Hormone Profiles in Hormone-dependent Cancers

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SUMMARY

Studies on the relationship of urinary excretion of androgen metabolites and estrogens to the natural history of breast cancer are reviewed. The importance of distinguishing between "within-population" studies (i.e., cancer patients versus normal controls) and "between population" studies (i.e., low-risk versus high-risk populations) is emphasized, and it is pointed out that "qualitative" agreement (i.e., the same direction of differences) between the two types of studies must be present in order to implicate a hormonal parameter as a determinant of the natural history of breast cancer. For reasons detailed in this paper, it is concluded that the reported relationship of low urinary androgen metabolite excretion to increased risk of developing breast cancer and poor response to adrenalectomy or hypophysectomy and the validity of the "estriol hypothesis," namely, that a high urinary ratio of estriol to estrone-plus-estradiol in early life is protective against subsequent development of breast cancer, are both dubious. A new hypothesis concerning the relationship of estrogens to breast cancer risk is presented: "A period of time, prior to age 30, during which the amount or biological availability of active estrogens (i.e., estrone and estradiol) is diminished, protects against subsequent development of cancer." This hypothesis is shown to be compatible with the epidemiological and biochemical data.

Reports concerning the influence of nutrition on endocrine parameters are reviewed. Inanition and obesity have been shown to alter steroid metabolism but it is not known whether nutritional "microdifferences" (i.e., differences between populations or individuals that are due to cultural, geographic, or socioeconomic factors, but that fall within the range of "normal" or adequate nutrition) can also alter steroid metabolism.

INTRODUCTION

Certain cancers, notably those of the breast, prostate, and endometrium, are described as hormone dependent on the basis of 2 findings: (a) that they arise from tissues that are normally responsive to endogenous hormones, and (b) that their course can often be influenced, favorably or unfavorably, by administration or removal of hormones, either through ablation of endocrine glands by surgery or radiation or through administration of antihormones (e.g., administration of estrogens or androgens in breast cancer; administration of estrogens in prostate cancer; administration of progestagens in endometrial cancer; orchiectomy for prostate cancer and male breast cancer; oophorectomy, adrenalectomy, and hypophysectomy for breast cancer; administration of antiandrogens in prostate cancer; administration of antiestrogens in breast cancer). Because of the therapeutic effect of alterations in the hormonal environment, considerable interest has developed in the possibility that the amounts or patterns of the secretion of endogenous hormones are related to either the induction or the untreated clinical course of such cancers. This approach has stimulated a large body of work which I should like to review for you today.

The basic methodology for studying the role of hormones in hormone-dependent cancer is what might be called "biochemical epidemiology," i.e., the cross-sectional study of hormonal differences between those who do and those who do not have a particular type of cancer. There are 2 ways in which such biochemical epidemiology can be applied: (a) by comparing hormone findings between populations, one of which has a low incidence of the cancer at issue, while the other has a high incidence (e.g., in the case of breast cancer, Chinese or Japanese women in Asia and Caucasian women in North America, respectively), and (b) within a population, by comparing hormonal findings of individuals who have the cancer at issue with those of individuals who do not (i.e., the "case-control" method). The distinction between these 2 approaches has not always been kept clearly in sight, but it is an important one; in order to implicate a particular endocrine parameter as a determining factor in a particular cancer, it would seem essential that the between-population and within-population comparisons of that parameter should yield the same directional or qualitative differences.

Since the overall topic of the present symposium is "Nutrition in the Causation of Cancer," it is clearly incumbent upon us to try to correlate nutritional differences with hormonal differences. In this connection, it might be pertinent to subdivide nutritional differences into 2 categories: (a) "microdifferences," comprising those differences between populations or individuals that are determined by cultural, geographic, or socioeconomic factors but are still within the overall range of normal or adequate nutrition; (b) "macrodifferences," consisting of the differences between adequate nutrition on the one hand and gross undernutrition (inanition) or overnutrition (obesity) on the other hand.

Gortner (35), Oiso (65), Hankin (38), and Fernandez (28)
have reviewed for us some of the between-population differences in the nutritional patterns of various countries. These fall essentially within the category of microdifferences. It has been relatively easy to show that gross undernutrition alters the metabolism of corticoids (10, 21, 48, 63), androgens (10, 21, 25, 48, 61, 63), and estrogens (70), but there is as yet no clear-cut evidence that micro-differences in nutrition can do so as well. After it has been demonstrated that consistent ipsidirectional between-population and within-population hormonal differences are correlated with cancer incidence, it should then be productive to focus research efforts on a study of the influence of nutritional microdifferences between the populations concerned upon those endocrine parameters that will have been implicated.

HORMONAL PARAMETERS THAT HAVE BEEN STUDIED IN CANCER

This discussion will be limited to breast cancer, since hormonal data concerning the other types of hormone-dependent cancer are as yet fragmentary. There have been interesting and extensive findings with respect to 2 sets of hormonal parameters, urinary androgens and urinary estrogens.

**Urinary Androgen Metabolites.** Extensive within-population studies of urinary androgen metabolism have been reported by Atkins, Bulbrook and Hayward and their coworkers in Great Britain, in a long series of papers from 1957 to date (2, 5, 6, 13, 14, 16). Basically, there were 2 major findings:

1. The daily urinary excretion of androgen metabolites (etiocholanolone and androsterone (Chart 1) was correlated with the responsiveness of metastatic breast cancer to adrenalectomy or hypophysectomy; more specifically, women with subnormal excretion of these metabolites usually showed a poor response to such surgery while women with normal metabolite excretion usually showed a good response. These findings have been confirmed by Juret et al. (47) in France, with respect to response to hypophysectomy, and by Kumaoka et al. (49) in Japan, with respect to response to adrenalectomy (Chart 2). On the other hand, Wilson et al. (76) in the United States have reported data that are in partial disagreement with those of Bulbrook et al. with respect to response to adrenalectomy, and Beck et al. (8) in Canada have failed to confirm a correlation between androgen metabolite excretion and response to hypophysectomy. More recently, unpublished studies monitored by the Breast Cancer Task Force of the National Cancer Institute have also failed to confirm the predictive value of urinary androgen excretion for response to endocrine ablation.

2. In a group of apparently normal women in Guernsey, the urinary excretion of androgen metabolites predicted, years in advance, the later appearance of breast cancer in certain individuals; more specifically, women who later developed breast cancer were found to have been excreting subnormal amounts of urinary androgen metabolites years earlier (15) (Chart 3). No other studies have been reported that either confirm or refute these findings.

Bulbrook et al. (17) also studied between-population differences in the excretion of urinary androgen metabolites. Healthy women from the British population (high risk for breast cancer) were compared with healthy Chinese and Japanese women in Asia (low risk for breast cancer); it was found that the Asian women had much lower urinary androgen metabolite excretion than did the British women, even after "correcting" for differences in body size (Chart 4). In other words, the directional difference between populations was opposite to the within-population directional difference. Lower excretion was associated with higher risk within a population but with lower risk between populations.

This latter finding is not reassuring concerning the possibility of a biological link, not to mention a cause-and-effect relationship, between urinary androgen metabolite excretion and breast cancer. Furthermore, it has been shown that subnormal urinary excretion of androgen metabolites...
Urinary Estrogens. Interest in the measurement of urinary estrogens in breast cancer is based on the widespread presumption that estrogens are carcinogenic with respect to the breast. This presumption, in turn, is based on the results of animal experiments and on human epidemiological data, as follows.

1. Of the 3 "classical" estrogens of human urine, estrone (E₁) and estradiol (E₂) cause breast cancer in rodents (54, 72) and estriol (E₃) does not (39).

2. Human females castrated prior to their late 30's have been shown to have about an 80% decrease in the incidence of breast cancer; no such decrease occurs in women castrated at a later age (27, 43). This has been generally interpreted to support the conclusion that estrogens are carcinogenic to the human breast also (20). Strictly speaking, it is more accurate to say that the studies show that the presence of something secreted by the ovary is necessary for the development of breast cancer; this "something" (presumably estrogen*) could be merely permissive and not causative. A more direct test of estrogen carcinogenicity is provided by the results of long-term administration of estrogens to intact women; all such studies to date (4, 11, 74) have shown no increase in the incidence of breast cancer, strongly suggesting that estrogens are not carcinogenic to the human breast.*

Despite these reservations, it has been considered to be of at least heuristic value to measure the urinary estrogens of various individuals and groups in order to determine whether the degree of "estrogenicity" is related to the development of breast cancer. Lemon et al. (51) and, more recently, Cole and MacMahon (20) have perceived an important problem in this approach. Not only do the 3 classical urinary estrogens differ in estrogenic potency, but estriol can be clearly shown, in rodents, to have antiestrogenic effects (44, 45, 75). For this reason, these authors have used the relative amounts of the 3 estrogens as a measure of "net estrogenicity." Lemon et al. (51) have expressed this relative amount as the "estriol quotient" [E₃/(E₁ + E₂)]. MacMahon et al. (56) have used the terms "estriol ratio" [E₃/(E₁ + E₂)] and "estriol proportion" [E₃/(E₁ + E₂ + E₃)] and have elaborated what has come to be known as the "estriol quotient."**

More recent studies have shown the presence of many other estrogens in human urine, among them 2-hydroxyestrone (31), 2-methoxyestrone (30), 16α-hydroxyestrone (80) and 16-epiestriol (81) (Chart 5). Few studies of these compounds in breast cancer have been done, although 2-hydroxyestrone is quantitatively the major estrogen in both plasma (77) and urine (79).

Grattarola et al. (36) have suggested that the ovarian secretion that favors the development of breast cancer is androgen, not estrogen.

It is still theoretically possible that estrogens might be carcinogenic if administered to very young females; against this possibility is the fact that administration of stilbestrol to fetal females in utero causes vaginal cancer (41) but has not been shown to cause breast cancer.

Estriol antagonizes the uterotrophic and transhydrogenase-stimulating effects of estrone and estradiol (44, 45, 75) but has not been shown to antagonize the carcinogenic effect (20).

* Estril antagonizes the uterotrophic and transhydrogenase-stimulating effects of estrone and estradiol (44, 45, 75) but has not been shown to cause breast cancer, so that studies can be carried out on

Chart 3. Comparison of the urinary excretion of etiocholanolone in women who later developed breast cancer with the excretion in women who did not develop cancer. The former show significantly lower values. Reproduced from Ref. 15 with the permission of the authors and the publishers.


(19, 32, 33, 58, 60, 67) and subnormal conversion of androgenic hormones to these metabolites (26, 78) occur in several other types of cancer and a wide variety of other chronic illnesses. A possible common factor may be the more or less marked undernutrition that is a feature of many of these diseases, since it has been shown that undernutrition, varying from simple "dieting" (25) to actual starvation (48, 63), can cause a decrease in the urinary excretion of androgen metabolites.

To summarize, therefore, the opposite directional findings of between-population and within-population studies in the hands of Bulbrook et al., the contradictory reports of other workers concerning within-population studies and the nonspecificity of subnormal androgen metabolite excretion in many unrelated diseases leave room for considerable doubt that androgen metabolite excretion is an important determinant of the natural history of breast cancer.
be known as the estriol hypothesis, namely, that "the relative levels of the estrogen fractions produced between puberty and about the 30th year of age are crucial determinants of a woman's life-time breast cancer risk" (20), more specifically that a higher estriol ratio is associated with a lower risk of developing breast cancer. The reference to "between puberty and about the 30th year of age" derives from another important piece of epidemiological data. Women who become pregnant prior to age 30 show a decreased risk of breast cancer (relative to nulliparous women) whereas women whose 1st pregnancy occurs after age 30 fail to show such a decreased risk and may indeed have an increased risk (55) (Chart 6). Cole and MacMahon (20) have concluded from this that some hormonal configuration characteristic of pregnancy must develop prior to age 30 in order to protect against breast cancer. Since these workers feel that it is the characteristic elevation of the estriol ratio in pregnancy that mediates its protective effect, they have generalized this to the conclusion that an elevated estriol ratio not due to pregnancy is also protective only if present prior to age 30.

A careful between-population study of the estriol ratio was carried out by MacMahon et al. (56), using Chinese and Japanese women in Asia as the low-risk population and Caucasian women in North American as the high-risk population. Table 1 shows that the Asian women had much higher estriol ratios than did the Caucasian women, as called for by the estriol hypothesis. Careful note should be made of the fact that the higher ratio was due to lower excretion of estrone and estradiol; the excretion of estriol was identical in the 2 populations. This important point will be discussed in more detail later in this paper.

Another between-population study by Dickinson et al. (24) provides further support for the estriol hypothesis. These workers found that Asian women living in Hawaii (intermediate-risk population) showed estriol ratios intermediate between those of Asian women in Asia and Caucasian women (Table 2). Once again, the excretion of estriol was identical in all 3 groups; it was the differences in excretion of estrone and estradiol that determined the differences in estriol ratio. Chart 7 shows the estriol proportion of these 3 populations. Each group showed essentially a normal distribution of this parameter, with no suggestion of bimodality.

There have been a number of within-population studies comparing the urinary estrogen excretion of Caucasian women with and without breast cancer. A serious potential problem exists in the interpretation of such studies, since Cole and MacMahon (20) have concluded that only an overnight urine sample instead of a 24-hr collection; this is the procedure that MacMahon's group has used for its studies.


Chart 6. Risk of developing breast cancer (relative to nulliparous women) in women who have their 1st full-term pregnancy at various ages. There is a linear increase in relative risk with age at 1st birth, from about 0.3 at age 15 to 1.0 in the early 30's to about 1.4 at age 40. Reproduced from Ref. 55 with the permission of the authors and the publishers.
Table 1  
Comparison of urinary estrogens in Asian and Caucasian women (56)

<table>
<thead>
<tr>
<th></th>
<th>Estrone (E₁)</th>
<th>Estradiol (E₂)</th>
<th>Estriol (E₃)</th>
<th>Estriol ratio</th>
<th>E₁ + E₂</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Follicular phase</td>
<td>Luteal phase</td>
<td>Follicular phase</td>
<td>Luteal phase</td>
<td>Follicular phase</td>
</tr>
<tr>
<td>Asian women (15–39)</td>
<td>4.5</td>
<td>6.9</td>
<td>2.4</td>
<td>3.7</td>
<td>8.3</td>
</tr>
<tr>
<td>Caucasian women (15–19)</td>
<td>6.9</td>
<td>12.8</td>
<td>3.5</td>
<td>6.9</td>
<td>6.1</td>
</tr>
<tr>
<td>Caucasian women (30–39)</td>
<td>10.6</td>
<td>12.4</td>
<td>5.5</td>
<td>5.9</td>
<td>9.6</td>
</tr>
</tbody>
</table>

Table 2  
Comparison of "estriol ratios" and estriol excretion between 3 populations of normal women (24)

<table>
<thead>
<tr>
<th></th>
<th>Estriol ratio</th>
<th>Estriol excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Follicular phase</td>
<td>Luteal phase</td>
</tr>
<tr>
<td>Asian women in Asia</td>
<td>1.33</td>
<td>1.53</td>
</tr>
<tr>
<td>Asian women in Hawaii</td>
<td>0.83</td>
<td>1.03</td>
</tr>
<tr>
<td>Caucasian women</td>
<td>0.06</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Chart 7. Distribution of the estriol proportion in 3 groups of normal women (drawn from data in Ref. 24). The 3 populations studied all show an essentially normal distribution of the estriol proportion. The mode shifts from about 0.6 for Asians in Asia to about 0.45 for Asians in Hawaii to about 0.35 for Caucasians. None of the 3 population distributions show evidence of more than 1 mode.

Table 3  "Estriol proportion" in Caucasian women of various ages (24)

<table>
<thead>
<tr>
<th>Age</th>
<th>Estriol proportion (follicular phase)</th>
<th>Follucular proportion (luteal phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15–19</td>
<td>0.40</td>
<td>0.44</td>
</tr>
<tr>
<td>20–24</td>
<td>0.39</td>
<td>0.43</td>
</tr>
<tr>
<td>30–39</td>
<td>0.38</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Chart 8. Women with breast cancer show about twice the normal excretion of endogenous estriol. Excretion of estrone and estradiol (not shown) is approximately normal, so that the estriol ratio is approximately twice normal. Reproduced from Ref. 40.

Estriol ratio that is present before age 30 influences the risk of developing breast cancer. Thus in order to interpret the estriol ratio in women of breast cancer age, it would have to be shown that this parameter does not change with age. A definitive study of this problem has not been reported, but Dickinson et al. (24) have shown that the estriol proportion based on endogenous estrogens does not change over the age range 15 to 39 (Table 3) and our group has shown (79) that the estriol proportion based on metabolites of radioactive estradiol tracers does not change over the age range 21 to 80. It appears not unreasonable, therefore, to examine whether the results of within-population studies are in agreement with those of between-population studies.

Unfortunately, the data are highly contradictory. Several groups (7, 46, 51, 71) have found lower estriol ratios in women with breast cancer than in controls, as would be expected from the estriol hypothesis, but several other groups (12, 40, 57, 64, 66), including Brown et al. (12) and our group (40), have found the opposite, namely, higher estriol ratios in women with breast cancer than in controls (Charts 8 and 9). Studies in Japanese women, cited by Lemon (50), showed no difference in estriol ratios between controls and patients with breast cancer. Without going into a detailed analysis of the relative merits of the various studies, it is certainly fair to say that the results of within-population studies of the estriol ratio in breast cancer are in considerable dispute and do not clearly support the estriol hypothesis.

An interesting side point concerns the findings of Lemon, who has reported lower estriol quotients in women with breast cancer than in controls (51). He has more recently reported (50) that normal women showed a polymodal distribution of the estriol quotient (Chart 10) and has interpreted this in terms of 3 genotypes, a homozygous
low-quotient group, a homozygous high-quotient group, and a heterozygous intermediate-quotient group. Chart 7, which presents data from the normal population studied by Dickinson et al. (24), fails to show any indication of more than 1 mode in the distribution of the estriol proportion.

It appears possible to reconcile the findings of the between-population studies with the epidemiological data concerning the protective effects of early castration and early pregnancy by a revised hypothesis that we would like to suggest: "A period of time, prior to age 30, during which the amount or biological availability of 'active estrogens' (i.e., estrone and estradiol) is diminished protects against subsequent development of breast cancer." The diminution may be due to surgical removal of the source of estrogens (castration), environmentally determined decreases in estrogen (Asian women in Asia) or the presence of an "antiestrogen" (pregnancy). From this point of view, emphasis upon the central role of estriol itself in all these situations appears somewhat misplaced. In the 1st 2 cases, simple decrease in the amounts of estrone and estradiol constitutes an adequate explanation; furthermore, the plasma levels of unconjugated estradiol are probably too low in nonpregnant individuals [ < 10 pg/ml, i.e., 3 to 5% of estradiol levels (53)] to exert any significant antiestrogenic effect.* Only in pregnancy do we need to invoke the existence of an antiestrogen; estriol may well fulfill this function, since breast cancer tissue has been shown to have 16α-hydroxylase activity (1).

ADDENDUM

Several very recent publications are highly pertinent to the discussion in this paper concerning the estriol hypothesis. The biological basis for this hypothesis consists largely of 2 observations in rodents: (a) that estriol has (e.g., 2-hydroxyestrone) may possess antiestrogenic properties should be kept in mind. This would seem to be a very productive area for further research.

The within-population studies that show an increased estriol ratio in cancer patients are difficult to fit into the estriol hypothesis but can be readily reconciled with the revised hypothesis, since the increased ratio in the cancer patients is on a different basis from that of the Asian women despite the comparable magnitude of the ratios in the 2 groups (Chart 11). In the Asian women, excretion of estriol is normal and excretion of estrone and estradiol is decreased, so that total estrogen excretion is decreased; in the cancer patients, excretion of estrone and estradiol is normal and excretion of estriol is increased, so that total estrogen excretion is increased.* Thus the directional change in the excretion of estrogens in the cancer patients (higher) is opposite to the change in patients with low cancer incidence (lower).

Three further caveats concerning estriol ratios must be expressed: (a) it is possible that the elevated estriol ratio in women with breast cancer is a nonspecific effect of illness, since an elevated ratio has also been reported in men who have had myocardial infarctions (9) or prostate cancer (59) as well as in patients with a variety of other illnesses (unpublished data from this laboratory); (b) the "low-T₃ syndrome" reported (68) in many illnesses including cancer might, if present, result in an increased relative excretion of estriol, since the latter is characteristic of hypothyroidism (30); (c) it is possible that the cancer itself may produce some of the excess estriol, since breast cancer tissue has been shown to have 16α-hydroxylase activity (1).
Chart 10. Distribution of estriol quotients (equivalent to estriol ratios) found by Lemon in normal Caucasian women. There is a suggestion of a polymodal distribution. Reproduced from Ref. 50 with the permission of the authors and the publishers.

Chart 11. Two different mechanisms for achieving an elevated estriol ratio. The values for normal Asian women are drawn from data in Ref. 24; the values for Caucasian women with breast cancer are drawn from a composite of the data from Ref. 40, 57, and 66. Both groups show an estriol ratio that is approximately twice normal, but in the normal Asian women this derives from normal estriol excretion and decreased estrone + estradiol excretion, whereas in the Caucasian women with breast cancer it derives from normal estrone + estradiol excretion and increased estriol excretion.

Minimal estrogenicity (i.e., uterotropic activity) of its own and acts as an antagonist to the uterotropic effect of estradiol (44, 45, 75); (b) that administration of estriol in contrast to administration of estradiol or estrone does not produce mammary cancers (39). Both of these observations have now been challenged. Anderson et al. (3) have reported that chronic administration of estriol, a more "physiological" approach than the acute administration used in previous studies, demonstrates that estriol has the same uterotropic activity as estradiol, mg for mg; Rudali et al. (69) have reported just as high an incidence of mammary cancer after estradiol administration as after estradiol administration. These new findings reinforce the doubts that we expressed earlier in this paper and suggest to us that the estriol hypothesis should probably be abandoned at this time.

Conversely, there is new evidence that 2-hydroxyestrone might well play the antiestrogen role currently ascribed (apparently erroneously) to estriol. Morishita et al. (62) have shown that administration of 2-hydroxyestrone to male rats, like clomiphene but unlike other natural estrogens or estrogen metabolites, causes increased luteinizing hormone secretion; this is generally considered to represent an antiestrogen effect, i.e., blockage of tissue uptake of estradiol in the hypothalamus. Studies from this laboratory (C. Martucci and J. Fishman, to be published) have shown that 2-hydroxyestrone binds well to the cytosol estrogen receptor, which necessarily endows this steroid with antiestrogen properties since it has no uterotrophic activity of its own (42) and it would compete with active estrogens for binding sites. Gelbke et al. (34) have shown that urinary excretion of 2-hydroxyestrone rises early and markedly during pregnancy, with the increase being disproportionately greater than that of other estrogens during early and midpregnancy; if 2-hydroxyestrone is indeed an antiestrogen, this observation may be related to the protective effect of pregnancy against subsequent breast cancer. Finally, Fishman et al. (29) have shown that the ratio of 2-hydroxyestrone to estradiol is greatly decreased in obese women; thus the increased risk of breast cancer in these women (23) might be related to the decreased relative amount of the protective antiestrogen, 2-hydroxyestrone.

REFERENCES


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