Effect of 17-Ketosteroids on Glucose-6-phosphate Dehydrogenase Activity (G6PD) and on G6PD Isoenzymes (37001)

A. LOPEZ-S. AND A. RENE

LSU Medical School, Department of Medicine, New Orleans, Louisiana 70112

Dehydroepiandrosterone (DHA) a potent inhibitor of glucose-6-phosphate dehydrogenase (G6PD), significantly decreases the activity of the G6PD of red blood cells of a normal adult man, when administered orally (1); furthermore, this enzyme, in a number of mammalian tissues, is potently inhibited *in vitro* by DHA (2–5) and has a high affinity for $\Delta 5$, 3β-hydroxy steroids (6). Disc electrophoresis permits the separation of different molecular forms of G6PD activity (7); Hori and Matsui (8) have shown specific isoenzyme inhibition of G6PD by dehydroepiandrosterone *in vivo*. The results of the *in vitro* effect of DHA and related steroids (androstenedione, etiocholanolone, and androsterone) on G6PD activity and the effect of DHA on the different G6PD isoenzymes isolated by disc electrophoresis are presented in this report.

Materials and Methods. Adult male rats of CDF strain were decapitated; their livers were removed, blotted with filter paper, washed in cold saline and immediately homogenized in 0.15 *M* KCl buffer (20%). The supernatant fraction was obtained by centrifugation at 66,000*g* for 60 min in an ultracentrifuge. The G6PD activity was assayed in the supernatant according to the procedure of Radack, Chisholm and Holten (9). One unit of G6PD activity is the amount of enzyme required to form 1 μmole of NADPH in 1 min. The assay consists of 120 μmoles of Tris–chloride buffer (pH 8.0) 0.2 μmole of glucose-6-phosphate, 0.9 μmole of $NADP^+$, 10.4 μmoles of $MgCl_2$, 0.6 μmole of 6-P-gluconate and enzyme per final volume of 1 ml. The OD changes were determined in a Gilford spectrophotometer Model 240 with a recorder attached and the changes in OD with time were recorded automatically. The inhibitory effect of different steroids was studied by adding them to the incubation media dissolved in ethanol in such amount as to obtain concentrations of 5×10^{-5}, 1×10^{-4}, and 5×10^{-4} *M* in 10% alcohol. Controls using only 10% ethanol were included in the experiments. Protein was measured by the biuret reaction and enzyme specific activity expressed as units of enzyme per milligram of protein.

Disc electrophoresis on acrylamide gels was carried out according to the method of DeFlora *et al.* (10), employing a 7.7% acrylamide in the lower gel and 4.1% acrylamide in the spacer gel. Samples of the hepatic homogenate in 2.9×10^4 *M* sucrose were applied to 5×80 mm gel columns beneath the buffer. After electrophoresis in Tris–glycine buffer (pH 8.3) with a constant current of 3 mA/column for 90 min, the gels were removed from the tubes, rinsed in distilled water and incubated at 37° for 2 hr in 0.1 *M* Tris HCl buffer (pH 8.6) containing 2×10^{-3} *M* glucose-6-phosphate, 5×10^{-4} *M* NADP, 0.07 m*M* Phenazine methosulfate and 0.5 m*M* *p*-iodonitrotetrazolium violet.

Results. Although DHA is the steroid with more potent inhibitory effect, androsterone, etiocholanolone and androstenedione are also significant inhibitors of G6PD under the conditions of our experiment (Table I). Since DHA was the most potent inhibitor, we studied the *in vitro* effect of dehydroepiandrosterone sulfate on the isoenzyme pattern of G6PD obtained by acrylamide gel electrophoresis. Figure 1 shows that no significant effect occurs on the G6PD zymogram at a DHA concentration of 10^{-5} *M*. At 5×10^{-5} *M* some uniform inhibition is observed in the G6PD zymogram and general inhibition with complete disappearance

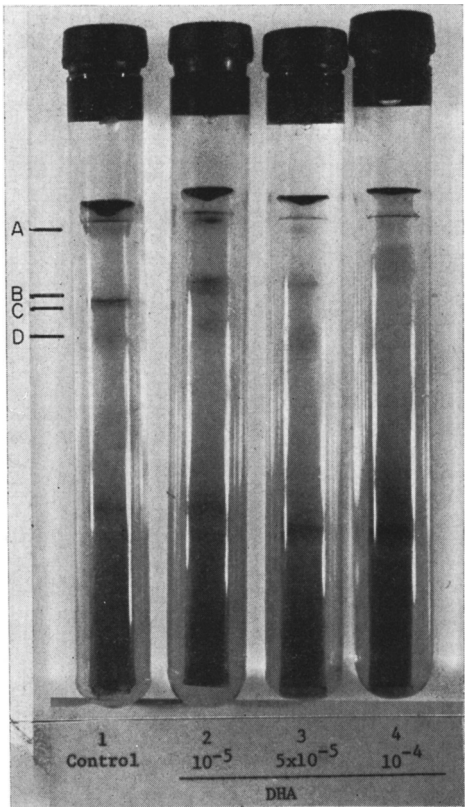

FIG. 1. Isoenzyme patterns of hepatic G6PD from normal adult male rats in the absence and presence of different concentrations of DHA. Anodal direction is downwards. Band D is particularly inhibited by DHA at a concentration of 1×10^{-4} *M*.

of band D can be observed in the zymogram at concentrations of DHA-sulfate of 10^{-4} *M*. No significant inhibition could be observed in the isoenzyme pattern (Fig. 2) when the DHA sulfate was added at a concentration of 10^{-4} to the staining media.

Discussion. From an experimental viewpoint the level of an enzyme activity can be related to the activity of the metabolic pathway in which it participates and any change in activity reflects alteration in the usage of that pathway. G6PD, rate-limiting enzyme in the hexosemonophosphate shunt, has been shown to play an important role in lipogenesis. Up to 40–57% of the NADPH required for the reductive systems of fatty acids can be contributed by the hexosemonophosphate pathway (11).

The potential biological significance of hormonal regulation of the enzymatic activity of G6PD in relation to lipogenesis and obesity has been previously pointed out by several investigators (12–17). As an extension of previous work we find in the present study that not only DHA but other steroids known to be metabolites of DHA (androsterone, etiocholanolone and androstenedione) have also a potent inhibitory effect upon hepatic G6PD. These findings confirm work by Ranieri and Levy (18) who have reported that the 17-ketosteroid group and a functional group in the C3 position of the steroids structure are essential for the inhibitory effect of the enzyme G6PD by steroids of the androstan family. They found that the inhibition was uncompetitive in the case of DHA with respect of G-6-phosphate and $NADP^+$. The potent *in vitro* inhibitory effect of the four interrelated steroids on enzyme G6PD may be of great biological significance in view of the finding that in normal individuals after a period of exercise there is an increase in the urinary excretion of these steroids, primarily etiocholanolone (19), and that in the male rat exercise produces a decrease in the enzymatic activity of the hepatic G6PD (20).

Of special interest is the finding of selective *in vitro* inhibitory effect by DHA of G6PD zymograms. Although very little is known re-

TABLE I. Inhibition of Hepatic G6PD by Different Steroids.

	% Inhibition[a]			
Concn	Dehydroepiandrosterone	Androstenedione	Androsterone	Etiocholanolone
5×10^{-5}	50.11	28.57	26.67	33.33
10^{-4}	65.74	57.14	46.67	54.00
5×10^{-4}	80.09	78.57	53.33	73.33

[a] Values for a representative experiment. Controls using only 10% ethanol were included and the corresponding inhibition values were subtracted.

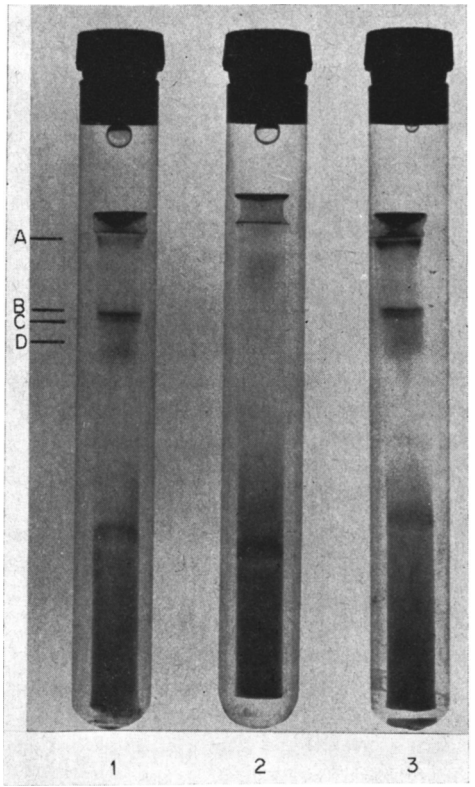

FIG. 2. Isoenzyme pattern of hepatic G6PD from normal adult male rats: (1) control zymogram of hepatic homogenate; (2) same as control plus 1×10^{-4} *M* DHA in the homogenate; (3) same as control plus 1×10^{-4} *M* DHA added to the staining media.

garding isoenzyme regulation in mammalian tissues, the catalytic properties of isoenzymes are ideally suited to the metabolic regulation of their respective tissues (21). Hori and Matsui (8) have shown a selective *in vivo* effect of hormones on hepatic G6PD similar to the one observed in Fig. 1. The findings in this study and others point out to the potential importance of hormonal regulation of the activity of G6PD in the synthesis of NADPH, a cofactor required for the reductive synthesis of fatty acids (22) and cholesterol (23). The criticism has been made that plasma concentration of these steroids is of two to three orders of magnitude below the concentrations required *in vitro* to produce inhibition of G6PD (11) and also that the DHA-sulfate has no *in vitro* inhibitory effect on G6PD activity (4). However, it has been shown that a DHA-sulfatide, another biological form of DHA, has about three times more potent inhibitory effect than free DHA and thus may play a significant biological role at low concentrations (24). In preliminary studies in our laboratory we have observed not only a decrease in the enzymatic activities of hepatic G6PD after exercise but a selective decrease in the same isoenzyme band shown in Fig. 2 to be inhibited *in vitro* by DHA (20).

Summary. *In vitro* experiments with rat hepatic glucose-6-phosphate dehydrogenase have shown that the activity of this enzyme is significantly inhibited by dehydroepiandrosterone, androstenedione, androsterone and etiocholanolone at concentrations of 10^{-4} and 5×10^{-4} *M*. At these concentrations, dehydroepiandrosterone has specific inhibitory effects on glucose-6-phosphate dehydrogenase isoenzymes separated by acrylamide gel electrophoresis.

Supported by funds from the National Heart and Lung Institute of U.S. Public Health Service (R01 HL 13205). The authors thank Mrs. Lillie Bell for her technical assistance and Mrs. Hedy Boelte for her secretarial assistance.

1. Lopez-S., A., and Krehl, W. A., Proc. Soc. Exp. Biol. Med. **126**, 776 (1967).

2. Marks, P. A., and Banks, J., Proc. Nat. Acad. Sci. USA **46**, 447 (1960).

3. McKerns, K. W., and Kaleita, E., Biochem. Biophys. Res. Commun. **2**, 344 (1960).

4. Tsutsui, E. A., Marks, P. A., and Reich, P., J. Biol. Chem. **237**, 3009 (1962).

5. Levy, H. R., J. Biol. Chem. **238**, 775 (1963).

6. Douville, A. W., and Warren, J. C., Biochemistry **7**, 4052 (1968).

7. Hori, S. H., and Kamada, T., Jap. J. Genet. **42**, 367 (1967).

8. Hori, S. H., and Matsui, S., J. Histochem. Cytochem. **15**, 530 (1967).

9. Rudack, D., Chisholm, E. M., and Holten, D., Jr., Biol. Chem. **246**, 1249 (1971).

10. DeFlora, A., Lorenzoni, I., Mangiarotti, M. A., Dina, D., and Bonsignore, D., Biochem. Biophys. Res. Commun. **31**, 501 (1968).

11. Ziboh, V. A., Dreize, M. A., and Hsia, S. L., J. Lipid Res. **11**, 346 (1970).

12. Weber, G., Advan. Enzyme Regul. **1**, 60 (1963).

13. Sonka, J., and Gregorova, I., Lancet **2**, 44 (1964).

14. Sonka, J., and Gregorova, I., Lancet **2**, 671 (1964).

15. Lopez-S., A., and Krehl, W. A., Lancet **2**, 485 (1967).

16. Leveille, G. A., Proc. Soc. Exp. Biol. Med. **125**, 85 (1967).

17. Tepperman, J., and Tepperman, H. M., Pharmacol. Rev. **12**, 301 (1960).

18. Raineri, R., and Levy, R. H., Biochemistry **9**, 2233 (1970).

19. Lopez-S., A., and Rene, A., Clin. Res. **20**, 1 (1972).

20. Lopez-S., A., and Rene, A., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **31**, 673 (1972).

21. Stifel, F. B., and Herman, R. H., Amer. J. Clin. Nutr. **25**, 606 (1972).

22. Katz, J., and Rogustad, R., J. Biol. Chem. **241**, 3600 (1966).

23. Gianelli, A. A., and Terner, C., Endocrinology **83**, 1311 (1968).

24. Benes, P., Simsony, R., and Ortel, G. W., Steroidologia **2**, 52 (1971).

Received Sept. 6, 1972. P.S.E.B.M., 1973, Vol. 142.