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Received June 17, 1958. P.S.E.B.M., 1958, v99.

Coenzyme Studies in Primaquine-Sensitive Erythrocytes.*† (24348)

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A severe, acute, genetically determined hemolytic anemia may be induced by primaquine, many other drugs of therapeutic importance, and fava beans, in certain otherwise healthy individuals(1-5). Because the biochemical and enzymic abnormalities which characterize erythrocytes of these drug-sensitive individuals were first investigated using primaquine, the syndrome precipitated by this drug as well as by toxicologically related agents has been designated "primaquine-sensitive hemolytic anemia"(5). Except for certain biochemical abnormalities in glutathione and carbohydrate metabolism, erythrocytes of primaquine-sensitive individuals appear to be normal(6). Reduced glutathione content of these erythrocytes is generally lower than normal; however, there is considerable overlapping of values (2). The reduced glutathione content falls still further *in vivo* during drug-induced hemolysis(7). Furthermore, reduced glutathione content is usually unstable when erythrocytes are incubated *in vitro* with acetylphenylhydrazine(2,8). The glutathione reductase activity of primaquine-sensitive erythrocytes is increased (Reaction 2)(9). Two enzymic abnormalities in carbohydrate metabolism of erythrocytes from primaquine-sensitive males have been reported by this laboratory. 1) Activity of glucose-6-phosphate dehydrogenase, the enzyme catalyzing initial oxidative reaction of the hexosemonophosphate shunt, is markedly diminished (Reaction 1)(10). In some sensitive females deficiency may be considerably less than in males(2). 2) The activity of aldolase is increased in erythrocytes of primaquine-sensitive males(11). Aldolase catalyzes the reversible splitting of FDP, supplying substrate for the oxidative reaction of the Embden-Meyerhof pathway (Reactions 3 and 4).

Enzymic abnormalities in primaquine-sensitive erythrocytes focus attention on the pivo-

Reaction 1:

$$\text{‡G-6-P} + \text{TPN}^+ \xrightarrow[\text{Dehydrogenase}]{\text{G-6-P}} \text{6-PG} + \text{TPNH} + \text{H}^+$$

Reaction 2:

$$\text{GSSG} + \text{TPNH} + \text{H}^+ \xrightarrow[\text{Reductase}]{\text{GSSG}} 2\,\text{GSH} + \text{TPN}^+$$

Reaction 3:

$$\text{FDP} \underset{\longleftarrow}{\xrightarrow{\text{Aldolase}}} \text{GA-3-P} + \text{DHAP}$$

Reaction 4:

$$\text{GA-3-P} + \text{Pi} + \text{DPN}^+ \underset{\longleftarrow}{\xrightarrow{\text{GAPD}}} \text{1,3-DPG} + \text{DPNH} + \text{H}^+$$

* This work was supported (in major part) by Office of Surgeon General, Dept. of Army, under contract with Dept. of Medicine, University of Chicago. Additional support was obtained from Winthrop Laboratories, N. Y., Parke, Davis & Co., Detroit, Mich., Eaton Laboratories, Norwich, N. Y., Abbott Labs., N. Chicago, Ill., and Douglas Smith Fn., University of Chicago.

† The investigations would not have been possible except for cooperation of inmates and administrative staff of Stateville Penitentiary, Joliet, Ill.

‡ DPN+, DPNH, oxidized and reduced diphosphopyridine nucleotide, respectively; TPN+, TPNH, oxidized and reduced triphosphopyridine nucleotide, respectively; GSH, GSSG, reduced and oxidized glutathione, respectively; G-6-P, glucose-6-phosphate; 6-PG, 6-phosphogluconate; FDP, fructose diphosphate; DHAP, dihydroxyacetone phosphate; GA-3-P, glyceraldehyde-3-phosphate; 1,3-DPG, 1,3 diphosphoglycerate; Pi, inorganic phosphate; GAPD, glyceraldehyde phosphate dehydrogenase.

tal position which the coenzymes TPN^+ and DPN^+ occupy in glutathione and carbohydrate metabolism. We have therefore determined the content of DPN^+ and TPN^+ in normal and primaquine sensitive erythrocytes prior to drug administration.

Materials and methods. Subjects were adult male volunteers who had previously been tested with standard dose of 30 mg of primaquine base daily for at least one week. Those who developed hemolytic anemia were designated "primaquine-sensitive"; those who were unaffected were called "normal" or "non-sensitive." No individual who had received drug within the previous 4 months was studied. TPN^+ specific glucose-6-phosphate dehydrogenase and DPN^+ specific alcohol dehydrogenase were obtained from Sigma Chemical Co. Venous blood was drawn into a chilled heparinized syringe and centrifuged at 1,800 x g for 15 minutes at 4°C in an International PR-2 refrigerated centrifuge. After removal of plasma and buffy coat, 6 ml of packed erythrocytes were extracted twice in the cold with 30 ml and 25 ml of 3% and 0.75% perchloric acid, respectively. The supernatants were combined and adjusted to pH 4.6 to 4.8 with 5 N KOH. The resulting precipitate of $KClO_4$ was removed by filtration through Munctells #OOH paper in a Buchner funnel. The filtrate was then frozen, lyophilized and dissolved in 4 ml distilled water and pH adjusted to 6.4 to 7.4 with either 0.2 N KOH or 3% perchloric acid. The chilled extract was filtered through a medium porosity sintered-glass funnel. Aliquots of this extract were assayed spectrophotometrically for TPN^+ with a TPN^+ specific glucose-6-phosphate dehydrogenase reaction in a Beckman DU spectrophotometer(12). Similarly, the extract was assayed for DPN^+ with a DPN^+ specific alcohol dehydrogenase reaction(13).

Results. The average content of TPN^+ in normal erythrocytes was 3.4 μmoles/100 ml erythrocytes, and average DPN^+ content was 5.3 μmoles/100 ml erythrocytes. These findings are consistent with the observations of Barnett(14). Primaquine-sensitive erythrocytes had greater amounts of TPN^+ than non-sensitive erythrocytes, with an average

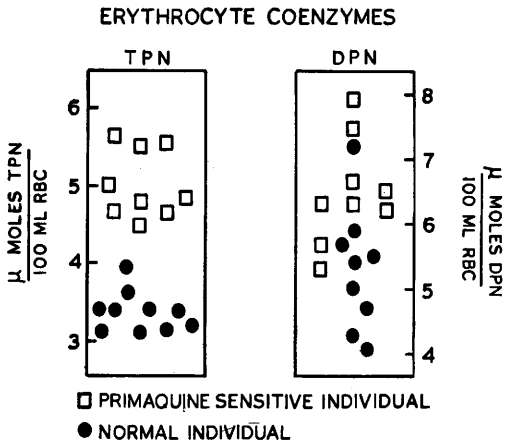

FIG. 1. Most of the points on the scattergram represent the average of repeated determinations on a single individual.

TPN^+ content of 5.0 μmoles/100 ml erythrocytes (Fig. 1). Although the mean value of DPN^+ content (6.5 μmoles/100 ml erythrocytes) in primaquine-sensitive erythrocytes was higher than that observed in non-sensitive erythrocytes, there was overlap in the values found in the 2 erythrocyte populations (Fig. 1).

Discussion. TPNH is the coenzyme for glutathione reductase which catalyzes the reduction of glutathione (Reaction 2). Reduced glutathione is necessary for protection of certain essential sulfhydryl containing proteins; *e.g.*, certain enzymes and hemoglobin(15). TPN^+ is the coenzyme for oxidation of glucose in the hexosemonophosphate shunt. Primaquine-sensitive erythrocytes deficient in glucose-6-phosphate dehydrogenase would reduce less TPN^+ to TPNH and, because they contain increased glutathione reductase, would oxidize more TPNH to TPN^+. Assuming a normal total TPN content (TPN^+ + TPNH) in primaquine-sensitive erythrocytes, the TPNH level would thus be correspondingly reduced. A decrease in TPNH in primaquine-sensitive erythrocytes could seriously affect reductive biosynthetic processes which may be necessary for the integrity of the erythrocyte(16).

Although the average DPN^+ content of primaquine-sensitive erythrocytes is slightly higher than normal, there is no clear division of values between the 2 populations. The in-

crease in aldolase activity in primaquine-sensitive erythrocytes could not explain the increase in their average DPN^+ content (Reactions 3 and 4). However, glutathione reductase can utilize DPNH as coenzyme in hemolyzates of human erythrocytes, though less effectively than TPNH(17). Whether the increased activity of glutathione reductase in primaquine-sensitive erythrocytes accounts for the increase in average DPN^+ content, can not, however, be substantiated by our present data.

Summary. TPN^+ content of erythrocytes of primaquine-sensitive males is greater than that of erythrocytes of normal males. The increase in TPN^+ may be explained by two known factors: 1) decrease in glucose-6-phosphate dehydrogenase activity, and 2) increased glutathione reductase activity in primaquine-sensitive erythrocytes. The mean values of DPN^+ content of erythrocytes in the two populations studied were different, but the values showed considerable overlap. Our current studies concern the effect of drug administration on both oxidized and reduced coenzymes in normal and primaquine-sensitive erythrocytes from both males and females.

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Received July 1, 1958. P.S.E.B.M., 1958, v99.

Liver Cholesterol Mobilization in Mice as Effected by Dietary β-Sitosterol and Cholic Acid.* (24349)

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The value of β-sitosterol as agent for lowering tissue cholesterol has been the basis of numerous investigations. A number of these reports have indicated that β-sitosterol fed together with cholesterol effectively prevents accumulation of tissue cholesterol in several species(1,2,3). This effect has been shown by Hernandez *et al.*(2) to be due to blocking of cholesterol absorption in the intestinal lymph. Since β-sitosterol is effective in preventing cholesterol absorption, one could assume that this substance would prevent absorption of recirculating cholesterol, and thus speed up the mobilization of accumulated tissue cholesterol. In a previous study on mobilization rate of accumulated plasma cholesterol in the rabbit (4), β-sitosterol had a slight, initial accelerating effect. Alfin-Slater *et al.*(5) have reported that soybean sterols, composed mainly of β-sitosterol and stigmasterol, have little ef-

* This investigation was supported in part by research grant from Michigan Heart Assn.