

Adrenalectomy Does Not Block the Inhibition of Mammary Carcinogenesis by Dietary Energy Restriction in Rats¹

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ABSTRACT Dietary energy restriction (DER) has been shown to reproducibly inhibit chemically induced mammary carcinogenesis. The inhibitory activity of DER has been reported to be associated with an increase in circulating corticosterone as well as a decrease in insulin-like growth factor 1 (IGF-1). To determine whether the adrenal glands are required for cancer inhibitory activity, the effects of DER were investigated in adrenalectomized (ADX) rats. Female Sprague-Dawley rats, 29–31 per group, were injected with 0.05 g 1-methyl-1-nitrosourea/kg body wt at 21 d of age, sham operated (SHAM) or bilaterally ADX at 24 d of age, and after 3 d adapted to meal feeding during which rats ate ad libitum (AL) or were restricted to 60% of AL energy intake. ADX resulted in a marked reduction in serum corticosterone in both AL and DER rats. Whereas the carcinogenic response in the mammary gland was not statistically different in SHAM-AL and ADX-AL rats, ADX did not block the cancer inhibitory activity of DER. In fact, cancer inhibitory activity was greatest in ADX-DER rats. Circulating levels of glucose, insulin, IGF-1, and IGF binding protein 3 also were reduced in DER rats. Collectively, these findings indicate that adrenal glands are not required for manifestation of the cancer inhibitory activity of DER. If circulation-borne factors such as corticosterone or IGF-1 are involved in the inhibition of mammary carcinogenesis by DER, IGF-1 is likely to play a greater role than corticosterone. *J. Nutr.* 134: 1152–1156, 2004.

KEY WORDS: • adrenalectomy • corticosterone • energy restriction • mammary carcinogenesis
• insulin-like growth factor

Dietary energy restriction (DER)³ inhibits the process of experimentally induced mammary carcinogenesis. Inhibition is accompanied by an increase in adrenal cortical steroid secretion as reflected by increases in both serum corticosterone and urinary cortical steroid excretion (1,2). As early as 1949 Boutwell and co-workers proposed that the protective effects of food restriction against experimentally induced cancer could be explained, at least in part, by hypertrophy of the adrenal glands (3). Since that time, there have been only a few tests of the original adrenal hypothesis in lung or skin carcinogenesis (4,5), and to our knowledge the effects of adrenalectomy on inhibitory activity of either food restriction or DER against mammary carcinogenesis have not been investigated.

Recent reports showed that besides the effects of DER on adrenal activity, concomitant changes occur in the synthesis of other factors that regulate glucose metabolism. Among the changes that accompany DER, the decrease in levels of insulin-like growth factor 1 (IGF-1) is particularly significant because reductions in circulating concentrations of this growth factor have been associated with a lower risk for cancer at

several sites including the breast (6,7). Consequently, in the absence of an experimental manipulation, it has not been possible in vivo to dissociate the effects of DER on adrenal cortical steroid secretion from those effects associated with changes in circulating IGF-1.

The primary objective of the experiments reported in this study was to determine how adrenalectomy would affect the inhibitory activity of DER against mammary carcinogenesis because increases in circulating corticosterone have been hypothesized to account for DER's cancer inhibitory activity. Moreover, because there is little information about the effects of adrenalectomy on levels of IGF-1 or its binding proteins during DER, this was also evaluated. As progress was made in investigating these effects, our analyses were expanded to ascertain how DER in combination with adrenalectomy affected other indicators of glucose homeostasis.

MATERIALS AND METHODS

Carcinogen administration, adrenalectomy, and diets. One hundred thirty-five female Sprague-Dawley rats were obtained from Taconic Farms at 20 d of age. At 21 d of age, rats were injected with 0.05 g 1-methyl-1-nitrosourea/kg body wt (i.p.) as previously described (8). For the first week of the study, rats were housed 3 per cage in solid-bottom polycarbonate cages equipped with a food cup. Thereafter, rats were individually housed for the duration of the study. Animal rooms were maintained at 22 ± 1°C with 50% relative humidity and a 12-h light/12-h dark cycle. The work reported was reviewed and approved by the Institutional Animal Care and Use Committee and conducted according to the committee guidelines.

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³ Abbreviations used: ADX, adrenalectomy; AL, ad libitum; DER, dietary energy restriction; DPC, days postcarcinogen; IGF-1, insulin-like growth factor 1; IGFBP, insulin-like growth factor binding protein; SHAM, sham-operated; TBS, Tris-buffered saline.

Four days following carcinogen injection, all rats were randomized into two groups: 76 rats were adrenalectomized (ADX) and 59 rats were sham-operated (SHAM). A larger number of rats were ADX in the event of unsuccessful surgery or the occurrence of surgery-associated mortality. No deaths occurred in sham-operated rats. Thirteen rats undergoing adrenalectomy died during surgery or due to postsurgical complications. Three rats were found to have adrenal tissue at necropsy and were eliminated from the study. Rats were administered liquid cherry-flavor children's Tylenol (1.0 g/L) for 24 h after surgery. All adrenalectomized rats were given 0.9% (wt:v) saline drinking water beginning 24 h after surgery and continuing for the duration of the study. All SHAM rats received deionized water during the study. Beginning at 7 d postcarcinogen (DPC), all rats within SHAM or ADX groups were randomized into two groups: rats either ate ad libitum (AL) or were fed 60% of the AL energy intake to make four groups in total, i.e., SHAM-AL, SHAM-DER, ADX-AL, and ADX-DER.

From 8 DPC, all rats were meal fed and given 2 meals/day (0600–0900 and 1400–1700), 7 d/wk, in order to avoid possible confounding due to intergroup variation of meal timing, meal number, and duration of food deprivation between meals. DER was started at 9 DPC with 10% DER, increased to 20% DER on the next day (10 DPC), and finally increased to 40% DER on 11 DPC. The level of 40% DER was maintained for the remainder of the study.

Rats in the AL-fed groups were allowed access to an unlimited amount of food at each meal while rats in the DER groups were given a restricted amount of their food at each meal. A modified AIN-93G diet (Table 1) and feeding protocol were used as previously described (1). The diet fed to 40% DER rats was formulated to ensure an intake of all nutrients equivalent to that of the respective control group, SHAM-AL or ADX-AL, while limiting total dietary energy by reducing carbohydrates. All rats were weighed 3 times/wk starting from 7 DPC. Rats were palpated for detection of mammary tumors 2 times/wk starting from 19 DPC.

Necropsy. Rats were killed between 56 and 58 DPC. An equal number of rats in each group were killed each day and a stratification procedure was used to ensure a similar distribution of the time (0900–1100) at which rats were killed in each group. Rats were killed via inhalation of gaseous carbon dioxide. After the rats lost consciousness blood was directly obtained from the retro-orbital sinus and gravity fed through heparinized capillary tubes (Fisher Scientific) into EDTA-coated tubes (Becton-Dickinson) for plasma or plastic tubes for serum. The bleeding procedure took ~1 min/rat and therefore reduced the likelihood that circulating corticosterone concentrations increased artifactually because these procedures can stimulate adrenocorticotrophic hormone release. Plasma or serum was isolated by centrifugation at $1000 \times g$ for 10 min at room temperature.

TABLE 1

Composition of diets

Component	AL	40% DER
	<i>g/kg</i>	
Cornstarch	325.0	208.4
Cerelose	325.0	208.04
Solka-Floc	50.0	83.3
Casein	200.0	333.3
DL-Methionine	3.0	5.0
Corn oil	50.0	83.3
AIN-93 G vitamin mix ¹	10.0	16.7
AIN-93 G mineral mix ¹	35.0	58.3
Choline bitartrate	2.0	3.3
Total	1000.0	1000.0
Energy, kJ/g	16.50	16.33
Protein, % energy	0.21	0.35
Carbohydrates, % energy	0.68	0.46
Fat, % energy	0.11	0.19

¹ The composition of the vitamin and mineral mixes is given in ref. (22).

Following blood collection and cervical dislocation, rats were then skinned and the skin was examined under translucent light. All grossly detectable mammary gland lesions were excised and processed for histopathological classification (9). Rats were also examined for completeness of the ADX procedure.

Assessment of circulating glucose, insulin, insulin-like growth factor 1, and corticosterone. Serum glucose was determined by the hexokinase/glucose-6-phosphate dehydrogenase method developed by the American Association of Clinical Chemistry and Centers for Disease Control. Briefly, 3.0 μ L of serum was mixed with 300 μ L glucose hexokinase reagent (ThermoDNA), incubated for 3 min at 37°C, and read at 340 nm. Serum insulin was determined by a commercial rat/mouse insulin ELISA kit (Linco Research) using rat insulin as the standard in the assay. Plasma IGF-1 was determined by a commercial rat IGF-1 enzyme immunoassay kit (Diagnostic Systems Laboratories) using the rat IGF-1 standards and controls in the assay. Serum corticosterone was determined by a commercial corticosterone enzyme immunoassay kit (Diagnostic Systems Laboratories) using the rat corticosterone standards and controls in the assay.

Assessment of circulating insulin-like growth factor binding proteins (IGFBPs). Serum IGFBPs were determined by Western ligand blotting using biotinyl-IGF-1 (GroPep). Two and one-half microliters of serum was run on a 12% Tris-glycine gel (Invitrogen Life Technologies) using the XCell Mini-Cell system (Novex Electrophoresis) under nondenaturing and nonreducing conditions. For ligand blots, proteins were transferred to a nitrocellulose membrane. Membranes were washed with Tris-buffered saline (TBS) containing 1% (v:v) Tween-20 for 30 min at room temperature and preblocked with 1% (v:v) bovine serum albumin in TBS containing 0.1% (v:v) Tween-20 overnight before incubation with 2 μ g of GroPep's biotinyl-IGF-1 in TBS for 90 min at room temperature. After sequential washes in TBS containing 0.1% (v:v) Tween-20, membranes were incubated in 1:1000 dilution of avidin-horseradish peroxidase [in TBS-0.1% (v:v) Tween-20] for 45 min at room temperature. After the membrane was developed with enhanced chemiluminescence reagent it was exposed to film. Digital images were captured from film using a Scanjet scanner (Hewlett-Packard) and Image Pro Plus software (Media Cybernetics) was used to quantify the signal intensity of the bands.

Statistical analyses. Differences among groups in cancer incidence were evaluated by χ^2 analysis (10). Differences among groups in the number of mammary carcinomas per rat (multiplicity) were evaluated by analysis of variance after square root transformation of the count data as recommended in ref. (11). Differences among groups in cancer latency were evaluated by the Mantel-Haenszel life table procedure (12). Circulating levels of glucose, insulin, IGF-1, and corticosterone were analyzed by ANOVA, and post hoc comparisons were made using the Bonferroni multiple-range test (10). Data derived from Western ligand blot analyses represent semiquantitative estimates of the amount of IGFBPs in serum. This fact was taken into account in the statistical evaluation of the data. The data are reported as means \pm SEM of the actual scanning units derived from the densitometric analysis of each Western ligand blot. However, for statistical analyses, the units of scanning density derived from the analysis of the Western ligand blots using Image Pro Plus were first ranked. This approach is particularly suitable for semiquantitative measurements that are collected as continuously distributed data and has the advantage of maintaining the relative relationships among data being compared without giving undue weight to outlying results. The ranked data were then subjected to multivariate analysis of variance (13). Statistically, this is a robust approach that takes into account the fact that levels of IGFBPs may not vary independently of one another, as well as the issues that exist when multiple comparisons are done using a particular set of data.

RESULTS

Body weight. As intended, DER significantly reduced the rate of body weight gain (data not shown) and final body weights, in the absence of weight loss, in both SHAM and ADX rats ($P < 0.001$, Table 2). However, rates of body weight gain or final body weights did not differ between SHAM-AL

TABLE 2

Effect of ADX and DER on the final incidence and multiplicity of mammary carcinomas and on final body weight in rats^{1,2}

Treatments	n	Cancer incidence	Carcinomas	Body weight
		%	n/rat	g
SHAM-AL	29	89.7 (26) ^a	2.4 ± 0.3 ^a	191 ± 2 ^a
SHAM-DER	30	40.0 (12) ^b	0.47 ± 0.1 ^b	139 ± 1 ^b
ADX-AL	31	93.6 (29) ^a	3.3 ± 0.4 ^a	192 ± 3 ^a
ADX-DER	29	17.2 (5) ^c	0.17 ± 0.1 ^c	136 ± 1 ^b

¹ Values are means ± SEM or % (number of rats with tumors). Values in columns without a common letter differ, $P < 0.05$.

² The incidence data were evaluated by χ^2 analysis. The multiplicity data, after square root transformation of the count data, and the body weight were evaluated by ANOVA and the Bonferroni multiple-range test.

and ADX-AL rats or between SHAM-DER and ADX-DER rats.

Cancer incidence, multiplicity, and latency. All grossly visible mammary lesions excised at necropsy were histopathologically classified, and only those lesions diagnosed as carcinomas were included in the data analysis; <5% of excised lesions were benign mammary pathologies, i.e., fibroadenomas or adenomas. Cancer latency was delayed by DER in both SHAM and ADX rats in comparison to AL rats that were either SHAM or ADX ($P < 0.01$, Fig. 1). In ADX rats, there was a trend ($P = 0.285$) for an earlier onset of carcinoma occurrence and an increased tumorigenic response in AL rats. On the other hand, ADX-DER rats tended to have a slower onset of tumor occurrence ($P = 0.052$) and had fewer palpable carcinomas per rat than SHAM-DER rats ($P = 0.046$). DER significantly reduced the incidence and multiplicity of mammary carcinomas (Table 2). The reduction in final cancer incidence attributed to DER was 55 and 82% in SHAM and ADX rats, respectively ($P < 0.001$). The final number of carcinomas per rat was reduced by 81% in SHAM rats and 95% in ADX rats ($P < 0.001$). The final incidence ($P = 0.054$) and multiplicity ($P = 0.038$) of carcinomas were lower in ADX-DER rats than in SHAM-DER rats. The incidence and multiplicity of cancer tended to be higher in ADX-AL than in SHAM-AL rats ($P = 0.151$).

Circulating glucose and related hormones. DER reduced the level of food-deprived serum glucose by 52% ($P < 0.001$) in SHAM-DER rats compared with SHAM-AL rats (Table 3). The level of food-deprived serum glucose was also decreased 60% in ADX-AL and ADX-DER compared with SHAM-AL rats ($P < 0.001$). DER reduced the level of serum insulin in SHAM-DER rats by 75% ($P = 0.009$) compared with SHAM-AL rats. The level of serum insulin was also decreased by 62% ($P = 0.034$) in ADX-AL rats and 84% ($P = 0.004$) in ADX-DER compared with SHAM-AL rats.

Plasma IGF-1 was lower ($P < 0.0001$) by 59% in SHAM-DER and 60% in ADX-DER rats compared to AL rats. Compared to SHAM rats, the plasma IGF-1 was lower in both AL ($P < 0.05$) and DER ADX rats by 17%. ADX dramatically reduced serum corticosterone; it was only 15% in AL rats and 6% in DER rats compared with SHAM rats. DER increased the level of serum corticosterone in SHAM rats by 100% ($P < 0.01$) and did not affect it in ADX rats.

Circulating IGF binding proteins. DER reduced the level of serum IGFBP-3 by 63% ($P < 0.001$) in SHAM-DER rats compared with SHAM-AL rats and by 51% ($P < 0.01$) in

ADX-DER rats compared with ADX-AL rats (Table 4). The level of serum IGFBP-3 was also decreased in ADX-AL rats by 48% ($P < 0.01$) and in ADX-DER rats by 31% ($P < 0.01$) compared with their SHAM controls. The changes in all other serum IGFBPs (30 and 24 kDa) showed a similar pattern of response. Dietary energy restriction reduced the level of these serum IGFBPs in SHAM-DER rats compared with SHAM-AL rats and in ADX-DER rats compared with ADX-AL rats ($P < 0.01$), while ADX enhanced the serum IGFBPs of 30 kDa in ADX-DER rats compared to SHAM-DER rats ($P < 0.01$).

DISCUSSION

Although the cancer inhibitory activity of DER is well established, the mechanisms by which the carcinogenic process is repressed have not been fully elucidated (14). In 1949 Boutwell et al. observed that the adrenal glands of food-restricted rats were enlarged relative to ad libitum controls in

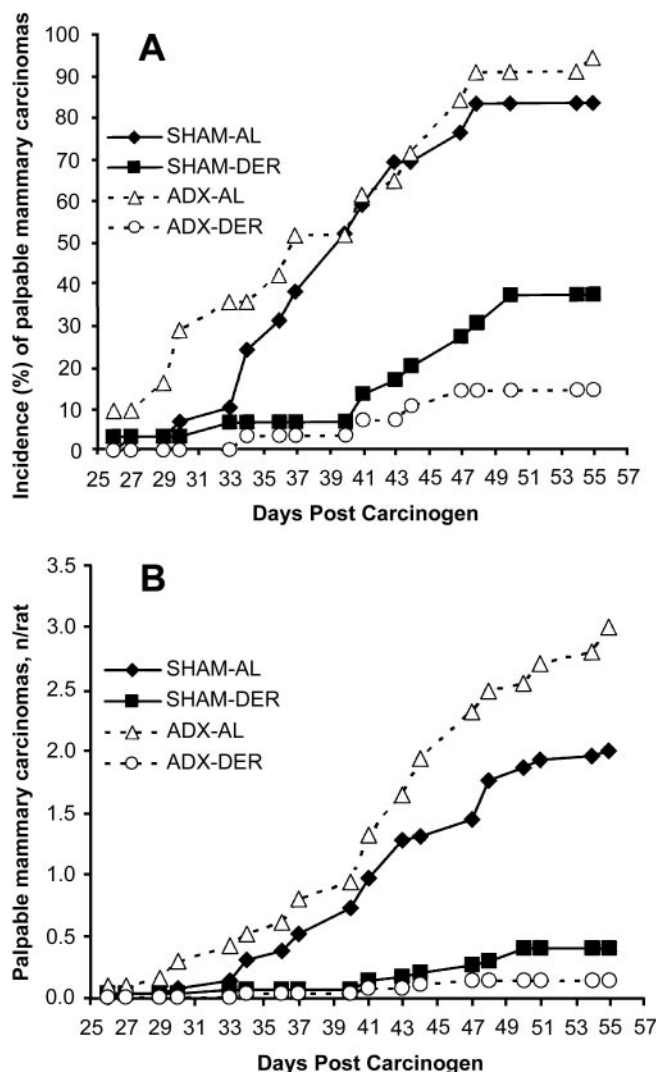


FIGURE 1 Effects of DER and/or ADX on palpable mammary adenocarcinomas. (A) Cumulative tumor incidence; (B) cumulative tumor multiplicity. The number of rats was 29, 30, 31, and 29 in the SHAM-AL, SHAM-DER, ADX-AL, or ADX-DER groups, respectively. Differences among groups in latency were evaluated by a life table procedure. DER was associated with the prolongation of cancer latency ($P < 0.01$).

TABLE 3

Effect of ADX and DER on circulating glucose, insulin, IGF-1, and corticosterone in rats^{1,2}

	SHAM-AL	SHAM-DER	ADX-AL	ADX-DER
<i>n</i>	29	30	31	29
Serum glucose, <i>mmol/L</i>	7.027 ± 0.329 ^a	3.409 ± 0.124 ^b	2.859 ± 0.117 ^b	2.823 ± 0.098 ^b
Serum insulin, <i>nmol/L</i>	0.484 ± 0.138 ^a	0.127 ± 0.020 ^b	0.185 ± 0.038 ^b	0.076 ± 0.008 ^b
Plasma IGF-1, <i>nmol/L</i>	55.9 ± 1.5 ^a	22.8 ± 1.3 ^b	46.2 ± 2.1 ^c	18.9 ± 1.1 ^b
Serum corticosterone, <i>nmol/L</i>	733 ± 30 ^a	1460 ± 61 ^b	107 ± 12 ^c	92 ± 11 ^c

¹ Values are means ± SEM. Values in rows without a common letter differ, *P* < 0.05.

² Data were analyzed by ANOVA and the Bonferroni multiple-range test.

an experimental model for breast cancer that was inhibited by food restriction. This led to the conjecture that the adrenal glands were involved in conferring protection against mammary cancer. Subsequent work demonstrated that adrenalectomy significantly increased both mammary tumor size and number (15), a finding that is consistent with the numerical differences in the carcinogenic response observed between SHAM-AL and ADX-AL groups (Table 2 and Fig. 1), although these differences were not statistically significant in this model system. However, the hypothesized relationship between DER-mediated protection against mammary cancer and the adrenal gland received only limited attention until recently. In a series of papers, our laboratory has reported evidence, albeit indirect, that adrenal cortical steroid secretion is stimulated by DER and that elevated levels of serum corticosterone are likely to account, at least in part, for the protective activity against cancer that is observed in response to DER (1,16–18). Consequently, we predicted that ADX would abolish the cancer inhibitory activity of DER in the proof-in-principle experiment reported in Table 2 and Figure 1 as it has been reported to do in food restriction in mouse model systems for skin cancer and lung cancer (4,5). However, contrary to expectation, ADX in DER-treated rats failed to alter the inhibitory activity of energy restriction (Table 2 and Fig. 1). While the reasons for this unanticipated observation are not immediately apparent, this surprising finding offers the opportunity to gain new insights about the mechanism(s) that underlies the cancer inhibitory activity of DER.

Two factors that could potentially confound the interpretation of the carcinogenesis data reported in this study are incomplete adrenalectomy or unplanned differences among groups in body weight gain. However, we confirmed the completeness of adrenalectomy at necropsy and eliminated 3 rats from the experiment that had adrenal tissue present. The serum corticosterone data presented in Table 3 provide further evidence that adrenalectomies were complete and resulted in

a dramatic reduction in circulating levels of corticosterone. The final body weights shown in Table 2 indicate that our feeding protocol resulted in essentially identical growth in AL rats that were intact (SHAM) or ADX as well as in the two DER groups (SHAM and ADX). Thus, the only differences in growth were those intentionally induced by DER.

As noted above, there are two reports that ADX abolished the protective effects of food restriction in model systems for lung cancer and skin cancer (4,5). However, a careful inspection of that work reveals a number of important differences in the experimental design and endpoints measured in those papers relative to that reported in this study. They include the investigation of food restriction (a reduction in total amount of food rather than only energy) in refs. (4,5) versus DER; the use of mice (4,5) versus rats; differences among experiments in the timing of carcinogen administration, adrenalectomy and the imposition of energy restriction; the observation of weight loss and significant differences in final body weights among groups in refs. (4,5) versus the absence of weight loss or differences in weight gain in this study; and the assessment of benign lesions, papillomas, and adenomas in refs. (4,5) versus using cancer as an endpoint in this study. This final point is of particular interest because we have shown that DER has differential effects on the occurrence of premalignant versus malignant mammary pathologies (1). Nonetheless, it is not possible to know whether any of these differences account for the apparently contradictory findings reported in refs. (4,5) versus the results reported here.

The hypothesis underlying the experiment reported in this study was that ADX would abolish the cancer inhibitory activity of DER. While we had intended to follow this experiment with another to demonstrate that glucocorticoid administration to ADX rats would restore the cancer inhibitory activity of DER, the findings reported did not warrant this approach. Nonetheless, it is important to emphasize that the adrenal glands are involved in glucose homeostasis and also

TABLE 4

Effect of ADX and DER on circulating levels of IGF binding proteins in rats^{1,2}

	SHAM-AL	SHAM-DER	ADX-AL	ADX-DER
<i>n</i>	29	30	31	29
	Scan unit (<i>pixel/cm</i>)			
IGFBP-3	0.807 ± 0.084 ^a	0.295 ± 0.030 ^b	0.416 ± 0.036 ^c	0.206 ± 0.032 ^d
IGFBPs of 30 kDa	0.457 ± 0.046 ^a	0.133 ± 0.014 ^b	0.529 ± 0.065 ^a	0.244 ± 0.029 ^c
IGFBPs of 24 kDa	0.023 ± 0.002 ^a	0.011 ± 0.001 ^b	0.024 ± 0.002 ^a	0.016 ± 0.002 ^b

¹ Values are means ± SEM. Values in rows without common letters differ, *P* < 0.05.

² Data were ranked and analyzed by ANOVA and the Bonferroni multiple-range test.

secrete several other factors, e.g., mineral corticoids and epinephrine, and thus the removal of these glands has effects that go beyond the observed reduction in circulating levels of glucocorticoids per se. Thus, the data support the conclusion that the adrenal glands are not required for mammary cancer inhibition by DER, but they do not contradict the recent report that high levels of corticosterone can inhibit mammary carcinogenesis when the adrenal glands are present (17).

In an effort to gain further insights into the effects of ADX in DER-treated rats, we analyzed plasma for IGF-1 since our recently published working hypothesis is that protection against cancer is the result of concomitant and reciprocal changes in circulating levels of corticosterone and IGF-1 (2,14). Consistent with our previous observations, in SHAM operated rats DER caused a reduction (59%) in levels of plasma IGF-1 while inducing approximately a twofold increase in serum corticosterone (Table 3). Interestingly, levels of plasma IGF-1 were reduced to a similar extent in ADX-DER, although serum corticosterone levels were very low. Taken at face value, these data are consistent with the interpretation that the reduction in plasma IGF-1 by DER is involved in cancer inhibition as has been hypothesized by others (19,20). Because the serum IGF binding proteins, particularly IGFBP3, bind IGF-1 and decrease its biological activity (7), and effects of DER on serum levels of IGFBPs during carcinogenesis have not been reported in rodents, levels of serum IGFBPs were assessed. Given that an ELISA is not currently available for measuring these binding proteins in the rat, we adapted the Western ligand blotting technique. Although a limitation with any Western blot assay is the semiquantitative nature of the data obtained, we judge the results shown in Table 4 to be very informative. Levels of serum IGFBPs were reduced to a similar extent by DER in both SHAM and ADX rats in comparison to SHAM-AL rats. Thus it appears that either DER or DER in combination with ADX reduced the absolute amount of IGF-1 in circulation, and this effect was not offset by differential changes in the levels of serum IGFBP3 or the other IGFBPs that were measured. Again, these effects were consistent with reduced availability of plasma IGF-1 in accounting for the cancer inhibitory effects of DER.

Our analyses also included the assessment of food-deprived levels of serum glucose and insulin (Table 3). Serum glucose levels were markedly reduced by both DER and ADX, a finding that was consistent with expectation, and the serum levels of insulin changed in a manner similar to the response observed for IGF-1, also as expected. However, what was surprising was that a robust carcinogenic response was observed in ADX-AL rats despite reduced levels of serum glucose and insulin. It will be important to determine the mechanisms by which carcinogenesis proceeds in rats that are ADX-AL versus ADX-DER since it is known that a hallmark of malignant transformation is a shift in metabolism that favors glycolysis for ATP production and consequently increases the requirement for glucose, i.e., the Walberg effect (21). Answers to such questions will only be obtained by studying effects occurring within the mammary gland and in premalignant and malignant pathologies. Such studies will undoubtedly benefit from the use of laser capture microdissection in order to investigate specific changes within epithelial and/or stromal cells resident in the mammary gland and mammary pathologies.

In summary, the results of this study failed to support the hypothesis that the adrenal gland is required for the cancer

inhibitory activity of DER. Moreover, because ADX effectively eliminated the increase in serum corticosterone that is usually induced by DER, but DER still inhibited carcinogenesis in the ADX rats, our findings do not support an obligatory relationship between serum corticosterone and the cancer inhibitory activity of DER. On the other hand, a dramatic reduction in circulating levels of IGF-1 and IGFBP3 was observed in DER-treated rats, and this effect was not altered by ADX. Thus, if circulation-borne factors such as corticosterone or IGF-1 are involved in the inhibition of mammary carcinogenesis by DER, IGF-1 is likely to play a greater role than corticosterone.

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