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Discussion

Dr. Judd: Dr. Zumoff, you have quoted me somewhat incorrectly. The paper that you quoted indicated that we did not find any difference between estrogen levels of fat and thin people. Indeed we did in our first paper in 1976, in which we studied 16 patients with endometrial cancer and 10 postmenopausal women without it. We expanded that study to include 35 patients with and 35 patients without endometrial cancer and published the results in 1979 in the *American Journal of Obstetrics and Gynecology*. We again found a striking correlation with body size not only for estrone but also for estradiol levels. We expanded our study further to 155 postmenopausal women and again in a 1980 publication reported finding a striking correlation between body size and both estrone and estradiol. And we expanded that even further in light of the observations of Dr. Siiteri that the delivery of estradiol may be somewhat different in obese and thin people because of SHBG[1] levels. In the paper that we published with Dr. Siiteri, we looked at total estradiol levels, SHBG levels, percentage non-SHBG-bound estrogen levels, and free estradiol levels, comparing fat and thin individuals. The difference in body size between our thin patients and fat patients was only about 50 pounds. In spite of this relatively small difference in body size, we observed quite large differences in circulating estradiol. The circulating estradiol level in our thin patients was approximately half of that of our heavy patients. When you add the issue of SHBG, if the free hormone has something to do with what is biologically available, there is clearly a 4-fold difference. As we look at the literature, not only our own work but also the work of others, we come up with an entirely different conclusion from yours. As observed originally by Siiteri and McDonald, there is a major difference in percentage of conversion of androstenedione to estrone with obese people, and this difference is absolutely seen in the circulation if you measure concentrations of these hormones.

Dr. Zumoff: Because of the demands of time, I did not go into a very elaborate discussion, and I apologize if Dr. Judd feels that he was somewhat taken out of context. My understanding on reading the earlier papers was that a positive correlation between blood levels and the degree of obesity exists, which indeed we too find. Everybody finds that. However, I was struck by the fact that even at the upper end of the correlation curve the values were not very high; they were within the generally accepted range of normal levels for both estrone and estradiol. I don't know whether that's still true in some of your recent papers, but the ones that I have seen both in the 1976 and 1979 publications and in your presentation at the Endocrine Society indicated to me that you were still talking about variations within the normal range. Of course, we did not look at all at the exceedingly intriguing problem of free *versus* total estradiol in the areas that Dr. Siiteri has talked about very recently with regard to breast cancer and endometrial cancer. He showed a very markedly increased fraction of free estradiol, which may change the whole complexion of the subject. I did not find mention of the gross elevation of plasma estrogens outside the normal range in obese women in the papers that you and your group published.

Dr. R. H. Purdy (Southwest Foundation for Research and Education, San Antonio, Texas): I would like to show a couple of slides of

[1] The abbreviation used is: SHBG, sex hormone-binding globulin.

mammalian cells.[2] These are normal BALB embryonic fibroblast cells which are used in many of the transformation assays that are being carried out in this country and elsewhere. These cells treated with estriol show no changes with amounts up to 50 μM at 72 hr. However, in the next slide I show cells taken from foci which have been transformed by either estrone or estradiol, and in the following slide is illustrated the ability of these chemically transformed cells to grow as multicellular tumor spheroids. On the last slide, I illustrate data from Dr. Brim's laboratory at the National Cancer Institute: 5 of 5 nude mice treated with such transformed cells developed tumors within a period of 7 to 10 days.

Question: Could Dr. Purdy tell us how he gave the estriol, because it is very important in understanding these data.

Dr. Purdy: These are cells grown in culture, treated with estriol.

Question: Was it put in repeatedly?

Dr. Purdy: It was put in at the beginning and 24 hr after the medium was first changed.

Question: It was not the same and constant?

Dr. Purdy: No; however, in our results we can change the media every 24 hr and see no effects of estriol.

Dr. Santen: Dr. Zumoff, Dr. Kirschner presented data on the conversion of androstenedione to estrone in premenopausal women. You have shown us plasma levels. Since the level of secreted hormone changes so much during the follicular phase of the menstrual cycle, could you tell us on what specific days of the menstrual cycle your studies were carried out, and whether this was the same in the control group and in the obese patients?

Dr. Zumoff: They were carried out between the second and ninth days of the cycle; when we plotted the values for each individual against day of cycle for both estrone and estradiol, we got an absolutely flat correlation. So levels were in a plateau period during that time, and the same thing was true for both the obese and nonobese women.

Dr. Judd: I need to respond to your comment, Dr. Zumoff. When you talk about the normal range of estrone and estradiol, you include obese people, thin people, etc. Consequently, the level that you see in an obese person will fall into the normal range. However, when you scrutinize the data on postmenopausal women, the levels of circulating estrone and estradiol are very different in fat people and thin people, and that is the whole point. Although you are stating that it makes no difference, it does make a difference. Obese women have much higher—twice as high—circulating levels of estrone and estradiol. And they have at least 4 times as much free estradiol as do thin people. Granted, they did not fall out of the normal range, because they are part of the normal range.

Dr. Zumoff: Even taking the matter of correlation at face value as the important parameter, I can only reiterate what I showed in one of my charts (Chart 1). When we plotted our data for total plasma estradiol against the percentage of deviation from ideal weight, we found no significant correlation. And when we did the same for estrone, we found a positive correlation which was very slight. When translated into numbers, this would mean that women at zero deviation would have a mean plasma estrone of 63 and women at 100% above ideal weight, namely twice normal weight, would have a plasma estrone of 75. So we do confirm a small positive correlation for estrone. It is indeed very small. We did not see a positive correlation with estradiol.

Dr. Siiteri: Since Dr. Zumoff mentioned my interest in availability of estrogens, I would like to amplify on that comment and perhaps destroy his argument. We developed methodology for determining precisely not only the percentage of free hormone for any steroid but, in particular, estrone or estradiol. We can also measure that fraction which is bound to albumin and also to the SHBG. We discovered that in fact the availability, if you include free and albumin-bound estradiol, varies 5-fold over the range of SHBG values that are found in various kinds of patients. That is, the SHBG concentration in obesity is very low and below, in fact, the level found in normal men. At that point, you have 90% of a given level of estradiol that is available, if we use that term. On the other hand, in somebody who is only slightly hyperthyroid, that percentage decreases to something like 20 or 30%. So the fact that you do not find a difference in a total estradiol level does not necessarily mean that the target tissue is not seeing a different amount of hormone at the level of the cytoplasmic receptor. It is terribly important, because we as endocrinologists have been measuring the total hormone concentration for many years, and it is only recently that we have started to address the question of what is available of a given amount of hormones. The point I want to make is that you can have 2 women with the same estradiol levels. In fact, the breast or endometrium in the one with a low SHBG can see 3 or 4 times more hormone than in the other with a high SHBG, even though the estradiol levels are the same.

Dr. Zumoff: I was exceedingly impressed when I first heard your data about the available estrogen fraction, and I am looking forward to seeing more. The point that I was hoping to make was that the concentration of appropriate effector in the body fluids is indeed the parameter to which we should pay more attention, rather than its synthesis rate. With the heretofore measurable parameter, namely, total estrogen, according to our data, little or nothing is visible. I would certainly not be a bit surprised, however, and I would be more than pleased if it turns out that there is indeed a detectable fraction of estrogen which is regularly and predictably elevated in women with breast cancer or at risk for breast cancer.

Dr. Sherman: I would just like to amplify Dr. Zumoff's consideration about the induction of carcinogenicity by estrogens as opposed to another potential action, the promoting effect of estrogens. The latter, I think, is perhaps even more relevant to the remainder of the discussion at this meeting. We looked within a group of patients with breast cancer to see if there was any relation between incidence and menopausal estrogen use, and like everybody else we could find no relationship. We asked another question, namely, was there an effect on age at diagnosis? We found a significant effect of menopausal estrogen use on the age at which patients got breast cancer; that is, the estrogen users developed carcinomas some 5 years earlier than the nonusers. We looked at the interaction of that with weight. In nonusers, we saw no effect of weight and age at diagnosis. But in the users, we showed that there was a more striking effect related to weight; that is, lighter women who took estrogens—we don't know the dose—had up to an 8-year difference in mean age at diagnosis than did their heavier counterparts. This may be a pharmacological effect, but these data from a small group of patients suggest that even endogenous estrogens might have an effect on promoting tumor as assessed by examining either the age at diagnosis or the stage at diagnosis. This effect of estrogens is above and beyond the effect that they might have on induction or as a carcinogen *per se*.

Dr. Zumoff: Is it not a relationship in the opposite direction that would suggest that the skinnier woman gets her promotional effect from the estrogen?

Dr. Sherman: Yes, as a dose response. We do not know what the doses are. I presume these are all doses given to a group of women. A given dose has a greater pharmacological effect on a woman of lighter weight and presumably has a greater effect in these women such that they would have the expression of an underlying tumor at an earlier age.

Dr. Joseph Thyssen (University of Utrecht, Netherlands): May I ask Dr. Sherman to comment on a recent finding that the hormone dependency as judged from estrogen receptor estimations is quite different between obese and thin women? In screening a large number of women, we found that the incidence of receptor-negative tumors was much higher in lean women than in fat women, the obese women having more than 75% estrogen receptor-positive tumors and the lean ones just over 50%. That is my first question. I do not know who can answer my second question at the moment, but several speakers have clearly indicated that the conversion of androstenedione to estrone is

[2] Slides not included.

influenced by obesity. I think we cannot question these findings, but it is quite clear at the moment that the estrogen that is really effective at the tissue level is estradiol. In a recent study, we were only able to find estradiol being picked up by the endometrium. So my question is, does anybody in the audience know which factors are influencing the peripheral conversion of estrone to estradiol in postmenopausal women?

Dr. Sherman: Actually, I do not have the answer to either one of your questions, nor does anyone else.

Dr. Thyssen: There are 2 papers on this issue, a recent paper by Ahweiss and a paper published a couple of years ago by McGuire. He mentioned the incidence of estrogen receptors in tumors in Japanese patients. That also fits in with observation of a higher incidence of negative tumors in lean women. He does not comment on this point, though.