sporadically in infants admitted to the hospital.

We believe that vitamin E has a significant though yet unexplained role in human hemopoiesis.

REFERENCES


To the Editor:

In reply to the letter from Drs. Frank A. Oski and Lewis A. Barness, we would like to point out the following:

In the first study reported by Drs. Oski and Barness the patients were given a total oral dose of 200–800 mg. of d-alpha tocopherol over 1–4 days. In the second study the children were given intramuscular injections of 1400 mg. d-alpha tocopherol over 7 days. The response in the first study (mean rise in hemoglobin 2.2 Gm. per cent and fall in retics 5.3 per cent) was much greater than in the second (mean rise in hemoglobin 0.4 Gm. per cent and fall in retics 1.3 per cent). As such, we believe we were correct in saying that the second study did not duplicate the response found in the first study. Dr. Barness and his colleagues have said, in their discussion on the second study, "In this series of patients the hemoglobin did not rise as much, nor the reticulocytes as much after treatment with Vitamin E, as in our previous study." Whether in fact this was due to the amount of blood drawn in the second study, as they suggest, remains to be proven but does not alter the correctness of our statement.

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REFERENCES

