# *INHIBITION OF MAMMALIAN GLUCOSE-6-PHOSPHATE DEHYDROGENASE BY STEROIDS**

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It has been considered for some time that steroid hormones may exert their regulatory effects by influencing certain enzymes.[1] However, our present knowledge of the mechanisms of steroid action upon enzymes is limited. Estrogens have been shown to stimulate the transfer of hydrogen between di- and triphosphopyridine nucleotides,[2, 3] although there is disagreement as to the role of steroids in this reaction.[4, 5] Recently, steroids were found to inhibit reduced diphosphopyridine nucleotide oxidation by enzyme preparations from mammalian and microbial sources.[6, 7] This action appears to be a general property shared by a large number of steroids.[7]

This study demonstrates a highly specific inhibitory effect on mammalian glucose-6-phosphate dehydrogenase (G-6-PD) of very low concentrations of dehydroisoandrosterone, pregnenolone, and certain related steroids. These steroids are not inhibitors of yeast or spinach G-6-PD or of mammalian 6-phosphogluconic dehydrogenase or isocitric dehydrogenase.

*Materials and Methods.—Preparation of enzymes:* G-6-PD was purified from human erythrocytes by a procedure described in detail elsewhere[8] involving ammonium sulfate fractionation followed by absorption on calcium phosphate gel, elution in a phosphate buffer, and a second series of ammonium sulfate precipitations. The final product generally had a specific activity 500-fold that of the crude hemolysate in a yield of about 40 per cent. This preparation was essentially free of hemoglobin and 6-phosphogluconic dehydrogenase activity.

Crude enzyme preparations were obtained from human and rat tissues and from spinach by homogenizing freshly obtained material in isotonic potassium chloride buffered at pH 7.4 (10%, weight/volume). The homogenate was centrifuged 30 minutes at 18,000 $\times$ *g* to sediment debris. The supernatant fluid was employed for assays of enzyme activity.

Partially purified yeast G-6-PD and pig heart isocitric dehydrogenase were obtained from Sigma Chemical Corporation. Partially purified 6-phosphogluconic dehydrogenase was prepared from rat liver by the method of Glock and McLean.[9]

*Enzyme assays:* Purified preparations of G-6-PD were assayed by the method of Kornberg and Horecker.[10] In measuring G-6-PD activity in crude tissue preparations, due to the presence of 6-phosphogluconic dehydrogenase, the rate of TPNH formation exceeds the rate of glucose-6-phosphate oxidation. Accordingly, in crude preparations, G-6-PD activity was assayed by the method of Kornberg and Horecker[10] determining the initial rate of TPNH formation by measuring the change of absorption at 340 m$\mu$ with an automatic recording Cary spectrophotometer or, alternatively, by the method of Glock and McLean.[9] In this latter method, G-6-PD activity is taken as the difference in the rate of TPNH reduction when the reaction mixture contains both 6-phosphogluconate plus glucose-6-phosphate and in the presence of 6-phosphogluconate alone. Comparable results with

regard to the effects of steroids were obtained with both methods of assay of G-6-PD activity. 6-Phosphogluconic dehydrogenase activity was determined by the method of Horecker and Smyrniotis.[11] Isocitric dehydrogenase activity was assayed by the method of Ochoa.[12] The steroids were added in 0.02 ml of dioxane. The total volume of the reaction mixture was 2.5 ml. Determinations of enzyme activity in the absence of steroids were performed in identical reaction mixtures containing 0.02 ml of dioxane. Addition of steroids in propylene glycol yielded results similar to those obtained when dioxane was used as the diluent.

*Sources of steroids:* All steroids were obtained from commercial sources with the following exceptions: dehydroisoandrosterone, androsterone, etiocholanolone, Reichstein's Compound "S," desoxycorticosterone and 17-hydroxypregnenolone were kindly supplied by Dr. Seymour Lieberman, and 3$\alpha$-OH-$\Delta^5$-androstene-17-one, epiandrosterone and androstenedione were kindly supplied by Dr. Michael Lombardo.

TABLE 1

EFFECT OF STEROIDS ON GLUCOSE-6-PHOSPHATE DEHYDROGENASES

| Compound Concentration ($M$) | Purified Human RBC G-6-PD | | | Crude Rat Adrenal G-6-PD | | |
|---|---|---|---|---|---|---|
| | $4 \times 10^{-5}$ | $1 \times 10^{-6}$ | $4 \times 10^{-7}$ | $4 \times 10^{-5}$ | $1 \times 10^{-6}$ | $4 \times 10^{-7}$ |
| | % Inhibition* | | | % Inhibition | | |
| Pregnenolone | 70 | 23 | 13 | 73 | 30 | 23 |
| 17-OH-Pregnenolone | | 21 | 8 | | 21 | |
| 3$\beta$-Hydroxyallopregnan-20-one | 61 | 13 | 3 | 78 | 20 | 10 |
| Pregnan-3,20-dione | 47 | 9 | 0 | 41 | 0 | |
| 17$\alpha$-OH-21-acetoxy-pregnenolone | 19† | 0 | | | 0 | |
| Pregnenolone-3-methyl-ether | 19 | 0 | | | 0 | |
| Progesterone | 23 | 0 | | 3 | 0 | |
| 17$\alpha$-OH-Progesterone | 18 | 0 | | | | |
| Dehydroisoandrosterone | 80 | 28 | 18 | 90 | 45 | 21 |
| 3$\alpha$-OH-$\Delta^5$-androstene-17-one | 80 | 30 | 15 | 90 | 41 | 18 |
| Epiandrosterone | 85 | 34 | | 95 | 45 | |
| Androstane-3,17-dione | 75 | 15 | | | | |
| $\Delta^4$-Androstene-3,17-dione | 55 | 10 | 0 | 52 | 3 | 0 |
| Androsterone | 52 | 11 | 0 | 50 | 10 | 0 |
| Etiocholanolone | 50 | 10 | 0 | 60 | 20 | |
| Estrone | 21† | 0 | | 15* | 0 | |
| Stilbesterol | 8 | 0 | | 5 | 0 | |

* Per cent inhibition of enzyme activity is expressed in terms of the rate of the reaction in the presence of the steroid compared to that in the absence of the steroid. The steroid was added to the reaction mixture in 0.02 ml of dioxane and all controls were run with 0.02 ml of dioxane.

† These compounds were insoluble at $4 \times 10^{-5}$ $M$ and the data are for the effect of the steroid at a concentration of $1 \times 10^{-5}$ $M$.

*Results.*—G-6-PD, purified from human red cells or in crude preparations of rat adrenal, is inhibited by pregnenolone and other $C_{21}$ steroids, 17$\alpha$-hydroxy-pregnenolone, 3$\beta$-hydroxyallopregnan-20-one, pregnan-3,20-dione, and by dehydroisoandrosterone, and other $C_{19}$ steroids, 3$\alpha$-OH-$\Delta^5$-androstene-17-one, epiandrosterone, androstane-3,17-dione, $\Delta^4$-androstene-3,17-dione, androsterone and etiocholanolone, at $1 \times 10^{-6}$ $M$ (Table 1). Certain of these steroids had inhibitory effects at $10^{-7}$ $M$ (Tables 1 and 2).

The following steroids were tested and found not to inhibit G-6-PD at concentrations as high as $4 \times 10^{-5}$ $M$: estradiol-17$\alpha$, estradiol-17$\beta$, estriol, hydrocortisone, cortisone, prednisolone, desoxycorticosterone, cortexolone (Compound "S"), cholesterol, stigmasterol, Na tauracholate, cyclohexanone, cyclopentanone, $\Delta^5$-androstene-3,17-diol, 17$\alpha$-methyl-$\Delta^5$-androstene-3$\beta$,17$\beta$-diol-17-benzoate, testos-

TABLE 2

EFFECT OF DEHYDROISOANDROSTERONE AND PREGNENOLONE ON DEHYDROGENASES OF VARIOUS SOURCES

| Enzyme* | Source | Dehydroisoandrosterone $1 \times 10^{-5}$ M % Inhibition† | Dehydroisoandrosterone $1 \times 10^{-7}$ M % Inhibition† | Pregnenolone $1 \times 10^{-5}$ M % Inhibition | Pregnenolone $1 \times 10^{-7}$ M % Inhibition |
|---|---|---|---|---|---|
| G-6-PD | Purified RBC—human | 60 | 10 | 58 | 5 |
| | Crude RBC—human | 69 | 16 | 55 | 10 |
| | Crude liver—human | 82 | 20 | 56 | 10 |
| | Crude adipose—human | 42 | 0 | 35 | 0 |
| | Crude adrenal—rat | 80 | 10 | 55 | 5 |
| | Crude liver—rat | 78 | 10 | 50 | 5 |
| | Crude testes—rat | 85 | 20 | 71 | 10 |
| | Purified yeast | 0 | | 0 | |
| | Crude spinach | 0 | | 0 | |
| 6-PGD | Crude RBC—human | 0 | | 0 | |
| | Crude adipose—human | 0 | | 0 | |
| | Purified liver—rat | 0 | | 0 | |
| | Crude adrenal—rat | 0 | | 0 | |
| | Crude liver—rat | 0 | | 0 | |
| | Crude testes—rat | 0 | | 0 | |
| | Crude spinach | 0 | | 0 | |
| ICD | Crude adipose—human | 0 | | 0 | |
| | Purified heart—pig | 0 | | 0 | |
| | Crude adrenal—rat | 0 | | 0 | |
| | Crude liver—rat | 0 | | 0 | |
| | Crude testes—rat | 0 | | 0 | |

* Abbreviations: G-6-PD, glucose-6-phosphate dehydrogenase; 6-PGD, 6-phosphogluconic dehydrogenase; ICD, isocitric dehydrogenase; RBC, red blood cell. For details of sources of enzymes see text.

† Per cent inhibition of enzyme activity is expressed in terms of the rate of the reaction in the presence of the steroid compared to that in the absence of the steroid. The steroid was added to the reaction mixture in 0.02 ml of dioxane and all controls were run with 0.02 ml of dioxane.

terone, pregnan-3$\alpha$,20$\alpha$-diol, ethisterone, 16,17-epoxy-21-acetoxy pregnenolone, 16,17-oxido-pregnenolone acetate, and pregnan-17$\alpha$21-diol-3,11,20-trione-21-acetate.

In addition to the effect of steroids on G-6-PD indicated in Table 1, dehydroisoandrosterone and pregnenolone were examined for their inhibitory action against a variety of G-6-PD from other sources and against two other dehydrogenases catalyzing reactions involving TPN reduction. It was found that the steroids inhibited G-6-PD from several human and rat tissues, but not from spinach or yeast and not 6-phosphogluconic or isocitric dehydrogenases from any of the several tissues examined (Table 2).

To determine whether dehydroisoandrosterone and pregnenolone are competitive or noncompetitive inhibitors of G-6-PD, the activity of the purified preparation of G-6-PD was measured in the absence and presence of the steroid over a wide range of substrate concentrations. These experiments were performed with an excess of TPN and limiting concentrations of glucose-6-phosphate on the one hand, and, with an excess of glucose-6-phosphate and limiting concentrations of TPN on the other hand[13] (Fig. 1). The data indicate that the steroids are noncompetitive inhibitors of G-6-PD with respect to either of its substrates, TPN or glucose-6-phosphate. The reciprocal plots of the enzyme activity in the presence of a constant amount of inhibitor resulted in straight lines which appear parallel to that obtained in the absence of inhibitor. This suggests that the steroid inhibition of G-6-PD may be an example of anti-competitive inhibition.[14, 15]

The degree of inhibition of G-6-PD by steroids is not related to the order of addition of the reactants. Thus, the same inhibition (70 to 80 per cent) was found when

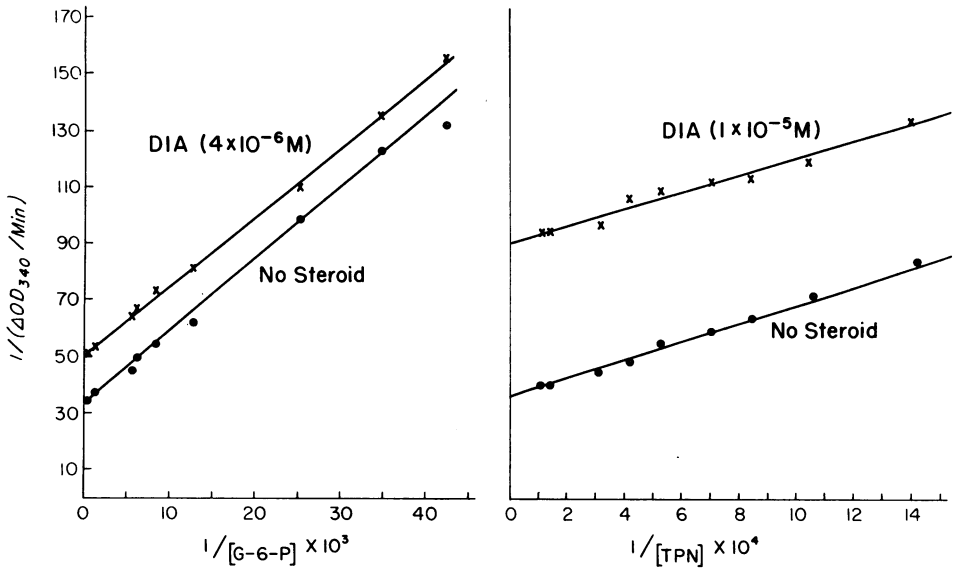


FIG. 1.—Relation between 1/v and 1/(S) for purified RBC glucose-6-phosphate dehydrogenase in the absence and in the presence of a constant amount of dehydroisoandrosterone. In the study plotted on the left, the concentration of glucose-6-phosphate was varied, and in the study plotted on the right, the concentration of TPN was varied. The steroid was added to the reaction mixture in dioxane. Studies without steroid were performed in reaction mixtures containing dioxane.[16]

G-6-PD was preincubated for 15 minutes at 25°C. with dehydroisoandrosterone or pregnenolone ($1 \times 10^{-5}$ *M*) in the presence or absence of TPN and/or glucose-6-phosphate.

The enzyme preparations used did not catalyze a TPN linked oxidation or reduction of the steroids employed. There was no detectable change in the level of TPNH or TPN, as measured spectrophotometrically, when the nucleotides, steroids and enzymes were combined. In addition, substitution of TPNH for TPN in the reaction mixture, in the presence of dehydroisoandrosterone, pregnenolone, or related steroids at $1 \times 10^{-5}$ *M*, was associated with no change in optical density measured at 340 $m\mu$.

*Discussion.*—Dehydroisoandrosterone, pregnenolone, and certain related steroids have been found to be potent inhibitors of mammalian G-6-PD catalyzed TPNH formation. The action of these steroids has a specificity with regard to both the dehydrogenase inhibited and the structure of the inhibiting compound. Thus, G-6-PD of yeast and spinach and mammalian 6-phosphogluconic and isocitric dehydrogenases are not inhibited. All the steroids with an inhibitory action on G-6-PD possess a ketone group in the $C_{17}$ or $C_{20}$ positions. Progesterone, corticosteroids, estrogens, and steroids with a hydroxyl rather than a ketone group at $C_{17}$ or $C_{20}$ have relatively little and, generally, no inhibitory effect on G-6-PD. On the other hand, the presence of a ketone or an $\alpha$ or $\beta$ hydroxyl group at $C_3$ or a saturated or unsaturated bond at $C_{4-5}$ or $C_{5-6}$ have little effect on the inhibitory action of the steroids tested. In addition, the steroid effect appears to be a catalytic one, since low concentrations of steroid inhibited the reduction of relatively large amounts

of TPN. Thus, as inhibitors of G-6-PD, these steroids have several characteristics compatible with a biologically significant enzyme inhibitor.

Recently, McKerns and Bell[17] reported that stilbesterol and, to a lesser extent, estrone, estradiol, pregnenolone, and progesterone, inhibited G-6-PD of rat or cow adrenal. The present results differ from these in several respects. The relative effects obtained with the compounds tested differ widely. Further, no preincubation was needed to obtain the steroid inhibition of G-6-PD in the present work. Preincubation of the enzyme preparations in the presence of the steroid for periods up to one hour did not enhance the inhibitory effects of the several compounds tested.

It has been demonstrated that some steroids may catalyze a pyridine-nucleotide transhydrogenation.[2-5] The presently described steroid inhibition of G-6-PD catalyzed TPN reduction is apparently unrelated to any such enhancement of TPNH oxidation. It is also unlikely that this steroid effect reflects a reaction with TPN, in view of the failure of the steroids to inhibit 6-phosphogluconic or isocitric dehydrogenases in the same preparations in which G-6-PD was markedly affected.

Our limited knowledge of the metabolism of the several steroids which inhibit G-6-PD makes it difficult to predict the possible physiological consequences of this steroid action. Nevertheless, it is reasonable to consider that such action of dehydrosterone, pregnenolone, and related steroids might function as a regulatory mechanism of significance in carbohydrate metabolism. Of particular pertinence to steroid metabolism is the generation of TPNH.[18] There is much evidence to indicate that TPNH is required at several steps in steroid biosynthesis, including the conversion of squalene to cholesterol,[19, 20] the conversion of cholesterol to pregnenolone,[21] steroid hydroxylations,[22-25] the conversion of androgen to estrogens[26] and cholesterol side chain cleavage.[27] The metabolic function of dehydroisoandrosterone is not well understood.[28] It is secreted by the adrenals in significant amounts and is believed to be the major precursor of the urinary 17-ketosteroid. The suggestion has been made that dehydroisoandrosterone is an intermediate in adrenal androgen synthesis.[29] More data[30, 31] are available on the biochemical role of pregnenolone. It appears that this steroid is a precursor common to progesterone, androgens, estrogens, and corticoid hormones and is derived, in part at least, from cholesterol. It has been suggested that the rate limiting step in the conversion of cholesterol to steroid hormones is involved in cholesterol transformation to pregnenolone.[21, 32, 33]

Inhibition of TPNH generation by pregnenolone or dehydroisoandrosterone could function as a control mechanism in the formation of these key intermediates in steroid hormone synthesis. For the steroid inhibitor of G-6-PD to effect a regulatory action of this type implies further that "intracellular geometry" is such that TPNH generated in the pentose phosphate pathway, but not that in the tricarboxylic acid cycle, is required for the rate limiting step in the synthesis of these intermediate steroids.

*Summary.*—Dehydroisoandrosterone, pregnenolone, and certain related steroids, at concentrations of $1 \times 10^{-6}$ *M* or less, inhibit mammalian glucose-6-phosphate dehydrogenase activity. These steroids do not inhibit 6-phosphogluconic or isocitric dehydrogenases or spinach or yeast glucose-6-phosphate dehydrogenase. A variety of other steroids, including estrogens, testosterone, corticosteroids, and

progesterone had little or no effect on glucose-6-phosphate dehydrogenase in concentrations as high as $4 \times 10^{-5}$ *M*.

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