models support this hypothesis. DHEA and synthetic analogues of DHEA have been proposed as possible chemoprotective agents in selected groups.

A nested case-control study was conducted using serum samples from a population-based serum bank to examine the association between serum DHEA and DHEAS levels and the risk of developing breast cancer. Blood samples were collected from 20,305 Washington County, MD residents. Incident breast cancer cases were identified through the Washington County Register. Fifteen premenopausal and 30 postmenopausal breast cancer cases were identified and matched to two controls by age and time since last menstrual period. Controls were alive and free of other cancers except for nonmelanoma skin cancer at the time the case was diagnosed. Serum steroid levels were determined by radioimmunoassays (RIAs). The associations between DHEA and DHEAS levels and the risk of developing prostate, bladder, and gastric cancers have also been examined.

Prediagnostic serum levels of DHEA were lower in women who developed premenopausal cancer compared to the controls, while postmenopausal cases had significantly higher prediagnostic levels of DHEA than the controls. DHEAS levels were not significantly different between cases and controls in either group. These associations between DHEA and the risk of developing breast cancer did not vary by time to diagnosis. This discrepancy between the results for premenopausal and postmenopausal women may result from the contribution of these steroids to the synthesis of estrogens in postmenopausal women. In similar nested case-control studies, we have shown low levels of DHEA to be a risk factor for subsequent bladder and gastric cancer, but not for prostate or ovarian cancer.

These associations between serum levels of DHEA and DHEAS and the risk of developing specific cancers should be considered in the decision to employ DHEA or a synthetic analogue in selected high-risk populations.

Chemoprevention of Rat Mammary Carcinogenesis: Exceptional Activity of Dietary Dehydroepiandrosterone (DHEA)

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Abstract A major determinant of progress in human breast cancer prevention is the identification of agents with significant anticarcinogenic activity and acceptable levels of toxicity in experimental animals. Over the past 20 years, more than 50 experimental regimens have been shown to have significant chemopreventive activity in the rat mammary gland. The most effective approaches to mammary cancer chemoprevention in rats involve surgical endocrine ablations such as bilateral ovariectomy. However, prophylactic surgical ablations are unlikely to be acceptable to the majority of the general public. All chemicals evaluated to date are less effective, and none has been shown to reduce mammary cancer incidence to zero. As a result, efforts continue to identify chemical agents whose protective activity is comparable to that of endocrine ablation. DHEA is an adrenal steroid with chemopreventive activity in several animal models for human cancer. In the present studies, the chemopreventive efficacy of DHEA was evaluated in rats exposed to the mammary gland carcinogen, *N*-methyl-*N*-nitrosourea (MNU). Groups of 20 female Sprague-Dawley rats were fed an AIN-76A diet supplemented with 0, 400, or 800 mg DHEA per kg diet; one week later, all rats received a single i.p. injection of 35 mg MNU per kg body weight. Animals were palpated weekly to monitor mammary tumor development, and all mammary tumors were histologically confirmed. When administered at 800 mg/kg diet, DHEA reduced

mammary cancer incidence in controls from 95% to 15%; carcinoma multiplicity in rats receiving 800 mg DHEA per kg diet was reduced by more than 85% from control levels. In a separate study, the 400 mg/kg diet dose of DHEA reduced the incidence of mammary cancer to 5% from 80% found in controls fed the basal diet. Reductions in mammary cancer incidence and multiplicity associated with DHEA administration were accompanied by large increases in cancer latency. Evaluation of mammary gland wholemounts from animals fed DHEA demonstrated a massive induction of lobuloalveolar differentiation. These results indicate the dietary supplementation with non-toxic dose levels of DHEA has chemopreventive efficacy approaching that of endocrine ablation. This protection may be mediated by the induction of differentiation in the mammary gland, during which sensitive mammary parenchymal structures (terminal end buds) are stimulated to develop into structures (alveolar buds) less sensitive to carcinogenic insult.

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Trapping of Genes Induced Upon Growth Arrest After Treatment With Antiestrogen or Retinoids Using Retroviral Promoter Trap

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Abstract Although chemopreventive anti-steroids such as the antiestrogens are thought to act through competitive inhibition of agonist binding to estrogen receptors, it has been postulated that the estrogen receptor changes its conformation when bound to a strong antiestrogen such as ICI-164,384. We hypothesized that such conformationally changed receptors could bind specific recognition sequences in the genome and activate specific genes that might be involved in growth arrest. In order to identify such genes with a functional assay, we used a retroviral gene trap U3*lac*Z. We have now isolated MCF-7 breast cancer cell line clones in which the *lac*Z reporter gene is inserted into the genes activated by either ICI-164,384 or retinoic acid. One such clone, B4, was further characterized. In B4, *lac*Z activity is induced by ICI-164,384 or *trans*-retinoic acid, and repressed after treatment with estradiol. Cloning of the 5'-flanking genomic sequence in this clone will be possible using polymerase chain reaction.

High Resolution Image Cytometry for Quantitative Assessment of Ductal Carcinoma *In Situ* of the Breast

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