INHIBITION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE BY HORMONES

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It has been reported previously that the metabolism of the adrenal cortex and the synthesis of corticoids are inhibited in vitro by female sex hormones but not by the male hormone testosterone (McKerns 1957). The administration of large doses of estrogens to the male rat was also inhibitory to adrenal function (McKerns et al. 1958). The site of this inhibition was found to be the enzyme glucose-6-phosphate dehydrogenase (G-6-PD) (McKerns 1959).

Many compounds of the estrogen, progesterone and thyronine series were found to be inhibitors of the activity of G-6-PD which was prepared from cow adrenal cortex and from rat adrenals (McKerns and Bell 1960). Activity of G-6-PD was also inhibited by these compounds in a crude adrenal homogenate preparation. The inhibition of G-6-PD which, of course, leads to a decrease in the hexosemonophosphate shunt and the decreased availability of TPNH, has profound significance to the metabolism of many tissues. Some of these metabolic effects, the type of inhibition, and some of the structural requirements for inhibitor compounds have been described (McKerns and Bell 1960).

In the past two years a great number of compounds have been tested against G-6-PD and other enzymes in order to determine those

structural requirements necessary for inhibition. Certain of these compounds with their relative inhibitory effects for G-6-PD are given in the table. The enzymes, 6-phosphogluconate, lactic, pyruvic and malate dehydrogenases were not inhibited by stilbestrol and certain other compounds under the assay conditions described. Slight inhibition of isocitrate dehydrogenase activity occurs with both androgenic and estrogenic compounds at 5 x 10^{-5} M. No trans-hydrogenases of the type described by Talalay and Williams-Ashman (1958) or by Villee and Hagerman (1958) were found in adrenal cortex tissue. Other enzyme systems are currently under study.

Relative Inhibition of Glucose-6-Phosphate Dehydrogenase by Various Hormones

Compound	% Inhibition At $5x10^{-5}M$	$1x10^{-6}M$
androstane	0	0
androsterone (3α-hydroxyandrostan-17-one)	50	10
androstanolone (17β-hydroxyandrostan-3-one)	15	0
androstane-3,17-dione	80	5
androstane-3α,17β-diol	20	0
17β-hydroxy-17α-methylandrosten-3-one	5	0
1-androstene-3,17-dione	70	25
4-androstene-3,17-dione	70	25
1-4-androstadiene-3,17-dione	60	20
dehydroisoandrosterone (3β-hydroxy-5-androsten-17-one)	80	20
testosterone	0	0
methyl testosterone	10	0
estrone	25	15
estradiol	15	5
estriol	0	0
stilbestrol	35	25
dimethyl ether stilbestrol	0	0
progesterone	15	0
17α-Hydroxy-progesterone	25	0
5-pregnenolone	60	25
17α-Hydroxy-pregnenolone	60	0
cortisone, hydrocortisone	0	0
hydrocortisone, deoxycorticosterone	0	0

The test system contained purified enzyme in Tris buffer (pH 8.0), umoles; $MgCl_2$, 10 μmoles; TPN, 0.2 μmoles; G-6-PD, 3.0 μmoles in a total volume of 3 ml. Compounds were added to the enzyme-substrate mixture, followed in 5 minutes by the addition of TPN to start the reaction.

EXPERIMENTAL AND RESULTS

Preparation of enzymes: Fresh adrenal cortex tissue obtained from cows or rats was homogenized and centrifuged at 700xg for 45 minutes. Mitochondria were removed by centrifuging at 23,000xg for 40 minutes. The activities of G-6-PD, 6-phosphogluconate, isocitrate, lactic, pyruvic and malate dehydrogenases were determined using the crude supernatant fraction. A purified G-6-PD preparation which was free of endogenous substrates and H-acceptors but which contained isocitrate dehydrogenase was prepared by ammonium sulphate precipitations similar to the method described by Kelly et. al. (1955).

Assay of dehydrogenases: The enzymatic activity of the dehydrogenases was assayed by following the reduction of TPN at 340 mμ by methods similar to those described in Colowick and Kaplan (1955). The compounds to be tested were dissolved in 0.01 ml. dioxane and dispersed into the Tris buffer solution (pH 8.0) which contained the enzyme and its appropriate substrate (total volume 3 ml.). TPN was then dispersed into the medium as a jet from a microsyringe and its reduction was followed on a model DU spectrophotometer or a Cary Recording spectrophotometer model 14 M to determine initial velocity.

Many compounds such as pregnenolone, androsterone, and 4-androstene-3,17-dione are more potent inhibitors of G-6-PD than compounds of the estrogen and progesterone series. It remains to be determined whether they are produced by the adrenal in amounts sufficient to function as local inhibitors or regulators of G-6-PD and thus of corticoid synthesis or are secreted by the gonads in amounts sufficient to inhibit. On the other hand, estrone, estradiol, progesterone, dehydroisoandrosterone and testosterone circulate in the blood in larger quantities. Testosterone, however, is inactive as an inhibitor. Therefore, the net effect may be a

sex difference in the metabolism of the adrenal cortex and a primary inhibitory effect on the adrenal by female sex hormones. It is interesting to note that estriol, the estrogenic hormone produced in large quantities during pregnancy, is inactive as an inhibitor of G-6-PD.

Our observations lead us to believe that the pituitary tends to compensate for the inhibition of adrenal cortex metabolism and the decrease in corticoid secretion caused by female sex hormones. There is an increase in corticotropin and prolactin and a decrease in growth hormone secretion (McKerns et. al., 1958). The increased corticotropin and prolactin secretions could account for the increased HMP shunt observed in liver and adipose tissue (McKerns and Bell, 1960). It has also been observed that adipose tissue from female rats has a much higher rate of oxidation of carbon 1 of glucose and a greater rate of synthesis of fatty acids than adipose tissue from littermate male animals (McKerns and Clynes). The results of further experiments which indicate the effects of these changes in adrenal-pituitary function on the intermediate metabolism of various tissues will be discussed in subsequent reports.

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