"CATALASE STUDIES IN THE HUMAN ERYTHROCYTE GHOST"

PETER BRIGHT-ASARE[1]

Ghana, Africa

Project Supervisor: Dr. J. G. Kaplan, A.B. (C.C.N.Y.), A.M., Ph.D. (Col.)[2]

The purpose of these studies was to determine the distribution of catalase in the human red blood cell stroma and membrane.

INTRODUCTION:

This project was performed with human erythrocyte ghosts prepared by the method of gradual osmotic lysis as developed by Robert Weed and associates. The catalase activity and hemoglobin concentration present in the ghosts were determined and compared with catalase activity and hemoglobin concentrations present in the intact erythrocyte.

METHOD.

Ghosts were prepared from washed human erythrocytes, (suspended to a hematocrit of 25%) by washing in a Servall refrigerated centrifuge, first with 30 m. osm phosphate buffer, then with 60 m. osm. tris buffer, and finally, twice with 30 m. osm. tris buffer. Ghosts wee then counted in a Coulter counter. Ghost hemoglobin was determined by the benzidine method; hemoglobin of intact red blood cells was determined from a standard curve by the cyan methemoglobin method. Catalase activity was determined in terms of μM of 0.04MH_2O_2 decomposed per min., per ml. of catalase solution (i.e. ghosts or intact erythrocyte suspension.)

RESULTS.

Average red cell catalase activity.

= 3.93 X10^{-7} μM H_2O_2 decomposed /min /ml /cell $\pm$ 0.59 S.D.

Average Ghost Catalase Activity	0.382X$10^{-7}$$\mu$M H_2O_2 decom/min/ml /Ghost
% Hemoglobin	0.089%
Average Ghost Recovery	98%

[1]IVth Year Medicine. 1964 Summer Research Project. Cell Physiology Lab.

[2]Department of Physiology, Dalhousie University.

$\therefore$ Average of catalase activity present in Ghost

= 9.8% of catalase activity in intact erythrocytes

CONCLUSION.

These results suggest that about 10% of the catalase activity in the intact erythrocyte is associated with the membrane while the remainder is intracellular and is thus, removed along with 99.91% of hemoglobin during the process of gradual osmotic lysis.

Further work is being done on these studies.

References

1. Danon et al: "Preparation of erythrocyte ghosts by gradual hemolysis in hypotonic aqueous solution". *Bull. Res. Coun. Israel E.*, 1956, 6, 36.
2. Hillier et al: "Ultrastructure of the plasma membrane as determined by the electron microscope." *Journal of comparative physiology*, 1953, 42, 203.
3. Hoffman, J. F.: "Physiological Characrerization of human red blood cell ghosts." *J. Gen. Physiol.* 1958, 42, 9.
4. Jorpes: "The protein component of the erythrocyte membrane or stroma". *Biochem. Journal* 1932, 26, 1488 C.
5. Ponder, Eric et al: "The behavior as regards shape, volume of human red cell ghosts in fresh and in stored blood". *Blood* 1957, 12, 1016.
6. Ponder: "The cell membrane and its properties". *The Cell*, Vol. 11, Ed. by Brachet and Mirsky.
7. Weed, R. I., and Berg: "Is hemoglobin an essential structural component of human erythrocyte membranes§" *Journal of Clinical Investigation*, Vol. 42, No. 4, 1963.
8. Bernstein, S. R. et al: "A method for the preparation of posthemolytic residue or stroma of erythrocytes." *J. Biol. Chem.* 1938, 122, 507.