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LETTERS TO THE EDITOR

Modified MENQOL

To the Editor:

I wish to point out an error in the article by Gelfand et al[1] that appeared in your January-February issue. Unlike the original Menopause Quality of Life (MENQOL) questionnaire, which asked participants to recall their experiences in the past month, the modified MENQOL-Intervention questionnaire, used in this study, has a recall period of one week.

Thank you for allowing us to bring this to the attention of your readers.

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Topical Progesterone

To the Editor:

The January-February 2003 issue of *Menopause* published an editorial by Dr. Gambrell and a paper by Dr. Wren and his colleagues concerning the use of topical progesterone and its purported lack of clinical effects.

Dr. Gambrell's editorial needs clarification, as in paragraph 2 he states that Pro-Gest is an extract of the Mexican wild yam. Pro-Gest is not a wild yam extract but is USP progesterone emulsified in aloe vera and vitamin E. Pro-Gest has been available in the United States through physicians, and later retailers, since 1978. It has also been the subject of several clinical trials.

Dr. Gambrell also stated that there are "no studies to even suggest a progestational effect." It is a widely held assumption that blood levels of progesterone in the range of 1 to 5 ng/mL as achieved through transdermal application are insufficient to cause a secretory change in the endometrium. Two studies have been completed to date that specifically looked at end-organ effects, with one demonstrating endometrial transformation, and one not.[1,2] Both studies were short-term studies and utilized different formulations. A prospective, randomized, crossover study comparing standard hormone replacement therapy [conjugated equine estrogen/medroxyprogesterone acetate (CEE/MPA)] to an estrogen (CEE)/progesterone cream regimen was presented by Dr. Jennifer Landes at this year's annual meeting of the American College of Obstetricians and Gynecologists. Study participants were evaluated by endometrial biopsy before treatment and after each 6-month treatment arm. The preliminary data presented indicated that estrogen/topical progesterone cream had a similar effect on the endometrium as the standard oral hormone replacement therapy. Additional findings indicated a strong patient preference for the progesterone cream-containing regimen ($P < 0.01$).

The current paper by Wren et al utilizes a formulation of progesterone (Pro-Feme) at a dose of 32 mg applied daily to soft tissue areas of the body (excluding the breasts). This is the same dose of progesterone that we used in our study using the formulation in Pro-Gest.[3] Our study resulted in higher blood levels of progesterone (1-2 ng/mL means) than are reported in this paper and also showed that twice-a-day application is likely to better sustain blood levels. Recent pharmacokinetic studies showed progressive uptake over 3 h to 19 ng/mL with Pro-Feme in rats.[4] Even though the Wren study was a negative study with a small number of participants for comparison, there may be trends toward significance in improving vasomotor symptoms ($P = 0.07$) and anxiety ($P = 0.10$). Would we see significance in a larger study? In support of this trend are results from a larger yearlong study that demonstrated a significant improvement in vasomotor symptoms ($P < 0.001$).[5] Differences can be expected because of the site of application, formulation, and excipients used to enhance penetration of progesterone. Sitruk-Ware highlights this point in her review of various progestins with respect to their route of administration.[6]

It is premature to conclude that there is no potential role for transdermal progesterone. There is evidence of beneficial effects of progesterone. Other mechanisms of coronary artery benefit from low levels of progesterone have been reported in monkeys[7-10] and should be evaluated in humans. We still need to effectively define

the threshold level of progesterone required to protect the endometrium and whether continuous low levels obtained transdermally can reliably offer this protection.

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To the Editor:

Congratulations to *Menopause* for opening the debate concerning the transdermal absorption of progesterone (and other steroid hormones) and its clinical significance. I am referring to two articles – an editorial by Dr. R. Don Gambrell, Jr., and an article by Dr. Barry G. Wren and associates in New Zealand. Both of these articles disparaged transdermal progesterone. I was somewhat surprised that the editors of *Menopause* did not take the opportunity for comment from transdermal progesterone advocates. Because my name was used explicitly throughout Dr. Gambrells' article as "one of the leading advocates," I feel obliged to respond.

Dr. Gambrell's argument rests on several erroneous assumptions and understandings. Curiously, he erroneously presumes that progesterone is *extracted* from wild yams. Plants do not make progesterone. Progesterone is synthesized commercially by a chemical process (first devised by Russell E. Marker in the late 1930s) using fats and oils from plants such as soybeans or wild yams. The resultant product is bioidentical to the progesterone that mammals make from cholesterol. USP progesterone is readily available to pharmacists and pharmaceutical companies for the making of progesterone creams, gels, capsules, troches, etc. The same or similar processes are used to synthesize bioidentical estrogens, corticosteroids, and testosterone, as well as progestins.

To his credit, Dr. Gambrell lists the basic facts that underlie the advantages of transdermal progesterone. For the interest of this issue's readers, I will reiterate them.

- Endogenous sex steroid hormones circulate in blood in two forms – protein-bound and "free." Sex hormone binding globulin (SHBG) is the binding protein for estradiol (E_2); and cortisol binding globulin (CBG) is the binding protein for progesterone. When protein-bound, the hormone is relatively nonbioavailable. Nonprotein-bound "free" hormone is the active, bioavailable form. In the case of E_2 or progesterone, more than 90% of the serum-borne hormone is protein-bound and nonbioavailable.
- When applied topically, most sex steroids (except for estriol) are well absorbed through skin into underlying body fat. Within 2 to 3 h, essentially all of the applied hormone is circulating in blood as "free" (nonprotein-bound) hormone. Being very fat-soluble, it is carried in blood by fatty components such as red blood cells, and very little of it is found in serum, per se.
- When ingested, sex hormones are subject to metabolization by enzymes in the intestine and the liver before entering the circulatory system. In this "first-pass-loss" process, more than 90% of the oral dose is converted into metabolites for excretion or becomes protein-bound, thus less bioavailable.
- When given orally in usual HRT doses, E_2 actually increases the production of SHBG, thus inhibiting E_2 effect. When given transdermally in physiological doses, there is no increase in SHBG; thus, the activity of the hormone is not inhibited. This again illustrates the advantage of transdermal application over oral dosages.
- Conventional serum hormone tests do not distinguish between "free" and protein-bound hormone.

Thus, the concentration of "free" hormone cannot be determined by this test. If one somehow tests for merely the "free" hormone in serum, the test ignores the fat-soluble "free" hormone being borne by red blood cells. Again, serum testing is not an accurate measure of the full concentration of active hormone present in blood.

- As blood circulates through tissue, "free" hormone molecules exit through capillaries into the extracellular fluid and then through cell membranes where, in target tissues, hormone molecules bond with specific protein receptors to carry their message to the nuclei of these cells.
- Protein-bound hormones, on the other hand, are water-soluble and accumulate in serum before being excreted in urine. Protein-bound hormones do not readily penetrate capillaries and are not capable of binding to intracellular receptors; therefore, they are relatively nonbioavailable. If one wishes to know the hormone status of an individual, it is important to know the concentration of "free" hormone, and this cannot be done with conventional serum testing.
- As blood circulates through saliva glands, the "free" hormone molecules, whether from serum or red blood cells, exit the blood through salivary capillaries and filter directly into saliva. Saliva is not water: it is a complex fluid into which the fat-soluble "free" hormones are readily soluble. Hormones that are bound to their large globulin-binding proteins do not filter into saliva. Thus, saliva hormone assay is the best test available to physicians to approximate hormone availability at target tissue sites.

When attempting to restore sex hormone balance or treat sex hormone deficiency, the level of tissue-available (ie, total "free") hormone is the operative factor. From the above important facts, it should be obvious that the goal of transdermal hormone dosages is to restore the total "free" hormone level to normal, healthy ranges, which can be measured by saliva testing. Serum hormone levels are irrelevant because their hormone contents are largely protein-bound and not bioavailable. The attempt to use transdermal hormones to raise serum levels to so-called normal or expected ranges is an obvious error and will lead to undesirable overdosing.

I will add a few other findings, not mentioned by Dr. Gambrell, that further show the irrelevance of conventional serum hormone levels.

When E_2 and progesterone are applied topically in physiologic doses to premenopausal[1] or postmenopausal women,[2] it is found that breast tissue levels of these hormones rise 80- to 100-fold, with physiological consequences. Transdermal E_2 in doses of just 1.5 mg/day doubled the rate of breast ductal cell proliferation. Progesterone in doses of just 25 mg/day greatly reduced the rate of breast ductal cell proliferation. Despite these obvious markers of hormone absorption and physiologic effects, serum levels of the hormones showed little or no difference from placebo controls. This is further proof that serum levels do not accurately reflect bioavailable hormone concentration at levels of powerful hormone activity.

In another example, Dr. SR Cummings and colleagues, in 1998, compared serum E_2 levels to bone resorption in postmenopausal women.[3] E_2 is known to inhibit osteoclast-mediated bone resorption, thus slowing bone loss. However, the rate of bone resorption did not correlate with serum E_2 levels. It did correlate well with levels of SHBG, the protein that binds to serum E_2, making it less bioavailable. The higher the levels of SHBG, the more E_2 becomes protein-bound and not bioavailable. With less "free" E_2 available, the greater is the bone resorption rate. The serum E_2 level was simply irrelevant.[1]

Similarly, conventional serum E_2 levels do not predict future breast cancer risk. Yet, it is widely acknowledged that E_2 is a major cause of breast cancer. In 2002, Dr. Cummings and colleagues found that a subfraction of serum E_2 (comprising only about 10% of the total serum E_2) using special technology not available to common practice, did, in fact, correlate positively with future breast cancer occurrence.[4] While not explicitly admitted by Dr. Cummings, it is safe to assume that the subfraction of serum E_2 that correlated with future breast cancer risk was the "free" (bioavailable) E_2.

In this matter, David Zava, PhD, an experienced saliva hormone researcher with a huge database from years of monitoring health patterns and saliva tests, finds a strong correlation between saliva E_2 levels and future breast cancer, especially if it is not balanced with progesterone. It turns out that the ratio between saliva progesterone and saliva E_2 is more important than the absolute values of either one.[3] When blood circulates through saliva cells, both "free" estrogen and "free" progesterone, whether from serum or red blood cells, filter directly into saliva. The scientific experience of using saliva tests is now enormous. There is no longer any doubt that, if one wished to measure total "free" (bioavailable) steroid hormone levels, saliva testing is far superior to conventional serum tests.

Dr. Gambrell argues that transdermal progesterone applications are insufficient if they do not raise hormone levels to normal or expected serum levels. As we have seen, serum testing cannot measure total "free" (bioavailable) hormone. More than 90% of serum progesterone is protein-bound and not bioavailable. There is no reason to raise the level of "free" progesterone obtained by transdermal absorption to levels higher than "free" progesterone found in healthy, normal, premenopausal women. Saliva tests can do that, but serum tests cannot. Comparing the level of "free" hormone from transdermal absorption to serum hormone levels is mixing apples with oranges.

Secondly, interpretation of saliva levels following topical application can be confusing unless one understands the pharmacokinetics. Fluctuations of endogenous hormone production are relatively minor over a 24-h period. Absorption from topical application is considerably more dynamic. If just 20 mg of progesterone is applied transdermally, peak saliva levels occur 2 to 3 h after topical application, reaching top levels of 16 ng/mL (16,000 pg/mL). After another hour or two, saliva levels begin to fall because of the normal excretion process. Eight hours after application, saliva levels have declined to 2 to 3 ng/mL (2000-4000 pg/mL), a drop of about 80%. By 24 h, saliva levels have fallen to 0.5 ng/mL (500 pg/mL). During this great surge of progesterone through the body, hourly serum levels show little or no change (personal communication from David Zava, PhD, ZRT Labs, Portland, OR, 1998).

The interpretation problem is widespread among physicians. Some tell their patients to wait 48 h, or even 2 weeks, to collect the saliva specimen. When the "treatment" levels appear no different from the pretreatment levels, the physicians mistakenly conclude that the progesterone was not absorbed! Conversely, I have received calls from physicians who obtain saliva specimens 3 to 4 h after application and become startled by the high progesterone levels found.

I recommend standardizing saliva collection at 10 to 12 h after application. By standardizing the saliva collection time with last application, one can make sense of the saliva levels found.

The Wren et al report suffers from the same error – trying to equate hormone activity with serum levels. One interesting admission stated that, instead of saliva levels being lower than serum levels, some saliva progesterone levels in women using transdermal progesterone were 1,000 times that found in serum. This means either gross overdosing or collecting the saliva at the peak of absorption 2 to 4 h after application.

The Wren et al report also mentioned a study of progesterone's effect on endometrium in women using E_2 patch. They found "failure" of secretory changes. The desired goal is not to restore menstrual periods in elderly women; the goal is to protect against estrogen-induced endometrial cancer. The marker for protection is not the presence of secretory changes; it is the absence of endometrial hyperplasia or any precancerous abnormalities of endometrial cells, which was the case in those women receiving transdermal progesterone. A study by Dr. Helene Leonetti[5] found that low dose transdermal progesterone was just as effective as medroxyprogesterone acetate [Provera (Pharmacia & Upjohn Company, Kalamazoo, MI, USA)] in preventing estrogen-induced endometrial abnormalities.

The error of assuming that serum levels are accurate or meaningful extends also to pharmaceutical preparations such as hormone patches. Not understanding the difference between oral and transdermal (skin patch) dosing, the early estrogen patches were all grossly overdosed, resulting in weight gain, swollen and tender breasts, water retention, hypertension, poor sleep, and all the symptoms associated with estrogen dominance. Over time, transdermal doses have been lowered in an attempt to limit these side effects. The new E_2 dose in patches is 10 times less than that of earlier patches. They now provide just 0.025-0.05 mg/day, achieving the same E_2 results as oral doses of 1 to 2 mg/day. Simple arithmetic tells us that transdermal dosing is 20 to 40 times more efficient than oral dosing. Why would it be any different for progesterone?

Dr. Gambrell begins his article with a story about the problem of creating a progesterone patch. Using the assumption that the transdermal dose would be that needed to achieve a serum level of 15 ng/mL, he calculated that the progesterone patch would have to be 30 times larger than an E_2 patch. However, bioavailable progesterone levels (as measured by saliva hormone assay) during the luteal phase in healthy premenopausal women is only 100 to 400 pg/mL. In progesterone-deficient premenopausal but anovulatory women, we find that just 15 to 20 mg of transdermal progesterone (even without the magic patch) will routinely restore normal saliva progesterone levels. In postmenopausal women, the optimal daily progesterone dose is only 10 to 12 mg/day. Given the experience of E_2 patches, Dr. Gambrell should be able to relax; there is no reason to assume that a good progesterone patch would be any larger than some of the earlier estrogen patches.

Transdermal progesterone dosing is alive and well. The sooner our physicians and pharmaceutical companies come to understand sex hormone kinetics, the

sooner we will be able to develop a safe, sane, and successful hormone balancing therapy that won't endanger the health of women, as our present hormone replacement therapy does.

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In reply:

In response to the letter from Dr. Lee, I regret that he interpreted my editorial as saying that progesterone could be converted through the skin from wild yams. The plant hormones, such as diosgenin, were extracted from wild yams and used in the laboratory to synthesize some of the original OC progestogens, such as norethynodrel and norethindrone. This was followed by the statement that it was unlikely that human skin could absorb and convert diosgenin into a biologically active progestogen. Unfortunately, most of the evidence to support efficacy of progesterone skin cream is anecdotal, and there are no long-term, randomized, clinical trials to confirm that salivary progesterone (the so-called free progesterone) provides proper measurements of effectiveness. One of the better studies was a crossover trial that was randomized between Pro-Gest cream and oral micromized progesterone.[1] However, the study involved only 20 participants for a length of only 33 days. One confounding factor in the problems with Prempro in the Women's Health Initiative (WHI) study was that it was approved by the Food and Drug Administration after only 1 year of study.[2] Although the WHI was randomized and intended for 8.5 years, it was cut short after 5.3 years.[3] Remember that the Scandinavian long-cycle study was intended for 5 years; however, it was stopped after 3 years because of increasing endometrial hyperplasia, including atypia, and a single case of adenocarcinoma.[4] The current study by Wren et al[5] of transdermal progesterone and its effect on symptoms, bone markers, and lipid levels, was randomized, double-blind, and placebo-controlled, but it had a length of only 12 weeks.[5] I take issue with Dr. Lee's statement that "...it is widely acknowledged that estradiol is a major cause of breast cancer." The estrogen-breast cancer debate remains just as controversial as the progesterone skin cream-salivary levels discussion.[6]

In response to Dr. Burry, I am relying on my memory of 20 years ago for the names of the "progesterone" creams brought to me from the health food stores by my patients. I am sure of the names "Born Again Wild Yam Cream" and the "Progesterone HP," and I thought that the third was "Progest," before progesterone was added to it; however, Dr. Burry is most likely correct. The year-long study presented to the American Council of Obstetricians and Gynecologists in April 2003, mentioned in Dr. Burry's letter, consisted of 20 women treated with conjugated estrogens 0.625 mg and medroxyprogesterone acetate 2.5 mg for six months. After an endometrial biopsy, these 20 women were crossed over to conjugated estrogens 0.625 mg and progesterone skin cream 20 mg for another six months when the endometrial biopsy was repeated. Although no cellular atypia was found in the endometrial biopsies, 12 of the specimens were proliferative endometrium, six in the hormone replacement therapy (HRT) group, and six in estrogen/progesterone skin cream group, while the remaining 28 were atrophic endometrium. Apparently, estrogen/progesterone skin cream had an effect on the endometrium similar to that of continuous-combined HRT in this short-term study. However, I again point out that a six-month study may not be long enough to show full endometrial protection.[7]

The most recent report of topical progesterone cream on estrogen-stimulated endometrium was conducted on 32 HRT users (after 5 dropped out) who discontinued their HRT just before entering the study.[8] These HRT users were selected so they could all have proliferative endometrium. Endometrial biopsies were obtained pretreatment and after 28 days of oral conjugated estrogen 0.625 mg and either 0%, 1.5%, or 4.0% progesterone cream twice daily. Numerical endometrial proliferation scores (EPS) were used and reviewed blindly by two pathologists, who concluded there was less proliferation in the progesterone cream users at the end of the 28-day study. The authors did not recommend progesterone cream as an alternative in HRT because of the short duration and limited number of patients in their study. Why don't some of these progesterone skin

cream advocates do the necessary long-term, randomized, placebo-controlled study and settle this issue of efficacy once and for all?

Following is the current position statement on progestogens from The North American Menopause Society: "Transdermal (topical) progesterone cream or gel preparations obtained either over-the-counter or custom-compounded by prescription may not exert sufficient activity to protect the endometrium from unopposed estrogen. These products should not be used for this purpose until optimal therapeutic doses and serum levels of topical progesterone are established and long-term trials are conducted that document endometrial protection."[9]

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In reply:

Dr. Lee is well known for his support of the use of transdermal progesterone cream. In an attempt to convince the medical community of the benefit of this route of administration of progesterone, he has produced a complex hypothesis to explain the many anomalies and then attacks any who dare to challenge his pet theory. However, he should remember that, of the many brilliant ideas that have been developed over centuries of scientific thought, there is only one criteria for success: Does the hypothesis work?

Dr. Lee has claimed that transdermal progesterone is the ideal treatment for the management of postmenopausal symptoms, but does it stop hot flushes or prevent night sweats? Does it reverse the dry, atrophic, alkaline vaginal epithelium? Does it prevent osteoporotic degeneration of bone? Does it improve mood changes and sexual discomfort? The answer to these simple questions is a resounding no! Transdermal progesterone does not improve these simple menopausal symptoms.

In his letter explaining the intricacies of transdermal progesterone, Dr. Lee has criticized our article, which presented the findings of a double-blind, randomized, placebo-controlled study that used a cream containing progesterone to be delivered in a dose of 32mg daily.[1]

The reason for performing this study was to ascertain if progesterone, delivered transdermally, had any effect on vasomotor symptoms, moods, sexual response, blood lipid values, or bone metabolic markers of postmenopausal women. We were unable to confirm any of the beneficial claims made by Dr. Lee. There was some evidence of absorption of progesterone, with markedly elevated saliva levels of all hormones, but the levels were insufficient to induce any change in any of the symptoms or parameters we had investigated.

Like Dr. Lee, we agree that lower levels of progesterone are required to inhibit mitosis than are required to induce a secretory change in the endometrium, and we have also investigated this aspect of transdermal progesterone therapy.[2] In one study, we showed that transdermal progesterone in doses ranging through 16 mg, 32 mg, and 64 mg daily had no inhibitory or secretory effect on proliferating endometrium when used in a sequential regimen.[2] In another study (as yet unpublished), we were not able to detect any evidence of inhibition of mitosis when 32 mg of transdermal progesterone was given at the same time as a transdermal estrogen gel.

It was difficult to reconcile some of the comments made by Dr. Lee with what is known of scientific research. One of the many unusual assertions by Dr. Lee was the statement that "it is widely acknowledged that estradiol is a major cause of breast cancer." Whereas there is little doubt that there is an association between cigarette smoking and lung cancer, there is considerable doubt about the association between estrogen and breast cancer. Is he claiming that estrogen is an oncogene? What is the scientific evidence for such a claim? Perhaps Dr. Lee is mistaking the slight increase in

spontaneous sporadic mutations during estrogen-induced mitosis with oncogenesis; if so, he is falling into the trap that epidemiologists often experience when trying to provide "evidence" for an association between a causative agent and a particular outcome. While hormones clearly influence the promotion and possibly the spread of breast cancer, there is no scientific evidence that estrogen causes breast cancer.

In his discussion on the use of saliva to monitor progesterone activity, Dr. Lee has selectively ignored research by other authors, including the important paper by Lewis et al from New Zealand, which clearly refuted the claims for the use of saliva as a monitoring device. In spite of the convoluted explanation put forward by Dr. Lee, a correlation between salivary levels of progesterone and biological cellular response has never been established. The levels of hormones found in saliva clearly have no relationship to clinical activity and should not be used to monitor hormonal levels when treating postmenopausal women.

Clearly the enthusiasm that Dr. Lee expresses and the selective use of references to support his hypothesis for transdermal progesterone has allowed wish and desire to cloud his scientific judgment. Unlike Dr. Lee, I do conduct research in a very strictly controlled environment, and our research demonstrates that the minute amount of progesterone being absorbed from transdermal cream does not have any beneficial effect on the well-being or the health of postmenopausal women.[3] It is dangerous to espouse a regimen that has no credible scientific evidence to support a beneficial clinical response.

Like his scientific knowledge, Dr. Lee's knowledge of geography also needs to be improved. I would like to point out to Dr. Lee that our research center is not in New Zealand. That country lies in the South Pacific, some 2,000 km to the east of Australia.

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Tibolone

To the Editor:

This letter is written in response to that of Drs. Herbert Kuhl and Inka Wiegratz, published in the January-February 2003 issue of *Menopause*, in response to our letter criticizing their article in the July-August 2002 issue of the journal, which purported to show that, during daily treatment of women with 2.5mg of Tibolone (Tib), a portion of the Tib was converted to 7α-methylethinylestradiol (7α-methyl EE), which has comparable estrogenic properties to 17αEE.

In the first place, we are pleased to note that Drs Kuhl and Wiegratz now acknowledge that Tib cannot directly be aromatized and that, in fact, three steps are required before an aromatized product could in principle be produced. Secondly, 19-norandrogens are less effective substrates of aromatase; for example, in the 1970s Harry Brodie reported that the aromatization of 19-nortestosterone proceeded at less than one-fifth the rate of androstenedione. Similar conclusions were reached by Engel and by Fishman. The issue of whether or not 7α-methyl-19-nortestosterone (MENT) is a substrate for aromatase has also been addressed using human placental microsomes as a source of enzymatic activity. Moslemi et al[1] reported that MENT was not an aromatizable substrate using this preparation. On the other hand, La Morte et al[2] claimed to show aromatization of MENT but did not actually measure the rate to compare with that of testosterone. As we indicated in our previous letter, the onus is on Drs. Kuhl and Wiegratz to prove that the Δ^4 isomer of Tib is aromatized by incubating it with placental microsomes and demonstrating the formation of ring A phenolic products.

The major issue, however, is whether adult human liver is capable of aromatizing anything. Kuhl and Wiegratz quote the paper by Harada et al (1998) in support of their contention. However, the authors of this paper found that the expression of aromatase in adult liver was highest in cells surrounding a tumor that was a secondary metastasis from another source. Expression at sites distal to the tumor was minute. The authors themselves state that the expression proximal to the tumor was likely caused by factors produced by the tumor. Therefore, it cannot be ruled out that the low expression at distal sites was also due to this concentration gradient of factors emanating from the tumor. On the other hand, we used RT-PCR, one of the most sensitive tools available, to amplify aromatase transcripts and could find none in adult liver, whereas they

were abundant in human fetal liver. Kuhl and Wiegratz also quote the paper of Smuk and Schwers in 1977 in which the authors incubated 18 g of liver homogenate with 80μCi of [7–^{3}H] androstenedione and recovered 3,000 cpm of tritiated product following recrystallization. The duration of incubation was not stated, and the authors did not estimate the fractional conversion, but it was clearly infinitesimal. On the other hand, Siiteri[3] utilized a double isotope product isolation assay and failed to find any radiolabel in estradiol or estrone. It should be pointed out that the tritium-release assay for aromatase cannot be applied to adult human liver because high rates of [3H] water formation are caused by another uncharacterized reaction. Taken together, these findings do not present a great case for the efficient conversion of Tibolone to an aromatized product in the adult human liver.

Another potential site where aromatized products could be formed, however, is the gastric mucosa, as recently reported by Ueyama et al.[4] This could account nicely for the conversion of oral noresthisterone to ethynylestradiol, but because the gastric mucosa does not express 3β-HSD,[4,5] then Tib would not be converted to ring A aromatic products in this tissue.

While a level of pharmacokinetic and pharmacodynamic knowledge clearly is useful in this context, as pointed out by Drs. Kuhl and Wiegratz, at the same time, knowledge of the cell biology of steroidogenesis would be a useful safeguard against hubris.

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In reply:

In his recent letter, Dr. Simpson again focused on in vitro experiments investigating aromatization of various compounds. This unavailing discussion may serve to divert attention from the formation of 7alpha-methyl-ethinylestradiol in women treated with tibolone. Irrespective of the way by which this potent estrogen is formed, the clinical facts are:

1. Within 2 h after intake of 10 mg norethisterone acetate, average peak serum levels of 200 pg/mL ethinylestradiol (EE) have been measured.[1]
2. Within 2 h after intake of 2.5 mg tibolone, average peak serum levels of 125 pg/mL of 7alpha-methyl-ethinylestradiol (MEE) have been measured.[2]
3. The measurement was carried out by an independent institution (AAI, Neu-Ulm, Germany), using a gas chromatography/mass spectrometry method, which is the gold standard for the measurement of estrogens in serum.
4. The time course of the pharmacokinetics of EE and MEE is nearly identical to that of EE in women after intake of an oral contraceptive, suggesting a rapid conversion either in the intestinal tract during resorption or in the liver during the first passage.
5. The results can be called in question only by repeating the clinical trials and not by sophisticated assumptions.

A last remark concerning the aromatization of nortestosterone derivatives: Norethynodrel was found to exert an estrogenic potency in the rat 100 times that of norethisterone (Table 16 on page 303),[3] even though it is a prodrug of norethisterone. This suggests that the aromatization does not occur via preceding transformation into norethisterone. Moreover, after treatment of women with 10 mg norethynodrel, which was the first oral contraceptive used more than 40 years ago, high levels of EE were found in serum, and it was supposed that the preparation was contaminated with high doses of EE. Probably, the EE levels were caused by aromatization in vivo.

The liver contains many CYP enzymes that are able to introduce hydroxy groups at various positions of the steroid molecule. If a steroid with a double bond between C5 and C10, like norethynodrel or tibolone, is hydroxylized at C1 or C2, one further step of oxidation/dehydration may cause a further double bond between C1 and C2, and the resulting structure will rapidly be converted to a phenolic ring A by keto-enol tautomery.

Concerning the term "hubris," which was used by Dr. Simpson in his letter, he should know that the tone of our letter was set by the tone of Dr. Simpson's first Letter to the Editor ("In conclusion, this paper is based on a series of misassumptions that seriously undermine its credibility").

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Nasal Administration

To the Editor:

Thank you for a very extensive and illustrative article on nasal application of drugs.[1] However, one aspect of nasal administration seems to be overlooked: The local effects on the brain.

Several animal experiments document an increased concentration of substances, including steroids, in the brain arterial blood or the brain tissues after nasal application or infusion into the nasal vein blood. The increase is correlated with concentrations in parallel-obtained blood samples from a peripheral artery or similar control samples. Hardly any evidence is available from investigations in humans. The mechanism is probably similar to the local transfer of steroids to uterus after vaginal application, which is well documented in clinical trials.

The observations raise two questions:

1. Does nasal application of steroids to menopausal women open a new alley for treatment because of local effects on the brain, eg, induction of changes in mood without systemic effects, or a targeted reduction in the releasing hormone, follicle-stimulating hormone or luteinizing hormonesecretion?
2. Does nasal application have the potential of inducing side effects?

A PubMed search under the names "Cicinelli E," "Skipor J," or "Einer-Jensen N" will reveal references related to the questions.

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In reply:

We appreciate the interest of Dr. Einer-Jensen in our review article. We would agree that, based on animal data, it is possible that there could theoretically be increased concentrations of sex steroids in the brain following intranasal administration.[1] This could also be similar to the local effects seen on the uterus with vaginal administration of sex steroids.[2] However, at this time, human data is lacking in this area.

Regarding differences in side effects using intranasal administration, our review of the literature seems to indicate an overall lower incidence of side effects compared with other routes of administration.[3]

The effect of estrogen replacement therapy/hormone replacement therapy on the brain is an important area of research. This is particularly true when considering other routes of administration. The intranasal route of administration should open up new areas of study for the treatment of menopausal disorders.

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