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THE RELATIONSHIP OF BIOCHEMICAL AND ENZYMIC ABNORMALITIES OF CELLS TO RADIATION SUSCEPTIBILITY AND TO THEIR INJURY BY OTHER AGENTS.

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ABSTRACT

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2. Title of Report: The Relationship of Biochemical and Enzymic Abnormalities of Cells to Radiation Susceptibility and to their Injury by Other Agents.
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The present data were obtained during our continuing investigations of primaquine sensitivity in relation to injury by various processes and their relation to disease. Combined in vitro and in vivo studies indicate that the mechanism of hemolysis involves inability of the cell to withstand oxidative stress in the presence of redox intermediates, such as methylene blue or ascorbic acid. By studies of glucose utilization and ribose formation, the site of action is shown to occur in the oxidative limb of the pentose phosphate pathway with its resultant complete shutdown. Studies are continuing to determine if this is by inactivation of the already deficient G-6-PD or by trapping of an insufficient amount of intermediate product. The injurious processes are analagous to those capable of being produced by X-radiation. Variable results obtained on radiation have helped focus attention on the erythrocyte membrane. Incubation of erythrocyte stromata in hemolyzate has revealed that significant changes in the activities of three of the chief enzymes of the pentose phosphate pathway can be induced simultaneously, both in primaquine-sensitive and normal erythrocytes. The results, with respect to normal erythrocytes, is to obtain a hemolyzate which is like that obtained from primaquine-sensitive erythrocytes. The three changes include inactivation of G-6-PD, activation of glutathione reductase, and, in the presence of TPN, partial inactivation of 6-phosphogluconic dehydrogenase.

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The basis for the studies being conducted on this project has been given in previous reports. Briefly, the relationships of cell metabolism to injurious processes and susceptibility to disease are being investigated. In particular, utilization of human erythrocytes deficient in glucose-6-phosphate dehydrogenase obtained from primaquine-sensitive individuals are being studied in comparison with erythrocytes from non-sensitive individuals, both healthy and with other diseases. Much of this work has been reviewed in a paper entitled, "Glucose-6-phosphate Dehydrogenase Deficiency in Hemolytic Anemia" by Dr. Carson; this perspective was published in the Federation Proceedings, Volume 19, p. 995, 1960.

Combined in vitro and in vivo studies indicate that the mechanism of hemolysis involves inability of the cell to withstand oxidative stress in the presence of redox intermediates such as methylene blue or ascorbic acid. By studies of glucose utilization and ribose formation the site of action is shown to occur in the oxidative limb of the pentose phosphate pathway with its resultant complete shutdown. Whether this occurs by complete inactivation of the already deficient G-6-PD or by trapping of an insufficient amount of intermediate product is being studied.

These injurious processes are analagous to those capable of being produced by X-radiation. Initial investigation of the effect of radiation on primaquine-sensitive cells, however, gave variable results and helped focus attention on the erythrocyte membrane. Incubation of erythrocyte stromata in hemolyzates has revealed that significant changes in the activities of three of the chief enzymes of the pentose phosphate pathway can be induced simultaneously: (1) G-6-PD is inactivated. The mechanism for this has been shown to be due to destruction of triphosphopyridine nucleotide, (TPN) necessary for the stability of

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G-6-PD; (2) Glutathione reductase is activated. The mechanism is as yet unknown. These first two effects make it possible to obtain, in hemolyzates from non-sensitive erythrocytes, an hemolyzate which mimics that obtained from primaquine-sensitive erythrocytes; (3) 6-phosphogluconic dehydrogenase is relatively unaffected except in the presence of TPN, when it also is inactivated by 50%. To exhibit these effects, the manner of preparation of the erythrocyte stromata and treatment of the erythrocyte is critical. They are related to the lytic process as shown by studies with ultraviolet radiation; typical changes in the activities of these enzymes are shown in the accompanying chart.

GSSG REDUCTASE, G-6-PD, and 6-P-GD in HEMOLYZATES

HEMOLYZATE - NORMAL	GSSG Red	G-6-PD	6-P-GD
A. Stromata Free Not Incubated	2.03	1.62	1.31
B. Stromata Free Incubated 45° C., 1 hr.	2.04	0.84	1.24
C. With Stromata Incubated 45° C., 1 hr.	2.59	0.29	1.16
D. With Stromata + TPN Incubated 45° C., 1 hr.	2.39	1.36	0.86
E. Stromata Free + TPN Incubated 45° C., 1 hr.	1.97	1.44	1.25

UNITS = $M \times 10^{-8}$ TPNH or TPN/ml/min. (final Hgb. = 0.21 Gms / 100 ml.)

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