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ABSTRACT

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1. Preparing Institution: The University of Chicago
2. Title of Report: The Relationship of Biochemical and Enzymic Abnormalities of Cells to Radiation Susceptibility and to their Injury by Other Agents (Including Observations on the Susceptibility of Primaquine-sensitive Cells to Radiation).
3. Principal Investigator: Paul E. Carson, M. D.
4. Number of Pages: [illegible]
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Continuing studies on the relationship of biochemical enzymic abnormalities of cells to radiation susceptibility and to injury by other agents have revealed integrated patterns of changes at several levels. Previous work has been primarily on the comparison of patients with glucose-6-phosphate dehydrogenase deficiency to normal individuals. During the period of this report we have discovered a patient with glutathione reductase deficiency and normal glucose-6-phosphate dehydrogenase who is also susceptible to primaquine-induced hemolysis. Whole body studies with injected glucose labeled with C^{14} in various positions have revealed identical deficiencies in the ability of the individual with the glutathione reductase deficiency and individuals with glucose-6-phosphate dehydrogenase deficiency to oxidized labeled glucose. This finding further confirms the relationship of glutathione reductase to carbohydrate metabolism, in general, and to the pentose phosphate pathway, in particular.

Studies on hemolysates have continued in terms of the interactions of the cell membranes with cytoplasmic constituents, namely, the ability of the membranes to alter the activities of the cytoplasmic enzymes. In effect, the cell membrane carries the capability of changing the metabolism of its cell and, in fact, leading to its own suicide. These factors correlate well with the whole body studies and lead to basic information regarding problems of cell aging, as well as susceptibility to injury before normal cell death.

Glutathione has been shown to be unstable in many laboratories, but the mechanism has not [illegible] clear. We have been able to show that this mechanism depends [illegible] on the intact pentose phosphate pathway and that changes in hemoglobin [illegible] are only incidental to the glutathione instability. This finding [illegible] and leads to a more direct approach to the study of injury [illegible].

Finally, [illegible] has been devised enabling cell metabolism to be studied in [illegible] including those producing hemolysis, in vivo, an arrangement [illegible] with either ultra violet or x-radiation during these incubations has been built in to this apparatus.

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ANNUAL PROGRESS REPORT

PERIOD COVERED: 1 JULY 1961 TO 30 JUNE 1962

PRINCIPLE INVESTIGATOR: PAUL E. CARSON, M. D.

INSTITUTION: THE UNIVERSITY OF CHICAGO DEPARTMENT OF MEDICINE

SUBJECT: THE RELATIONSHIP OF BIOCHEMICAL AND ENZYMIC ABNORMALITIES OF CELLS TO RADIATION SUSCEPTIBILITY AND TO THEIR INJURY BY OTHER AGENTS (INCLUDING OBSERVATIONS ON THE SUSCEPTIBILITY OF PRIMAQUINE-SENSITIVE CELLS TO RADIATION. AUTHOR: PAUL E. CARSON, M. D.

CONTRACT NO. DA-49-007-MD-968

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This report covers the period from July 1, 1961 to June 30, 1962. In addition, an interim report will be presented in December.

During this period, work concerning the relationship of red cell metabolism and genetically determined enzyme deficiencies to injury produced either in vivo or in vitro by drugs, by radiation, or radiation-like effects, or by disease, has enabled us to see certain patterns which, although obtained on different levels, permit an integrated picture.

1. Whole body.

In patients with fully expressed glucose-6-phosphate dehydrogenase deficiency, whole body studies of the conversion of injected glucose labeled with C^{14} in either the 1 or 6-position have revealed significant inability on the part of these subjects to produce $C^{14}O_2$ at a rate approaching that of people who do not have the deficiency. This significant finding was expected for the glucose labeled in the 1-position, but quite unexpected for the glucose labeled in the 6-position. Glucose labeled in the 1-position is presumably oxidized by way of the pentose phosphate pathway with an intact glucose-6-phosphate dehydrogenase required so that in the absence, glucose-1-oxidation would be greatly diminished. Glucose labeled in the 6-position, however, presumably is oxidized by way of the glycolytic pathway, in which (in these subjects with G-6-PD deficiency) all of the enzymes are intact. The full significance of this finding is not yet understood. One interpretation is, of course, that all of carbohydrate metabolism is involved because of the

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single enzyme deficiency. In addition, the same studies performed in our case of glutathione reductase deficiency, first discovered in our laboratory and with no other cases as yet reported, has shown a similar failure to oxidize the labeled glucose compounds. Since this subject is also susceptible to primaquine-induced hemolysis, we can suggest that this failure to utilize glucose labeled in the two terminal positions is also related to susceptibility to hemolysis, and presumably to injury by other agents or in other ways. Additional studies are being carried on in this area.

II. Hemolysate studies.

We have continued our studies on the interactions of the red cell membrane structure, which we usually call ghosts or stromata, with the cytoplasmic enzymes which regulate metabolism of the cell. We have found it particularly useful to study some of these activities simultaneously, especially glutathione reductase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconic dehydrogenase. Not only have we confirmed the inactivation of G-6-PD by the TPN-ase activity of the red cell ghosts with concomitant activation of glutathione reductase but also we have now discovered another ghost effect requiring TPN specifically as a cofactor which inactivates 6-phosphogluconic dehydrogenase. These findings have been found in normal red cells and their effect is to produce a primaquine-sensitive like hemolysate. The significance of these findings is not yet clear beyond the evident fact that the cell membrane is capable of altering its own metabolic enzymes and in a sense contributing to its own suicide. The cofactors,

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especially the metals and possible protective agents concerned with these phenomena, are being studied

III Intact cell studies.

The findings described in the previous section have been obtained using hemolysates but not intact cells. We have devised a new instrument in order to study these effects in intact cell incubations. Provision has been made for radiation of intact cell incubations by either ultra-violet light or a cobalt 60 core. The cell incubations are performed in compartmentalized flasks which have been designed to provide constant motion to allow for isotopic exchange studies and which are connected to manifolds, allowing various gases to provide different atmospheres for the cells. With this apparatus many of the in vivo conditions under which hemolysis occurs can be mimicked and when necessary altered in a controlled manner.

IV. Chemotherapy.

Several studies have appeared in the literature during the past few years ascribing rather exotic functions to the glutathione of the red cell and to its stability. We have been able to show that the maintenance of the glutathione activity in normal red cells is really quite simple and requires merely an intact glucose-6-phosphate dehydrogenase to provide reduced coenzyme, TPNH, for the activity of glutathione reductase. Hemoglobin is not necessary and changes in hemoglobin are incidental rather than primary events. This has been demonstrable by utilizing the stromal effects described in II above and

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should simplify and clarify the role of glutathione in red cell metabolism to a great extent; a more direct understanding of radiation effects is now possible.

All of these patterns provide an integrated approach to further study of several problems including conditions necessary for in vivo hemolysis and relationships to other diseases.

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BIBLIOGRAPHY

Carson, Paul E.; Long, W.K.; and Ickes, C.E.: Activation of Glutathione Reductase (GSSGR) in Hemolyzates by Stromata. Federation Proceedings, 20, 64. (1961)

Kellermeyer, Robert W.; Tarlov, A.R.; Brewer, G.J.; Carson, P.E.; and Alving, A.S.: The Hemolytic Effect of Therapeutic Drugs: Clinical Considerations of the Primaquine-Type Hemolysis. J.A.M.A., 180, 388, (1962)

Long, W.K., and Carson, P.E.: Erythrocyte Glutathione Reductase Activity in Diabetes. Biochem. & Biophys. Res. Comm., 5, 394, (1961)

Carson, Paul E.; Brewer, George J.; and Ickes, Charles: Decreased Glutathione Reductase with Susceptibility to Hemolysis. J. Lab. & Clin. Med., 58, 804, (1961)

Carson, Paul E. and Tarlov, A.R.: Biochemistry of Hemolysis. Ann. Rev. of Med., 13, 105, (1962)

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