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In vivo Effect of Dehydroepiandrosterone on Red Blood Cells Glucose-6-Phosphate Dehydrogenase. (32567)

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The biological mechanism of action of steroid hormones still remains unknown. Reports from the literature suggest that they may exert their regulatory effect by an effect on certain enzymatic functions. That specific enzymes are influenced by certain steroids has been reported by McKerns(1) and others (2-3). With regard to dehydroepiandrosterone (DHA) it has been shown by different investigators that this steroid hormone is a potent *in vitro* inhibitor of the enzyme glucose-6-phosphate dehydrogenase. This information is summarized in Table I.

Such a metabolic inhibition *in vivo* has interesting implications, particularly if the inhibition of glucose-6-phosphate dehydrogenase resulted in a decreased availability of TPNH, which is an important co-factor in the synthesis of fatty acids.

Materials and methods. The present study was designed to test the *in vivo* effect of dehydroepiandrosterone on the enzyme glucose-6-phosphate dehydrogenase of red blood cells. For this, two similar experiments were carried out. In the first study, 100 mg of pure DHA (dehydroisoandrosterone, Sigma Chemical Co.) were administered for 3 consecutive days to a healthy adult male engaged in normal professional activities. Red blood cell glucose-6-phosphate dehydrogenase activity was measured every other day for one week, before the administration of DHA (control periods), during the 3 days of administration, and thereafter; each time the determination of G-6-PD was done in triplicate. The urinary excretion of 17 ketosteroids and DHA was also measured daily during the same period of time, in duplicate. A similar experiment was repeated 9 months later on the same subject but using a more accurate method of measuring dehydroepiandrosterone; a total of 500 mg of DHA in 4 consecutive days was administered in this second experiment.

Glucose-6-phosphate dehydrogenase was determined using the method of Kornberg and Horecker(4) by measuring the increase of TPNH absorbance at 340 millimicrons per unit of time in a medium consisting of enzyme (r.b.c.) substrate (glucose-6-phosphate), buffer (triethanolamine) and TPN (as a co-factor). All these reagents were obtained from C. F. Boehinger and Soehne, GMBH, Mannheim, Germany.

Dehydroepiandrosterone: In the first experiment urinary DHA was hydrolyzed by the method of Burstein and Lieberman(5) and then separated by column chromatography by the method of Fotherby(6). The isolated DHA was then measured colorimetrically by a modification of the Petenkofer reaction(7) described by Inagaki(8). In the second experiment, the DHA, after hydrolysis,

TABLE I. *In vitro* Effect of Dehydroepiandrosterone on Glucose-6-Phosphate Dehydrogenase.

System	Concentration	% Inhibition	Ref.
Adrenal cortex (cows and rats)	5×10^{-5}M	80	(15)
" "	1×10^{-6}M	20	(15)
Human erythrocytes	4×10^{-5}M	80	(12)
" "	1×10^{-6}M	28	(12)
" "	4×10^{-7}M	18	(12)
Rat adrenals	4×10^{-5}M	90	(12)
" "	1×10^{-6}M	45	(12)
" "	4×10^{-7}M	21	(12)
Lactating guinea pigs (mammary gland)	3×10^{-4}M		(16)
Rat adrenal	5×10^{-5}M	80	(17)

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was separated by thin layer silica gel(18), then diluted with isopropanol, the trimethylsylylether formed and quantitatively determined by gas-liquid chromatography. A Barber Coleman Model 5000 with argon ionization detector was used with a U-glass column 6 feet long with an ID of 4 millimeters with QFI-1, 3% coating and gas-chrom-Q support (800-100 mesh). Argon is used as carrier gas at an inlet pressure of 18 psi. Column temperature 218°C, detector temperature 248°, and 280° injector temperature. This method was found to be more specific than the colorimetric one. Several determinations by both methods performed on the same samples showed an average of 12% higher values by the colorimetric method. 17 ketosteroids were determined by the method of Robbie and Gibson(9).

Results and discussion. Fig. 1 shows the response in urinary steroid excretion and red blood cell levels of glucose-6-phosphate dehydrogenase to the oral administration of DHA. It can be seen that after the administration of 300 mg of DHA there seems to be a decrease in the glucose-6-phosphate dehydrogenase activity of the red blood cell (13 ± 0.36†). This decrease was found to be statistically significant ($P<.01$). The G-6-PD activity returned to control values after 8-10 days. That the orally administered DHA was at least partially absorbed is demonstrated by the marked rise in the urinary excretion of total 17 ketosteroids and DHA. (DHA usually constitutes 15-26 percent of the urinary 17 ketosteroids in normal individuals(10)). Essentially similar findings were observed in the second experiment using the more specific gas-liquid chromatography method for the determination of DHA (Fig. 2). The decrease in G-6-PD activity was also statistically significant ($P<.01$) (7.3% ± 0.62‡).

One can predict an increase of approximately 0.8 micromols per 100 cc of plasma for each milligram of orally injected DHA (11). The percent inhibition which could be expected by such an increase was calculated to be of about 6-10%(12). It is interesting to

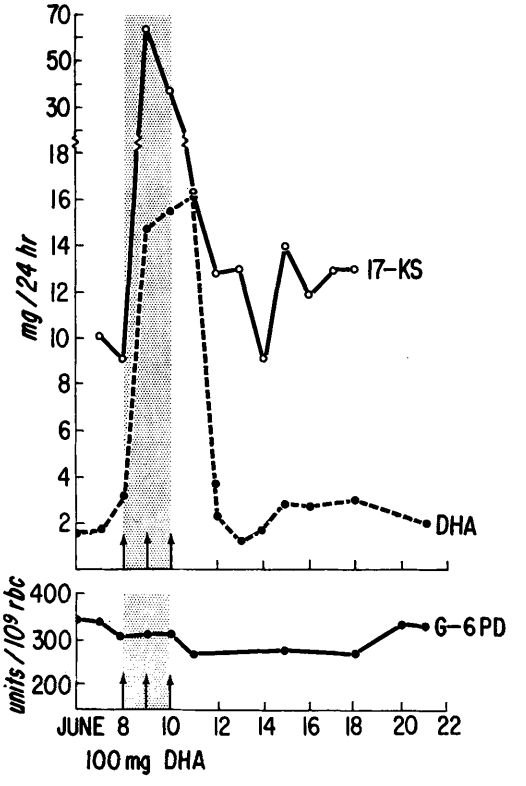

FIG. 1. Changes in the red blood cell glucose-6-phosphate dehydrogenase after the oral administration of 300 milligrams of dehydroepiandrosterone.

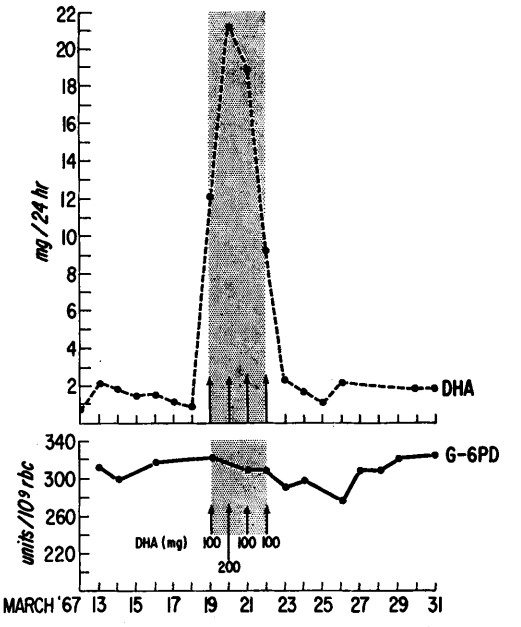

FIG. 2. Changes in the red blood cells glucose-6-phosphate dehydrogenase after the oral administration of 500 milligrams of dehydroepiandrosterone.

† S. E.

‡ S. E.

note that the mean decrease in the activity of the G-6-PD of the red blood cell(13 and 7.3%) observed in our experiment is of an order of magnitude similar to the one expected using the calculation described above.

Obviously the changes observed are still within the normal range and any specific interpretation of the biological significance of these changes should be approached with caution. Nevertheless, it seems possible that *in vivo,* DHA or one of its metabolic products may function as a factor in regulating the activity of the enzyme glucose-6-phosphate dehydrogenase in red blood cells. If this also occurs in other tissues as well, DHA could play a very important role in influencing the rate of oxidation of glucose *via* the hexosemonophosphate shunt. The possible importance of this regulation in obesity has been pointed out by us(13) as well as others(14).

Summary. Dehydroepiandrosterone, a potent *in vitro* inhibitor of the enzyme glucose-6-phosphate dehydrogenase, produced a significant decrease in the activity of the glucose-6-phosphate dehydrogenase of the red blood cells of a normal adult man when administered orally in doses of 100 mg for 3 consecutive days.

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Tumor Induction in Thymectomized Rats by Murine Sarcoma Virus (Moloney) and Properties of the Induced Virus-free Tumor Cells. (32568)

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A murine sarcoma virus (MSV) isolated by Moloney from BALB/c mice inoculated with Moloney leukemogenic virus (MLV) induces tumors rapidly in most strains of newborn mice(1), but rarely induces tumors in intact newborn rats. Rat embryo cells in culture can be transformed by MSV and these transformed cells will induce tumor growth in young syngeneic hosts(2). Tumors have also been induced in Osborne-Mendel and Sprague-Dawley rats when injected intracerebrally at 3 days of age with MSV(3). It is known that thymectomy can influence the frequency of neoplasms induced by several tumor viruses (5,6). Thymectomized rats were therefore used to test their susceptibility to MSV. The biological properties of one of the rat tumors which does not produce infectious virus will be discussed here in detail.

Materials and methods. Rats. Inbred rat strains BN, Lewis and an Fl hybrid BN x Lewis obtained from Microbiological Associates, Walkersville, Md. were used.

Viruses. MSV, a tumor extract of BALB/c