Dehydroepiandrosterone Feeding and Protein Phosphorylation, Phosphatases, and Lipogenic Enzymes in Mouse Liver (43010)

MARIO MARRERO,*,† RUSSELL A. PROUGH,‡ RENE A. FRENKEL,§ AND LEON MILEWICH*,†

The Cecil H. and Ida Green Center for Reproductive Sciences and Departments of Obstetrics-Gynecology† and Biochemistry,§ The University of Texas Southwestern Medical Center at Dallas Dallas, Texas 75235 and the Department of Biochemistry ‡ School of Medicine, University of Louisville, Louisville, Kentucky 40292*

Abstract. **Dehydroepiandrosterone (DHEA) treatment is effective in preventing or delaying the onset of various genetic and induced disorders of mice and rats. Associated with the beneficial therapeutic effects exerted by action of this steroid is the development of hepatomegaly. To determine whether the changes associated with hepatomegaly also involve alterations in activities of tissue enzymes, we evaluated the effects of DHEA (0.45% in food, w/w) on hepatic protein kinases, phosphatases, and lipogenic enzymes in mice of various strains. The rates of fatty acid and cholesterol syntheses also were evaluated. DHEA administration resulted in profound changes in the sodium dodecylsulfate-polyacrylamide gel electrophoresis patterns of endogenous radiophosphorylated proteins obtained by incubation of liver homogenates with (γ-^{32}P]ATP. These changes were dependent upon the medium used for homogenization. Thus, when homogenates of liver tissue of DHEA-treated mice were prepared in Tris buffer containing sucrose (0.25 *M*) there was a marked decrease in phosphorylation of the proteins of relative molecular weight ~116,000 (M_r ~116,000), ~82,000, ~80,000, ~58,000, ~56,000, ~48,000, ~34,000, ~32,000, and ~31,000 compared with controls. With liver homogenates of DHEA-treated mice prepared in Tris buffer alone, there was a marked increase in phosphorylation of the proteins of M_r ~70,000, ~49,000, ~34,000, ~31,000, and 28,000 compared with controls. Moreover, the specific activity of kinases for endogenous protein acceptors in liver of control mice was higher than that in liver of DHEA-treated animals. The specific activities of casein kinase, cAMP-dependent protein kinase, and cGMP-dependent protein kinase remained unchanged with DHEA treatment, but the specific activity of histone kinase was increased approximately 30%. Long-term administration of DHEA also was associated with increases in the specific activities of liver AMPase and GTPase (approximately two times), but not of other nucleotidases, alkaline phosphatase, acid phosphatase, glucose-6-phosphatase, or phosphotyrosine phosphatase. The activity of hepatic NADP-linked malic enzyme was increased significantly (two to three times) by DHEA treatment of female mice of three different strains, but was unchanged in male C57BL/6 mice. The specific activities of hepatic glucose-6-phosphate dehydrogenase, NADP-linked isocitrate dehydrogenase, and ATP-citrate lyase were not affected significantly by DHEA treatment of mice. The rate of hepatic lipogenesis, determined by incorporation of tritium from ^3H_2O into fatty acids, was decreased approximately 70% in DHEA-treated mice, while the rate of cholesterol synthesis was increased approximately 44% compared with controls.**

[P.S.E.B.M. 1990, Vol 193]

Dehydroepiandrosterone (DHEA) treatment of mice and rats confers protection against diabetes (1), obesity (2–4), lupus (5), hemolytic anemia (6), spontaneous breast tumors (3), and chemically induced tumors (7–9). The administration of DHEA also results in decreased body weight gain (2, 10, 11), hepatomegaly (2, 10–12), lowered triglyceride synthesis in liver of mice that are destined genetically to become obese (2), inhibition of hepatic glucose-6-phosphate dehydrogenase (G6PD) activity in obese rats (11), and inhibition of G6PD activity and pentose phosphate production in isolated hepatocytes of DHEA-treated rats (13) compared with controls. Taken together, these observations have led to a hypothesis to

Received March 14, 1989. [P.S.E.B.M. 1990, Vol 193]
Accepted October 13, 1989.

0037-9727/90/1932-0110$2.00/0

explain the mode of action of DHEA that involves diminished hepatic lipogenesis due to inhibition of G6PD (2, 4) with a concomitant decrease in intracellular levels of NADPH, a cofactor that is required for fatty acid synthesis. This hypothesis, however, was challenged by Casazza *et al.* (14) who demonstrated that G6PD activity and the [$NADP^+$]/[NADPH] redox couple in liver of rats treated with DHEA were not changed significantly compared with controls, thus supporting the concept that NADPH levels in liver were not decreased. Moreover, these investigators did not find an antiweight effect with DHEA treatment. It should be emphasized that Casazza *et al.* (14) used a regimen of meal feeding of 3 hr/day in which the rats consumed an average of 13.9 g of food. With this regimen the rats were in a state of caloric deficiency and, thus, this study cannot be compared directly with those of other investigators in which animals were fed *ad libitum* (2, 4). At the present time it has not been possible to ascertain whether the molecular mechanisms by which DHEA acts to exert its beneficial therapeutic effects in mice and rats (1–9) are the same or even related. It seems reasonable to entertain the possibility that there are common biochemical phenomena that underlie the chemopreventive effects of DHEA.

Because DHEA is highly effective by oral administration, we assumed that livers of DHEA-treated animals are exposed to relatively high levels of this compound and that the beneficial effects are mediated, at least in part, by direct action of this steroid (or some of its metabolites) on the liver.

HL-60 human promyelocytic leukemia cells can be differentiated into cells with the mature myeloid phenotype by chemopreventive agents such as retinoic acid: changes in protein phosphorylation have been shown to be associated with this differentiation (15). We used such an approach to evaluate whether DHEA treatment of mice results in alterations of hepatic protein kinase activities toward endogenous and exogenous protein acceptors. In addition, to establish whether other enzymes involved in phosphate metabolism in liver are altered by DHEA action, we evaluated the activities of various phosphatases, including alkaline phosphatase, acid phosphatase, glucose-6-phosphatase, phosphotyrosine phosphatase, and various nucleotidases.

Whereas liver cytosolic malic enzyme is inducible *in vivo* by treatment of rats with DHEA (11, 13–17), there are conflicting reports in regard to the *in vivo* effects of DHEA on hepatic glucose-6-phosphate dehydrogenase (11, 13, 14, 18–21). To find out whether mice of various genetic backgrounds respond to DHEA with changes in the activities of cytosolic lipogenic enzymes, we determined G6PD, NADP-linked malic enzyme, NADP-linked isocitrate dehydrogenase, and ATP-citrate lyase. In addition, the rates of hepatic fatty acid and cholesterol syntheses in liver also were determined.

Materials and Methods

Chemicals. Acrylamide, *N,N'*-methylene-bis-acrylamide. *N,N,N',N'*-tetramethylethylenediamine, sodium dodecyl sulfate, molecular weight protein standards, Coomassie blue R-250, and protein assay dye reagent were purchased from Bio-Rad Laboratories (Richmond, CA). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Tritiated water (5 Ci/ml) was purchased from Amersham Corp. (Arlington Heights, IL). [γ-^{32}P]ATP was a gift from Dr. Melanie Cobb (University of Texas Southwestern Medical Center, Department of Pharmacology). Deionized double distilled water was used in the preparation of solutions.

Animals. Mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and kept at the Animal Resources Center of The University of Texas Southwestern Medical Center. (NZW × BXSB)F_1 mice were bred at the same animal facility. The animals were given food and water *ad libitum*. The diets consisted of either AIN-76A (ICN Biochemicals, Cleveland, OH) (control) or the same diet that was supplemented with DHEA (0.45%, w/w). In this study, mice were kept on either diet for various times, up to 7 months.

Subcellular Fractions. At selected times, mice were killed by cervical dislocation under ethyl ether anesthesia. Liver was removed, weighed, and homogenized at 4°C (with 5 ml of medium/g of tissue) in a Potter glass homogenizer fitted with a Teflon pestle. The homogenizing medium consisted of either Tris buffer (50 m*M*, pH 7.4) or Tris buffer (50 m*M*, pH 7.4) containing sucrose at a final concentration of 0.25 *M*. Freshly prepared liver homogenates were used in studies of endogenous and exogenous protein phosphorylation and for determination of phosphatase activities.

Homogenates were fractionated by differential centrifugation at 4°C first at 200*g* for 10 min to give "nuclear pellets." The 200*g* supernatant fractions were centrifuged at 8000*g* for 15 min to give "mitochondrial fractions." The supernatants were centrifuged at 14,000*g* for 15 min to give "lysosomal-light mitochondrial" fractions, and the supernatants were centrifuged at 105,000*g* for 1 hr to give "microsomal" and "cytosolic" fractions. Protein concentrations were determined according to the method of Bradford (22) using bovine serum albumin as standard.

Endogenous Protein Phosphorylation Determined by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Autoradiography. The effect of DHEA treatment on liver protein kinases was evaluated in female (NZB × NZW)F_1 mice by radiophosphorylation of endogenous liver proteins. Liver homogenates were prepared in either Tris buffer (50 m*M*, pH 7.4) or Tris buffer (50 m*M*, pH 7.4) containing sucrose (0.25*M*). The incubation mixture consisted of liver homogenate (10 μg of protein, $MgCl_2$ (10 m*M*), β-mercaptoethanol (5 m*M*), and [γ-^{32}P]ATP

(100 μM, 1 μCi) in a total volume of 110 μl. Incubations were conducted at 25°C for 5 min. The reactions were terminated by addition of Tris buffer (25 μl; 150 mM, pH 6.8) containing sodium dodecyl sulfate (3%), 2-mercaptoethanol (1 M), glycerol (15%), and bromophenol blue (0.1%) and by heating in boiling water for 5 min. Proteins were separated by SDS-PAGE on 10% polyacrylamide gels as described by Laemmli (23) and were stained with Coomassie blue. Thereafter, the gels were mounted on filter paper, dried *in vacuum*, and subjected to autoradiography (Kodak XR-2 film; Eastman, Rochester, NY) to visualize the radiophosphorylated proteins.

Protein Kinase Activities. The specific activities of protein kinases in liver of DHEA-treated and control mice were determined by use of endogenous and exogenous protein substrates as described by Fontana *et al.* (15), with slight variations. The reaction mixture used to study endogenous phosphorylation consisted of liver homogenate (10 μg of protein), Tris buffer (50 mM, pH 7.4), β-mercaptoethanol (5 mM), $MgCl_2$ (10 mM), and [γ-^{32}P]ATP (100 μM; 1 μCi) in a total volume of 110 μl. Cyclic AMP- and cGMP-dependent protein kinases were assayed in a total volume of 110 μl by use of the same mixture that also contained cAMP (10 μM) or cGMP (10 μM). Protein kinase C was assayed by use of the mixture described for endogenous phosphorylation that also contained $CaCl_2$ (1 mM), 1,2-diolein (5.9 mM), and phosphatidyl serine (55 mM) in a total volume of 110 μl. For assay of histone kinase, calf thymus histone type III-S (44 μg) was added to the mixture used in the study of endogenous phosphorylation and, for the assay of casein kinase, casein (440 μg) and KCl (0.1 M) were added to the mixture used to study endogenous phosphorylation in a total volume of 110 μl. All incubations were conducted at 25°C for 10 min either in duplicate or triplicate. The reaction mixtures (90-μl aliquots) were spotted on Whatman 3 MM filter paper disks (2.3 cm in diameter) and thereafter immersed in cold 10% trichloroacetic acid. The filters were washed three times in 5% trichloroacetic acid, twice in 95% ethanol, and, finally, air dried. The radioactivity associated with radiophosphorylated proteins were assessed by use of a Packard liquid scintillation spectrometer model 3300 after addition of scintillation fluid (ACS II; Amersham, Arlington Heights, IL).

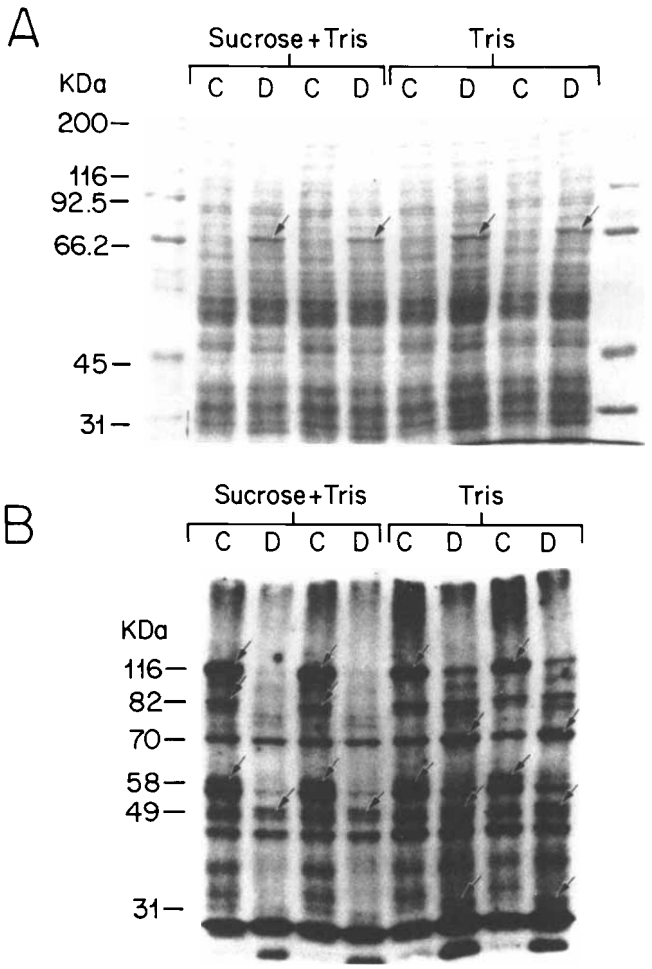

Figure 1. (A) Separation of liver proteins by SDS-PAGE. Female (NZB × NZW)F_1 hybrid mice (2 months old) were treated with DHEA (0.45% in food, w/w) for 6 months. Liver tissues were homogenized either in Tris buffer (50 mM, pH 7.4) containing sucrose (0.25 M) or Tris buffer alone (50 mM, pH 7.4). Incubations of liver homogenate (10 μg of protein), Tris buffer (50 mM, pH 7.4), $MgCl_2$ (10 mM), β-mercaptoethanol (5 mM), and [γ-^{32}P]ATP (100 μM, 1 μCi) in a total volume of 110 μl were conducted at 25°C for 5 min as described in Materials and Methods. The proteins were separated by SDS-PAGE on a 10% polyacrylamide gel and stained with Coomassie blue. The gel was mounted on filter paper and dried *in vacuo*. C, Control; D, DHEA treated. Note the striking increase in the concentration of the protein of M_r ~72,000 (indicated by the arrow) in liver of DHEA-fed animals. (B) Autoradiography of radiophosphorylated proteins obtained by use of the gel described in (A). Kodak XR-2 film was exposed to the gel for 48 hr with cooling.

Phosphatases. Phosphatase activities were determined in liver of DHEA-treated and control mice by use of the following substrates: AMP, ADP, ATP, GMP, GDP, GTP, *O*-phospho-L-tyrosine, glucose-6-phosphate, and *p*-nitrophenylphosphate, in a manner similar to that described (24). The incubation mixtures consisted of liver homogenates or subcellular fractions (prepared in either Tris buffer [50 mM, pH 7.4] or Tris buffer containing sucrose [0.25 M]), $MgCl_2$ (10 mM), KCl (0.1 M), β-mercaptoethanol (5 mM), and appropriate phosphate esters (5 mM). The incubations were conducted at 25°C for 15 min in a total volume of 1 ml, with shaking. Phosphate liberated in these reactions was measured according to the method of Fiske and Subbarow (25).

Lipogenic Enzymes. The specific activities of the lipogenic enzymes, G6PD, NADP-linked malic enzyme, NADP-linked isocitrate dehydrogenase, and ATP-citrate lyase were determined by use of cytosolic fractions prepared from liver of DHEA-treated and control mice. Enzyme activities were determined spectrophotometrically by linking the enzymatic reactions to the oxidation or reduction of pyridine nucleotides, as described (26–29). A Gilford model 240 recording spectrophotometer was used in these determinations.

Hepatic Lipogenesis. *De novo* syntheses of fatty

Table I. Effect of DHEA Treatment on Protein Kinase Activities of Mouse Liver[a]

Treatment	pmol ^{32}P incorporated/mg protein · min					
	Endogenous protein phosphorylation	cAMP-dependent protein kinase (endogenous proteins)	cGMP-dependent protein kinase (endogenous proteins)	Protein kinase C (endogenous proteins)	Histone kinase	Casein kinase
Control	375 ± 2	212	66	110	306	1060
DHEA	248 ± 16*	210	65	117	406	1002

[a] Female (NZW × BXSB)F_1 hybrid mice (2 months old) were treated for 3 months with either a control diet or a diet that contained DHEA (0.45%, w/w). Liver homogenates were prepared in Tris buffer containing sucrose (0.25 *M*) and protein kinase activities were determined as described in Materials and Methods. Average rates of ^{32}P incorporation from [γ-^{32}P]ATP (100 μM; 1 μCi) into protein obtained in duplicate determinations are given for all kinases except for endogenous protein kinase where the mean ± SD of triplicate determinations are presented. * $P < 0.001$.

Table II. Phosphatase Activities in Liver of DHEA-Treated DBA/2 Mice[a]

Phosphatase	Phosphate released (nmol/mg protein · min)			
	Tris buffer		Tris buffer plus sucrose	
	Control	DHEA	Control	DHEA
AMPase	65	199	88	197
ADPase	14	21	18	19
ATPase	45	40	35	33
GMPase	53	51	60	72
GDPase	35	36	24	24
GTPase	106	249	91	217
Acid phosphatase	92	82	69	74
Alkaline phosphatase	199	198	197	152
Glucose-6-phosphatase	17	26	17	15
Phosphotyrosine phosphatase	11	23	16	17

[a] Female DBA/2 mice (2 months old) were treated for 6 months with either the control diet or the same diet containing DHEA (0.45%, w/w). Liver tissue was homogenized either in Tris buffer or Tris buffer that contained sucrose (0.25 *M*), and phosphatase activities were determined as described in the text. The rates of inorganic phosphate released are the average values of duplicate determinations.

acids and cholesterol in liver of DHEA-treated and control female BALB/c mice were determined. In brief, the animals were placed under a tungsten lamp for approximately 40 min to dilate the tail veins of each animal. Heparin (10 IU) in phosphate-buffered saline solution (0.25 ml, pH 7.4) was injected intraperitoneally and mice were left under the tungsten lamp for another 15 min. Thereafter, tritiated water (250 μCi) in phosphate-buffered saline solution (0.5 ml, pH 7.4) was injected into one of the tail veins of each animal. One hour later the animals were killed under ethyl ether anesthesia, blood was collected into heparinized syringes to prepare plasma, and livers were excised and weighed. The livers and plasma were kept frozen at −70°C until the time of assay. Cholesterol was separated by digitonin fractionation after saponification of the chloroform-methanol-extracted lipids. The rates of fatty acid and cholesterol syntheses were determined as described by Lowenstein *et al.* (30).

Statistics. The data were analyzed by Student's *t* test.

Results

Endogenous Protein Phosphorylation Determined by SDS-PAGE and Autoradiography. The patterns of liver proteins of DHEA-treated female (NZB × NZW)F_1 mice obtained by SDS-PAGE and Coomassie blue staining were the same whether the homogenates were prepared in Tris buffer alone or in Tris buffer containing sucrose (0.25 *M*): there was a marked induction of a protein of relative molecular weight of approximately 72,000 (M_r ~72,000) (Fig. 1A and reference 10), which was identified as peroxisomal enoyl CoA hydratase/3-hydroxyacyl CoA dehydrogenase (31). The protein patterns in liver of control mice also were identical whether the homogenates were prepared in Tris buffer or Tris buffer containing sucrose (Fig. 1A). The action of DHEA, however, was manifested by profound differences in the patterns of the radiophosphorylated proteins obtained in incubations of liver homogenates with [γ-^{32}P]ATP (Fig. 1B). These differences were evident by either decreases or increases in the relative levels of radiophosphorylation, which depended upon the media used for homogenization. Thus, when homogenates were prepared in Tris buffer-sucrose, DHEA treatment resulted in a marked *decrease* in phosphorylation of the proteins of M_r ~116,000, ~82,000, ~80,000, ~58,000, ~56,000, ~48,000, ~34,000, ~32,000, and ~31,000 compared with controls (Fig. 1B) but, relative *increases* in the levels of the phosphorylated proteins of M_r ~70,000, ~49,000, ~34,000, ~31,000, and ~28,000 in liver of DHEA-treated mice compared with controls resulted when liver tissue homogenates were prepared in Tris buffer alone (Fig. 1B). The nature of the phosphorylated proteins has not been established.

Protein Kinase Activities.. The activity of hepatic protein kinases for endogenous protein acceptors was approximately 33% lower in female (NZB × NZW)F_1 mice treated with DHEA compared with control animals (300 vs 448 pmol of ^{32}P incorporated/mg protein ·

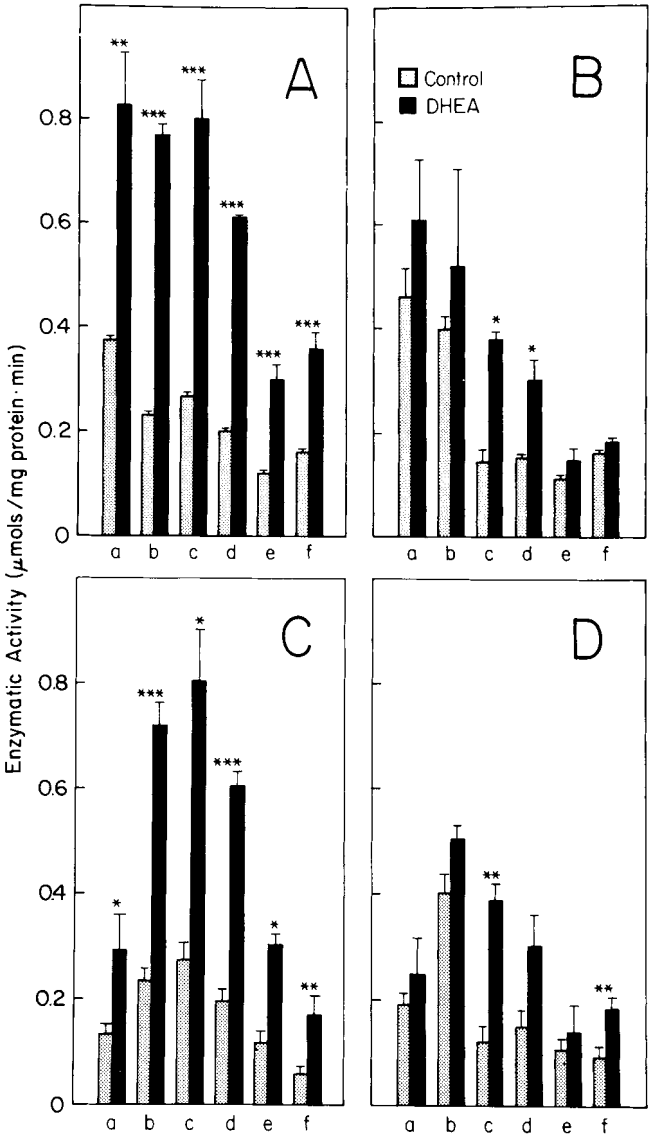

Figure 2. GTPase and AMPase activities in liver tissue. The specific activities of GTPase and AMPase were determined in homogenates and subcellular fractions prepared from liver tissue of male and female C57BL/6 mice treated with DHEA (0.45% in food, w/w) (three animals per group). DHEA feeding was commenced at 1 month of age and continued for 6 months. (A) GTPase in liver of female mice. (B) GTPase in liver of male mice. (C) AMPase in liver of female mice. (D) AMPase in liver of male mice. a, homogenate; b, nuclear pellet; c, mitochondria; d, lysosomes-light mitochondria; e, microsomes; f, cytosol. The mean ± SD are presented: * < 0.05, ** < 0.01, *** < 0.001.

min). This decrease in phosphorylation of endogenous proteins also became apparent by visual observation of the autoradiograms presented in Figure 1B (compare Lanes C [control] and D [DHEA] obtained with sucrose-containing homogenates). A decrease in phosphorylation of endogenous proteins of approximately 34% by DHEA treatment also was demonstrated in liver of female (NZW × BXSB)F_1 mice (Table I). There were no differences in the specific activities of cAMP-dependent protein kinase, cGMP-dependent protein kinase, protein kinase C, and casein kinase in liver of DHEA-treated and control mice, but the specific activity of histone kinase was increased approximately 33% by treatment with the steroid (Table I).

Table III. Effect of DHEA Treatment on GTPase Activity in Liver of Female (NZB × NZW)F_1 mice: Effects of Freezing and Thawing[a]

Liver homogenate	Phosphate released (nmol/mg protein·min)		
	Control	DHEA	*P*
Freshly prepared	422 ± 49	608 ± 44	<0.001
Frozen-thawed	281 ± 24	479 ± 96	<0.001

[a] Female (NZB × NZW)F_1 mice (2 months old) were treated with DHEA for 6 months. Liver tissue was homogenized in Tris buffer. GTPase activity was determined in freshly prepared homogenates and in frozen-thawed preparations (−20°C for 48 h). Liver tissues of five control animals and five animals treated with DHEA were used in this study. Values are mean ± SD.

Phosphatases. Treatment of female DBA/2 mice with DHEA for 6 months resulted in approximately two-fold increases in the activities of liver AMPase and GTPase when compared with controls (Table II). No differences in the activities of phosphotyrosine phosphatase, ADPase, ATPase, GMPase, GDPase, glucose-6-phosphatase, acid phosphatase, and alkaline phosphatase in liver of DHEA-treated mice were observed when compared with controls. Liver phosphatase activities were not affected in the presence of isotonic sucrose (0.25 *M*) (Table II). The specific activities of AMPase and GTPase also were increased significantly in liver of C57BL/6 mice treated with DHEA for 6 months: the relative increases in the activities of these phosphatases were demonstrated by use of homogenates and subcellular fractions of liver and were higher in female mice than in male mice compared with the corresponding controls (Fig. 2). Similar increases in the activities of AMPase and GTPase were obtained by use of homogenates prepared from liver obtained from female (NZB × NZW)F_1 mice that were treated with DHEA for 5 months (data not shown), but changes were not observed in liver of female (NZB × NZW)F_1 mice fed DHEA for 1 to 14 days (data not shown). The activity of GTPase in freshly prepared liver homogenates (female [NZB × NZW]F_1 mice) was compared with that in frozen-thawed homogenates: freezing and thawing resulted in a decrease in the specific activity of the enzyme of approximately 25% (Table III).

Lipogenic Enzymes. The administration of DHEA to female mice of the strains (NZB × NZW)F_1, (NZW × BXSB)F_1, and C57BL/6 resulted in a significant increase (two to three times) in the activity of hepatic NADP-linked malic enzyme, except for male C57BL/6 mice (Table IV). A slight decrease in specific activity of hepatic G6PD occurred with DHEA treatment of female (NZB × NZW)F_1 mice and female (NZW × BXSB)F_1 mice, and a slight increase was observed by treatment of male and female C57BL/6 mice (Table IV), but these changes were not significant.

Table IV. Lipogenic Enzymes in Liver of Mice Treated with DHEA[a]

Strain (sex)	Treatment	Specific activity (nmol/mg protein · min)			
		Malic enzyme (NADP linked)	Glucose-6-phosphate dehydrogenase	Isocitrate dehydrogenase (NADP linked)	ATP-citrate lyase
(NZB × NZW)F_1[b] (female)	Control	14.3 ± 5.2	11.1 ± 2.0	164 ± 22	—[c]
	DHEA	43.1 ± 2.2**	9.5 ± 1.0	190 ± 17	—
(NZW × BXSB)F_1[d] (female)	Control	12.1 ± 5.1	10.3 ± 2.2	148 ± 20	—[c]
	DHEA	28.1 ± 3.2**	8.7 ± 1.4	134 ± 19	—
C57BL/6[e] (female)	Control	25.3 ± 4.8	8.7 ± 1.3	154 ± 10	3.8 ± 0.4
	DHEA	47.8 ± 14.5*	11.1 ± 3.1	165 ± 41	3.9 ± 0.3
C57BL/6[e] (male)	Control	40.2 ± 6.2	9.1 ± 2.3	157 ± 8.0	4.0 ± 0.5
	DHEA	47.0 ± 3.7	11.9 ± 1.0	169 ± 32	4.0 ± 0.6

[a] Malic enzyme, glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, and ATP-citrate lyase were determined in supernatant fractions obtained by centrifugation at 105,000*g* for 1 hr as described in the text. The specific activities are expressed as the mean ± SD. * $P = 0.05$; ** $P < 0.001$.
[b] Mice ($n = 5$) were 1.5-month old at the initiation of DHEA treatment. The treatment was continued for 4.5 months.
[c] Not determined.
[d] Mice ($n = 5$) were 2 months old when started on DHEA treatment, which was administered for 3 months.
[e] Mice ($n = 4$) were 1-month old when started on DHEA treatment, which was continued for 6 months.

Table V. Rates of Fatty Acid and Cholesterol Syntheses in Liver of DHEA-Treated and Control Mice[a]

Treatment	Liver weight (g)	μmol tritium incorporated into fatty acids (g wet liver · hr)	μmol tritium incorporated into cholesterol (g wet liver · hr)
Control	0.93 ± 0.15	34.5 ± 7.78	0.84 ± 0.13
DHEA	1.13 ± 0.082	10.3 ± 2.54**	1.21 ± 0.16*

[a] Two-month-old female BALB/c mice were treated with DHEA for 4 months. Thereafter, the mice were given an intravenous bolus of ^3H_2O (250 μCi) and, after 1 hr, the animals were killed and blood and liver were collected as described in the text. The rates of hepatic fatty acid and cholesterol syntheses were determined as described by Lowenstein *et al.* (30). The mean ± SD of three animals per group are presented. * $P < 0.05$; ** $P < 0.01$.

The activity of NADP-linked isocitrate dehydrogenase in liver of DHEA-treated mice was slightly higher than that in controls except for female (NZW × BXSB)F_1 mice in which a small decrease in activity was observed (Table IV). The activity of ATP-citrate lyase remained unchanged with DHEA treatment in liver of both male and female C57BL/6 mice (Table IV).

Rates of Hepatic Fatty Acid and Cholesterol Syntheses. The rate of fatty acid synthesis in liver of female BALB/c mice, determined by incorporation of tritium from intravenously administered ^3H_2O, was reduced approximately 70% in mice treated with DHEA when compared with control animals. In the same study, the rate of hepatic cholesterol synthesis increased approximately 44% with DHEA treatment (Table V). Liver weights of DHEA-treated female BALB/c mice were slightly increased over those of control animals (Table V), and the color of the liver was much darker (mahogany) in DHEA-treated mice than in controls (data not shown) as demonstrated previously (10, 31).

Discussion

Since the discovery of the role of protein phosphorylation and dephosphorylation in the regulation of glycogen metabolism (32), endogenous protein phosphorylation has been shown to serve a key role in the regulation of a variety of cellular processes (e.g., 33–41). We identified changes in endogenous protein phosphorylation by kinases in liver of mice treated with DHEA, both by SDS-PAGE and biochemical determinations. The presence or absence of isotonic sucrose in the homogenization medium had an effect on the activity of protein kinases or on the availability of endogenous liver protein substrates, or both, as demonstrated by differences in the patterns of radiophosphorylated proteins. A possible explanation for this finding is that, depending on the homogenization medium, different proteins are exposed for interaction with cellular kinases; alternatively, it is possible that the kinases, which are originally present in defined cellular organelles, become available for reaction with proteins after disruption of these organelles by the action of hypotonic buffer (without sucrose). Thus, the sucrose effect possibly is a reflection of organelle integrity. Although the specific activity of protein kinases for endogenous proteins in liver of DHEA-treated mice was decreased approximately 33%, it is possible that the levels of phosphorylated proteins, determined with the methodology described in this study, may actually represent a composite of protein kinase and phosphatase activities.

The specific activity of hepatic G6PD in DHEA-treated mice of various genetic backgrounds was not changed significantly when compared with controls. It appeared, however, that the activity of this enzyme was

either decreased or increased slightly, depending on the strain of mice evaluated. Although it is not possible to ascertain the *in vivo* activity of G6PD by determinations conducted *in vitro* at V_{max} conditions, it is evident that G6PD levels in liver of lean rats (11, 14) and mice (this study) were not decreased significantly by treatment with DHEA and, thus, we would expect that hepatic lipogenesis would remain unchanged in these animals. Chronic treatment of female BALB/c mice with DHEA, however, resulted in a significant decrease of the rate of fatty acid synthesis of approximately 70% when compared with controls. Plasma triglyceride levels determined in mice of the same strain and sex have been shown previously to be markedly elevated with DHEA treatment when compared with controls (179 ± 24 mg/dl [mean ± SD] vs 93 ± 15 mg/dl) (42). These apparently contradictory findings may be explained by a possible decrease in uptake and hydrolysis of triglycerides at tissue sites other than the liver due to changes in lipoprotein lipase activity; this possibility, however, has not been investigated. In the present study, we also found that the rate of cholesterol synthesis in liver of DHEA-treated female BALB/c mice was increased approximately 44%, a finding that correlates well with increases in the levels of plasma cholesterol reported previously for animals of the same strain and sex (control, 52 ± 8 mg/dl (mean ± SD]; DHEA, 97 ± 11 mg/dl) (42).

A significant increase in the specific activity of hepatic NADP-linked malic enzyme was observed in DHEA-treated female mice of the various strains studied, a finding that is similar to that demonstrated previously in rats (4, 13, 14, 17). However, there was no significant increase in malic enzyme activity in liver of male C57BL/6 mice, where the basal level of this enzyme was considerably higher than that in liver of corresponding females. In this study we did not find significant changes in the specific activities of NADP-linked isocitrate dehydrogenase and ATP-citrate lyase in mouse liver.

GTPase is associated with intracellular membranes and AMPase has been reported to be present in plasma membranes and cytosols of eucaryotic cells (43, 44); the finding that these enzymatic activities are present in all subcellular fractions of liver, as demonstrated in this study, suggests cross-contamination of these fractions by membrane and/or cytosolic associated enzymes. The significance of the increases in AMPase and GTPase activities (approximately two times) induced by long-term DHEA treatment of mice is unknown.

We have demonstrated that treatment of mice with DHEA results in changes in the activities of liver protein kinase(s) for endogenous substrates, AMPase, GTPase malic enzyme, and in rates of fatty acid and cholesterol syntheses. It is possible that these changes in enzymatic activity play a role in some of the beneficial chemotherapeutic effects exerted by DHEA in susceptible mice and rats and, also, in the development of hepatomegaly. Identification of the phosphorylated liver proteins that are modulated by DHEA action may provide important insights into the mode of action of this steroid.

This investigation was supported in part by USPHS Grant UO1 CA43311.

1. Coleman DL, Leiter EH, Schwizer RW. Therapeutic effects of dehydroepiandrosterone (DHEA) in diabetic mice. Diabetes **31**:830–833, 1982.
2. Yen TT, Allan JA, Pearson DV, Acton JM, Greenberg MM. Prevention of obesity in A^{vy}/a mice by dehydroepiandrosterone. Lipids **12**:409–413, 1977.
3. Schwartz AG. Inhibition of spontaneous breast cancer formation in female C3H (A^{vy}/a) mice by long-term treatment with dehydroepiandrosterone. Cancer Res **39**:1129–1132, 1979.
4. Cleary MP, Seidenstat R, Tannen RH, Schwartz AG. The effect of dehydroepiandrosterone on adipose tissue cellularity in mice. Proc Soc Exp Med **171**:276–284, 1982.
5. Lucas JA, Ahmed SA, Casey ML, MacDonald PC. Prevention of autoantibody formation and prolonged survival in New Zealand Black/New Zealand White F_1 mice fed dehydroepiandrosterone. J Clin Invest **75**:2091–2093, 1985.
6. Tannen RH, Schwartz AG. Reduced weight gain and delay of Coombs positive hemolytic anemia in NZB mice treated with dehydroepiandrosterone (DHEA). Fed Proc **41**:463, 1982.
7. Nyce JW, Magee PN, Hard GC, Schwartz AG. Inhibition of 1,2-dimethylhydrazine-induced colon tumorogenesis in BALB/c mice by dehydroepiandrosterone. Carcinogenesis **5**:57–62, 1984.
8. Pashko LL, Harel GC, Rovito RJ, Williams JR, Sobel EL, Schwartz AG. Inhibition of 7,12-dimethylbenz[*a*]anthracene-induced skin papillomas and carcinomas by dehydroepiandrosterone and 3β-methylandrost-5-en-17-one in mice. Cancer Res **45**:164–166, 1985.
9. Schwartz AG, Tannen RH. Inhibition of 7,12-dimethylbenz[*a*] anthracene and urethan-induced lung tumor formation in A/J mice by long-term treatment with dehydroepiandrosterone. Carcinogenesis **2**:1335–1337, 1981.
10. Marrero M, Snyder JM, Prough RA, MacDonald PC, Milewich L. Influence of orally-administered dehydroisoandrosterone (DHA) on mouse liver protein synthesis and liver mitochondria morphology. The Endocrine Society Abstracts of Papers, 69th Annual Meeting, p208, 1987.
11. Shepherd A, Cleary MP. Metabolic alterations after dehydroepiandrosterone treatment of Zucker rats. Am J Physiol **246**:E123–E128, 1984.
12. Prassana HR, Schwartz A, Hacobian N, Magee PN. Effects of feeding dehydroepiandrosterone (DHEA) on the hepatic metabolism of 7,12-dimethylbenzanthracene (DMBA) in rats. Fed Proc **46**:2274, 1987.
13. Garcea R, Daino L, Frassetto S, Cozzolino P, Ruggiu ME, Vannini MG, Pascale R, Lenzerini L, Simile MM, Puddu M, Feo F. Reversal by ribo- and deoxyribonucleosides of dehydroepiandrosterone-induced inhibition of enzyme altered foci in the liver of rats subjected to the initiation-selection process of experimental carcinogenesis. Carcinogenesis **9**:931–938, 1988.
14. Casazza JP, Schaffer WT, Veech RL. The effect of dehydroepiandrosterone on liver metabolites. J Nutr **116**:304–310, 1986.
15. Fontana JA, Emler C, Ku K, McClung JK, Butcher FR, Durham JP. Cyclic AMP-dependent and -independent protein kinases and protein phosphorylation in human promyelocytic leukemia (HL-60) cells induced to differentiate by retinoic acid. J Cell Physiol **120**:49–60, 1984.
16. Tepperman HM, De La Garza SA, Tepperman J. Effect of

dehydroepiandrosterone and diet protein on liver enzymes and lipogenesis. Am J Physiol **214**:1126–1132, 1968.
17. Su C-H, Hardy H. Effect of dehydroepiandrosterone on mitochondrial glycerophosphate dehydrogenase and malic enzyme activities. FASEB J **2**:A581, 1988.
18. Willmer JS, Foster TS. The influence of dehydroepiandrosterone and pregnenolone upon adaptation of hepatic glucose-6-phosphate dehydrogenase to refeeding a high-glucose diet after fasting. J Biochem **43**:1375–1377, 1965.
19. Granholm NH, Staber LD, Wilkin PJ. Effects of dehydroepiandrosterone on obesity and glucose-6-phosphate dehydrogenase activity in the lethal yellow mouse (strain 129/Sv-A^y/A^w). J Exp Zool **244**:67–74, 1987.
20. Cleary MP, Zisk JF. Anti-obesity effect of two different levels of dehydroepiandrosterone in lean and obese middle-aged female Zucker rats. Int J Obes **13**:193–204, 1986.
21. Schwartz AG, Hard GC, Pashko LL, Abou-Ghorbia M, Swenn D. Dehydroepiandrosterone: An anti-obesity and anti-carcinogen agent. Nutr Cancer **3**:46–53, 1981.
22. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72**:248–254, 1976.
23. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**:680–685, 1970.
24. Motoyama N, Takimoto K, Okada M, Nakagawa H. Phosphotyrosine phosphatase: A novel phosphatase specific for phosphotyrosine, 2′-AMP and p-nitrophenylphosphate in rat brain. J Biochem **101**:939–947, 1987.
25. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. J Biol Chem **66**:375–400, 1925.
26. Stark MJ, Thompson B, Frenkel R. Possible alternative functions of rat liver malic enzyme. Arch Biochem Biophys **166**:174–180, 1976.
27. Glock GE, McLean P. Further studies on the properties and assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver. Biochem J **55**:401–408, 1953.
28. Kornberg A. Isocitric dehydrogenase of yeast (TPN). Methods Enzymol **1**:705–707, 1955.
29. Cottam GL, Srere PA. The sulfhydryl groups of citrate cleavage enzyme. Arch Biochem Biophys **130**:304–311, 1969.
30. Lowenstein JM, Brunengraber H, Wadke M. Measurement of rates of lipogenesis with deuterated and tritiated water. Methods Enzymol **34**:279–287, 1974.
31. Frenkel RA, Prough RA, Hicks SH, Milewich L. Dehydroepiandrosterone induces peroxisomal enzymes in rodent liver. J Cell Biol **107**:122a, 1988.
32. Krebs EG, Strell JT, England PJ, Hirazo TS, Bronton CO, Vandenheede JR. Protein Phosphorylation in Control Mechanisms. New York: Academic Press, pp31–45, 1973.
33. Greengard P. Phosphorylated proteins as physiological effectors. Science **199**:146–152, 1978.
34. Krebs EG, Beavo JA. Phosphorylation-dephosphorylation of enzymes. Annu Rev Biochem **48**:923–959, 1979.
35. Jungmann RA, Kranias EG. Nuclear phosphoprotein kinases and the regulation of gene transcription. Intl J Biochem **8**:819–830, 1977.
36. Levinson AD, Opperman H, Levintow L, Varmus HE, Bishop JM. Evidence that the transforming gene of avian sarcoma virus encodes a protein kinase associated with a phosphoprotein. Cell **15**:561–572, 1978.
37. Levinson AD, Oppermann H, Varmus HE, Bishop JM. The purified product of the transforming gene of avian sarcoma virus phosphorylates tyrosine. J Biol Chem **255**:11973–11980, 1980.
38. Hunter T, Sefton BM. Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. Proc Natl Acad Sci USA **77**:1311–1315, 1980.
39. Sefton BM, Beemon K, Hunter T. Comparison of the expression of the *src* gene of Rous sarcoma virus in vitro and in vivo. J Virol **28**:957–971, 1978.
40. Kramer G, Henderson AB, Pinphanichakarn P, Wallis MH, Hardesty B. Partial reaction of peptide initiation inhibited by phosphorylation of either initiation factor eIF-2 or 40S ribosomal proteins. Proc Natl Acad Sci USA **74**:1445–1449, 1977.
41. Levin DH, Ramu RS, Ernst V, London IM. Regulation of protein synthesis in reticulocyte lysates: phosphorylation of methionyl-t-RNA_f binding factor by protein kinase activity of translational inhibitor isolated from heme-deficient lysates. Proc Natl Acad Sci USA **73**:3112–3116, 1976.
42. Milewich L, Bennett M, Putman RS, Frenkel RA. Effects of dehydroepiandrosterone on hepatic lipogenic enzymes and plasma lipids in mice. J Cell Biol **107**:645a, 1988.
43. Itoh R. Purification and some properties of cytosol 5′-nucleotidase from rat liver. Biochim Biophys Acta **657**:402–410, 1981.
44. Headrick JP, Willis RJ. 5′-Nucleotidase activity and adenosine formation in stimulated, hypoxic and underperfused rat heart. Biochem J **261**:541–550, 1989.