Characterization of Serum Dehydroepiandrosterone Secretion in Golden Hamsters (44542)

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Abstract. **Dehydroepiandrosterone (DHEA) is an adrenal androgen whose function is poorly understood. Although DHEA and DHEA sulfate (DHEAS) are secreted in relatively high quantities by the human adrenal, the laboratory rat secretes very little, thus hindering experimental studies of the hormone. In this paper, we measured the changes in serum DHEA and DHEAS under various physiological conditions in golden hamsters. Evening serum DHEAS fell from 6.30 ± 0.78 μg/dl (mean ± SE) before surgery to 3.03 ± 0.23 μg/dl 12 days after bilateral adrenalectomy. Hamsters had higher levels of DHEA and DHEAS in the evening than in the morning, but removal of the gonads did not consistently decrease serum DHEA or DHEAS in males or females. Evening levels of DHEA and DHEAS reached a peak around 7 weeks of age and then gradually decreased to about one-third of these levels by one year of age. These results suggest that DHEA and DHEAS are secreted at least in part from the hamster adrenal, that they do not originate from the gonads, and that there is a daily rhythm with peak levels at a time of day just preceding the active phase. In addition, the levels of these hormones decrease with aging.** [P.S.E.B.M. 2000, Vol 224:278–284]

Dehydroepiandrosterone (DHEA) is a hormone secreted from the human adrenal gland in large quantities, and to a lesser extent, from the gonads. It is a weak androgen, but its physiological role is poorly understood (1–6). Most circulating DHEA comes from the conversion of dehydroepiandrosterone sulfate (DHEAS) in peripheral tissues (7–9). In most *in vitro* studies, DHEA is more active than DHEAS (1). On the other hand, DHEAS has a longer serum half-life and provides an indicator of DHEA activity (1, 2, 4, 7). DHEA and DHEAS secretion can be stimulated by ACTH, but other factors may also be involved in regulating their levels (10, 11).

Whereas DHEA and DHEAS are secreted in relatively high quantities by the human adrenal (1, 10), the laboratory rat secretes very little (12–14), thus hindering experimental studies of hormone secretion (15). The hamster adrenal is similar to the human in that it secretes cortisol as a major glucocorticoid (16–21), and hamster cytochrome P450C17 exhibits 17,20-lyase activity that catalyzes the formation of DHEA at the adrenal level (13, 14, 19).

One objective of the present work was to determine whether the hamster secretes quantifiable levels of serum DHEA and/or DHEAS that originate from the adrenal gland. We also studied the influence of time of day and the effect of castration and gonadal hormone replacement on hormone levels in male and female hamsters.

DHEA levels decline with age in humans (3, 20–24), and this has been correlated with an increase in the incidence of various disease conditions including cancer (25), cardiovascular disease (1, 26, 27), and autoimmune disease (28). In an additional experiment described here, we studied the influence of age on DHEA and DHEAS levels in hamsters.

Materials and Methods

General Methods. All protocols and procedures in this study were approved by the Institutional Animal Care and Use Committee and are consistent with the U.S. Public Health Service and National Institutes of Health policies and guidelines.

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Golden hamsters (*Mesocricetus auratus*, LAK:LVG), obtained from Charles River Laboratories, were used in these experiments. They were maintained on an LD 14:10-hr light:dark cycle (lights on 0200–1600 hr for Experiments 1 and 4 and 0600–2000 hr for Experiments 2 and 3). All animals were maintained in polycarbonate cages with wood-chip bedding and were fed *ad libitum*. Blood samples were obtained from the retro-orbital sinus (0.5 ml) under light carbon dioxide anesthesia. The samples were obtained within 2 min of disturbing the animals to minimize any influence from the stress of handling.

Protocol Experiment 1. Ten 12-week-old male hamsters arrived from Charles River and were individually housed. Two weeks later the animals were given drinking water containing 1% saline and 5% sucrose and maintained on this formula for the duration of the study. One week later, all animals were bled within 30 min of lights out. Adrenalectomy was done in two stages to minimize trauma and increase survival. Four days after obtaining the blood samples, all animals were anesthetized with sodium pentobarbital (35 mg/kg ip), and the right adrenal gland was surgically removed using a lateral approach. Three days later, the left adrenal was excised using a similar procedure. Twelve days after both adrenals had been removed, all animals were again bled from the retro-orbital sinus. Five of the animals died of adrenal insufficiency before the end of the experiment so the results represent data from five animals.

Protocol Experiment 2. This experiment involved 30 male hamsters that were 9 weeks old on arrival, and they were individually housed. One week later, 20 animals were bilaterally castrated under sodium pentobarbital anesthesia (35 mg/kg ip) whereas the other 10 received sham surgery. One-half of the castrated hamsters were implanted with a blank silastic capsule (0.078′ i.d. × 0.125′ o.d.), whereas the other half were implanted with a similar capsule filled with 20 mm of testosterone as previously described (29).

Starting 1 week later, all animals were bled from the retro-orbital sinus once per week for 9 weeks under light carbon dioxide anesthesia. The samples were taken at a different time of the day each week to determine the diurnal rhythm in hormone secretion.

Protocol Experiment 3. The protocol for this experiment was identical to the one for Experiment 2 except that female hamsters were bilaterally ovariectomized, and the steroid replacement group was implanted with silastic capsules (0.078′ i.d. × 0.125′ o.d.) containing 10 mm of estradiol 17β as previously described (30).

Protocol Experiment 4. Eighteen male hamsters arrived from Charles River at 23 days old and were group housed in polycarbonate cages on an LD 14:10 photoperiod as described above. They were bled at approximately monthly intervals starting at 26 days of age through 432 days of age. All samples were taken within 30 min before lights out (time of peak in serum DHEAS, see Fig. 2B). One hamster died at 285 days of age. Between Day 314 and the end of the study, animals died at a rate of one per month so there were 11 animals for the last sample on Day 432 of age.

Hormone Levels. Serum DHEA levels were assessed using a double-antibody RIA kit from Diagnostic Systems Laboratories, Inc. (Webster, TX). The sensitivity (minimal detectable level) was 31 pmol/ml, and the average intra-assay coefficient of variation was 7.8%. All samples for each experiment were done in one assay. The cross-reactivity with DHEAS, isoandrosterone, and androstenedione was 0.02%, 0.73%, and 0.46%, respectively.

Serum DHEAS was measured with a Coat-A-Count RIA kit from Diagnostic Products Corporation (Los Angeles, CA). The lowest standard in the kit was diluted 1:2 to obtain a 67.8 nmol/l calibrator (≈ 87% binding). The sensitivity was 29.8 nmol/l, and the average intra-assay coefficient of variation was 7.4%. The cross-reactivity with DHEA, estrone-3-sulfate, testosterone, and androstenedione was 0.08%, 0.56%, 0.10%, and 0.12%, respectively.

Total serum testosterone was determined using a Coat-A-Count direct RIA kit (Diagnostic Products, Los Angeles, CA) as previously described (29). The sensitivity of the testosterone assay was 140 pmol/l, and the intra-assay coefficient of variation was 6.4%.

Total serum corticosterone was assessed using a double-antibody RIA kit for rats and mice from ICN Biomedicals, Inc. (Costa Mesa, CA). Serum samples were diluted 1:20 with the steroid diluent provided in the kit. The percentage cross-reactivity with desoxycorticosterone, testosterone, and cortisol was 0.34%, 0.10%, and 0.05%, respectively. The intra-assay coefficient of variation was 5.2%, and the sensitivity was 72 pmol/l.

Total serum cortisol was measured using a double-antibody radioimmunoassay kit from Diagnostic Systems Laboratories (Webster, TX). The sensitivity was 3.04 nmol/l, and the intra-assay coefficient of variation was 6.7%. The cross-reactivity with prednisolone, corticosterone, and 11-deoxycortisol was 33.3%, 9.36%, and 3.8%, respectively.

Serum adrenocorticotropic hormone (ACTH) was assessed using Diagnostic Products Corporation's double-antibody kit. The sensitivity was 8 pg/ml (1.76 pmol/l), and the intra-assay coefficient of variation was 11.3%. The standard curve of this human assay kit was parallel to serially diluted hamster serum.

Statistics. Data from Experiment 1 were analyzed by paired *t* test. Data from Experiments 2 and 3 were analyzed by multivariate analysis of variance (MANOVA) for repeated measures. Where significance was indicated using this test, the data were analyzed *post hoc* with Student-Newman-Keuls Multiple Comparisons test to determine which groups differed. The change in hormone levels with age (Experiment 4) were analyzed with MANOVA and *post hoc* analysis with Student-Newman-Keuls Multiple Comparisons Test. Data from Experiment 4 were also analyzed by simple regression. $P < 0.05$ was considered significant for all analyses.

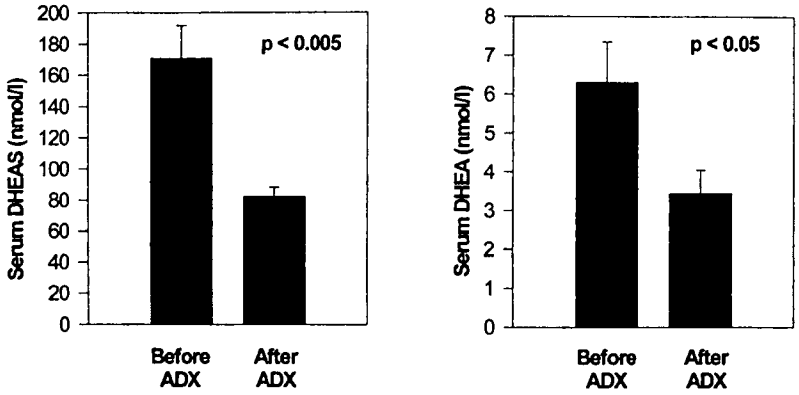

Figure 1. Serum DHEA and DHEAS before and 12 days following bilateral adrenalectomy of five hamsters. Bars and vertical lines represent mean ± SE.

Results

Experiment 1. There was a marked decrease in serum DHEA and DHEAS 12 days after adrenalectomy (Fig. 1), but there was still a measurable amount of both hormones in the serum.

Experiment 2. In male hamsters, serum testosterone was very low in the castrate group, but the testosterone capsules elevated serum levels to a physiologic range, close to those of the control group (Fig. 2C).

The sham control group exhibited an obvious daily rhythm in serum DHEA and DHEAS (Figs. 2A & 2B). Compared with 1200 or 1700 hr levels, DHEA values were elevated starting at 1800 hr, and the levels of DHEAS were higher starting at 2000 hr. The castrated hamsters without testosterone had higher DHEAS levels at 0800, 1200, 1700, 1800 and especially at 2000 hr than the control group (Fig. 2B), but testosterone-replaced animals were similar to the control group except at 1700 and 1800 hr (Fig. 2B). Serum DHEA was similar in the three groups except at 2000 and 2100 hr when the castrate group had lower levels than the controls (Fig. 2A).

In the sham control group, serum cortisol and corticosterone were elevated in the evening, starting at 1800 hr and declining by 2200 hr (Figs. 3A & 3B). The surge in serum cortisol resulted in much higher levels than the surge in serum corticosterone. The evening surge in both hormones was inhibited by castration but was similar to control animals if they were replaced with testosterone. Serum ACTH was also elevated in the evening in the control group starting at 1600 hr and falling to baseline values by 2200 hr (Fig. 3C). There was no consistent effect of castration or testosterone on serum ACTH.

Experiment 3. Serum DHEA was similar in the three groups of female hamsters except at 1200 and 2400 hr when the ovariectomized animals had higher levels than the control group (Fig. 4). Serum DHEAS was higher in the ovariectomized animals than in the control group at 1200 hr and 2400 hr, and lower in the ovariectomized animals replaced with estradiol than the control group at 1600, 1800, and 2000 hr. Serum DHEA and DHEAS exhibited a daily rhythm in the female hamsters with peak levels at 1800 hr for DHEA and 1600 hr for DHEAS.

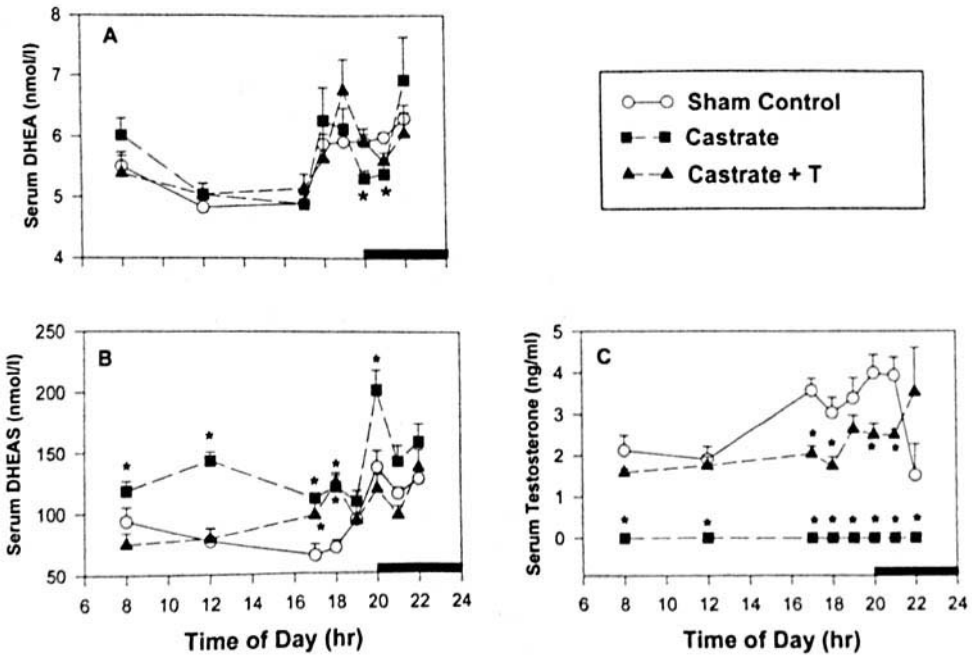

Figure 2. Serum (A) DHEA, (B) DHEAS, and (C) testosterone in male hamsters that were either castrated alone or castrated and implanted with a 20-mm silastic capsule of testosterone, and in control hamsters undergoing sham surgery. There were 10 animals per group, and they were bled weekly at various times of the day. The filled horizontal bar represents the dark period. *$P < 0.05$ compared with the control group at the same time point.

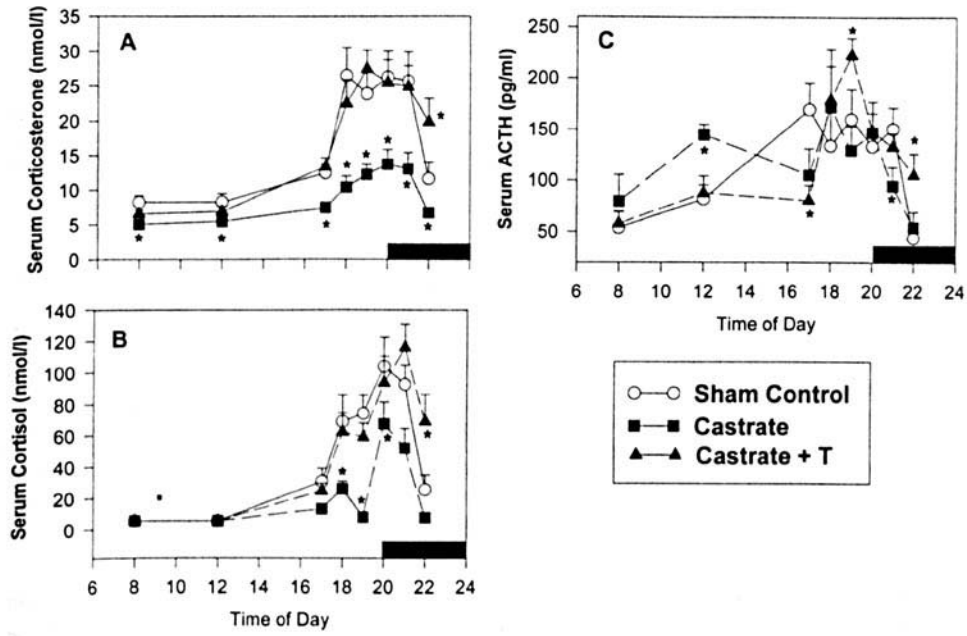

Figure 3. Serum (A) corticosterone, (B) cortisol, and (C) ACTH in the same animals as in Figure 2. See Figure 2 for further details.

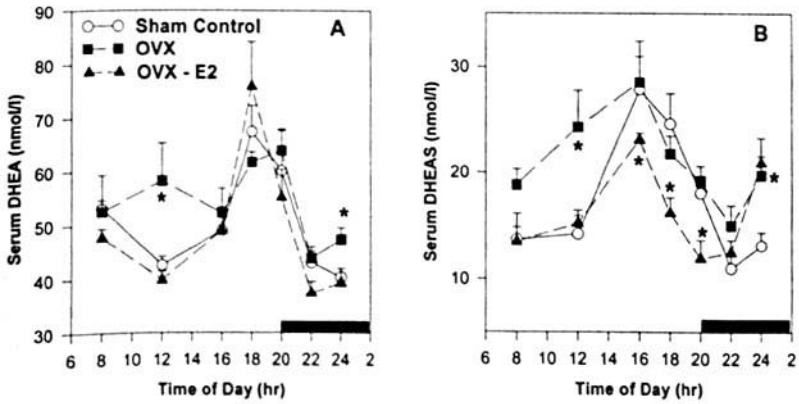

Figure 4. Serum (A) DHEA and DHEAS (B) in female hamsters that were either ovariectomized alone or ovariectomized and implanted with estradiol and in control hamsters undergoing sham surgery. There were 10 animals per group, and they were bled weekly at various times of the day. The filled horizontal bar represents the dark period. $^*P < 0.05$ compared with the control group at the same time point.

Experiment 4. Serum DHEA reached peak levels at the Day 52 sample time and then gradually decreased through the first year of life of these animals (Fig. 5A). MANOVA for repeated measures indicated an effect of age at $P < 0.001$ and *post hoc* analysis with the Student-Newman-Keuls test indicated all the other samples were different from the Day 52 sample: Day 141 at $P < 0.05$ and all other sampling times at $P < 0.01$. A regression analysis of the values from Day 52 to the end of the study indicated a linear relationship of hormone concentration and age with a correlation coefficient of –0.85.

Serum DHEAS also exhibited peak levels at 52 days of age and then gradually decreased for 6 months before leveling off at about 8 months of age (Fig. 5A). MANOVA for repeated measures indicated an effect of age at $P < 0.001$ and *post hoc* analysis with the Student-Newman-Keuls test indicated all the other samples were different from the Day 52 sample: Days 72, 107, and 141 at $P < 0.05$ and the other time points at $P < 0.01$. Regression analysis of the values from Day 52 to Day 285 indicated a linear relationship of hormone concentration and age with a correlation coefficient of –0.92.

Serum cortisol increased with age and reached peak levels at 197–314 days of age before declining. MANOVA for repeated measures indicated an effect of age at $P < 0.001$ (Fig. 5B). There was no influence of age on serum corticosterone levels (Fig. 5B).

Discussion

The results of Experiment 1 showing that the levels of DHEA and DHEAS decrease by about 50% after adrenalectomy indicate that a large percentage of the plasma levels of these hormones originate from the adrenal gland. It appears unlikely that a large percentage of the DHEA/DHEAS remaining after adrenalectomy originated from the gonads, since castration resulted in no change in serum DHEA (Fig. 2A) and an increase in serum DHEAS (Fig. 2B). The adrenal glands of this species are encapsulated, and we believe the organs were completely removed in the adrenalectomy group. We therefore propose that the DHEA/DHEAS re-

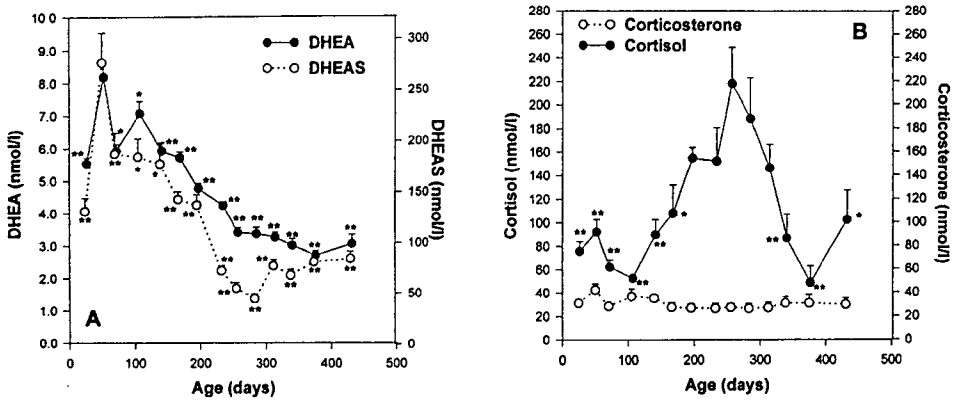

Figure 5. (A) Serum DHEA and DHEAS at various ages in 18 male hamsters in samples obtained within the 30 min preceding lights out. Note that DHEAS and DHEA are represented with different scale so that even though DHEAS is present in much higher concentrations in the serum, the plots look similar. Beginning after Day 314, animals began dying at the rate of one per month so there was an *n* of 11 for the last sample. $^{*}P < 0.05$; $^{**}P < 0.01$ compared with the Day 52 sample. (B) Serum cortisol and corticosterone levels at different ages in the same hamsters described in (A). MANOVA for repeated measures indicated a change of serum cortisol over time ($P < 0.001$). $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the Day 257 sample. SE bars for corticosterone were within the circles.

maining in the serum after bilateral adrenalectomy originated from an unknown extra-adrenal tissue.

The levels of DHEAS reported for the serum of men are 30–50 times higher than we measured in male hamsters (20, 23, 31). Our assay was not able to detect measurable levels of DHEAS in serum from laboratory rats (unpublished observation), but Baulieu and Robel (12, 32) have reported values ≈ 1/100th of those we report here for hamsters. Although nonhuman primates have higher levels of DHEAS than most other species, the levels are still much lower than humans (33). DHEA and/or DHEAS levels have been reported for many other species including guinea pigs, rabbits, dogs, sheep, pigs, goats, horses, cows, and chickens (34–38). Of these species, the levels are highest for rabbits and dogs, but most of the DHEA and DHEAS in these animals is eliminated following castration (35, 36).

The diurnal rhythm in serum DHEA and DHEAS in male (Figs. 2A & 2B) and female (Figs. 4A & 4B) hamsters was similar in the three conditions tested (intact, castrate, and castrate plus steroid replacement) with peak levels occurring around the time of the surge in glucocorticoids (Figs. 3A & 3B) and ACTH (Fig. 3C). The results with respect to glucocorticoids are consistent with the literature, and the peak is near the time of lights off when these nocturnal animals start exhibiting an increase in locomotor activity (16, 18). The peak levels of serum DHEAS seemed to occur a few hours earlier in female than male hamsters (Figs. 2B & 4B). Serum DHEA and DHEAS levels are highest in men (11, 39) and women (39, 40) in the morning hours which is also near the time of maximal adrenal glucocorticoid secretion and the onset of locomotor activity. An evening increase in plasma DHEAS has also been reported in rats (41). Conversion of DHEA/DHEAS to testosterone may account for some of the nocturnal rise in serum testosterone levels (Fig. 2C).

Mean serum DHEA levels were much higher in female than male hamsters at all times of the day, whereas mean serum DHEAS was higher in males (compare data of Fig. 2 with Fig. 4). This pattern is similar to humans where men have higher DHEAS levels (1, 20, 23, 42), and women have higher levels of DHEA (42). Stage of the estrous cycle may have influenced individual differences in adrenal androgen secretion in the female hamsters, but the data were the mean from animals at random stages of the cycle.

The level of serum DHEA and DHEAS in castrated hamsters was at least as high as for intact animals suggesting that the testes did not contribute a large amount of these hormones or precursors to the circulating levels. The effect of ovariectomy was analogous to male castration in that serum DHEA and DHEAS were at least as high as the intact controls (Fig. 4), suggesting that the hormones are not derived from the ovaries. Exogenous estradiol decreased the magnitude of the evening surge of DHEAS (but not DHEA) compared with the nonreplaced animals (Fig. 4). Although the assays measured the total hormone in the serum, it is possible that the gonadal steroids altered metabolism of DHEA and DHEAS by affecting enzymes involved in the degradation of the hormones or by influencing serum-binding protein levels, thus making more available for degradation. Gaskin and Kitay (43) did studies on the influence of gonadal hormones on adrenocortical function in golden hamsters and reported that gonadectomy decreased adrenal weight in males and females and decreased glucocorticoid secretion in males.

The changes in evening levels of DHEA and DHEAS with age (Fig. 5A) seem to be somewhat analogous to those reported for men and women (3, 20, 22, 23, 44). There was an increase in the levels around the time of reproductive maturation compared with prepubertal animals, and then the levels gradually decreased for the next 3–4 months. As for humans, part of the decrease may be related to the decline in overall health that is associated with aging. In hamsters, serum cortisol levels peaked at middle age, and there was no change in serum corticosterone with age (Fig. 5). These results are consistent with those reported by Ottenweller *et al.* (17) in hamsters bled around noon. Similarly, there is

also no large decrease in serum glucocorticoids with age in humans (45, 46). It is possible that at least part of the decline in DHEA and DHEAS levels with age in our study was due to a change in the time of day peak levels occur (47); however, we have also examined 8 AM levels (lights on 0600–2000 hr) in male hamsters, and these values also declined with age (Pieper DR and Lobocki CA, unpublished data).

An interesting, serendipitous finding in the present work, unrelated to the DHEA results, was that castration inhibited the evening surge of glucocorticoids (Figs. 3A & 3B) in male hamsters. This was especially true for serum corticosterone. The levels returned to normal when the animals were implanted with testosterone, suggesting that this hormone enhances the evening glucocorticoid surge in these animals.

The present paper is the first to characterize serum DHEA and DHEAS levels in the hamster except that we have recently reported that DHEA and DHEAS decrease with exercise in this species (59). It is also the first report of a fall in DHEA levels with age in rodents. The regulation of adrenal steroid secretion in hamsters may be more similar to primates than the rat in that hamsters secrete easily measurable quantities of DHEA and DHEAS that increase at about the same time as the glucocorticoids and decrease with age.

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