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LETTERS TO THE EDITOR

EXPERIMENTS ON PROLIFERATION OF NORMAL HUMAN BREAST TISSUE IN NUDE MICE DO NOT SHOW THAT PROGESTERONE DOES NOT STIMULATE BREAST CELLS

In the recent paper by Laidlaw and colleagues (1) entitled "The proliferation of normal human breast tissue implanted into athymic nude mice is stimulated by estrogen but not progesterone," they claimed that "E_2 is sufficient to stimulate human breast epithelial cell proliferation at physiologically relevant concentrations and that P does not affect proliferation either alone or after E_2 priming." Taken at face value these results strongly suggest that the increased breast cell proliferation seen in the luteal phase of the menstrual cycle compared with the follicular phase is solely an estrogen effect. This would be an important finding for a number of reasons, in particular because it would remove any doubts regarding possible adverse effects on breast cancer risk by adding progestins to estrogen replacement therapy. The results presented in Laidlaw *et al.*'s paper do not, however, support such a strong conclusion.

The results in the paper that are relevant to this issue are shown in Table 1. The results relating to E_2 dosing alone show that cell proliferation (TLI) was approximately proportionately related to E_2 dose up to a dose of a 2-mg pellet, but a further increase to a 6-mg pellet had no additional effect. A 2-mg pellet appears thus to be the maximally effective dose of E_2 as regards breast cell proliferation.

The P pellet alone produced no breast cell division, possibly solely due to the fact that no PR was present. This is not relevant to the human menstrual cycle situation since the progesterone in the luteal phase is preceded by follicular levels of E_2.

When a [1]P pellet was added to the maximally effective dose of E_2 (*i.e.* the 2 mg pellet) no further increase in cell

TABLE 1. E_2 and P dose and serum levels, breast cell TLI and PR levels[a]

E_2 dose (mg pellet)	E_2 serum (pmol/liter)	P dose (mg pellet)	P serum (nmol/liter)	Breast cell TLI (cf baseline)	PR (% cells)
0	~100	0	~4	0	0
0.5	~400	0	~4	+1.0	–
1.0	~600	0	~4	+1.2	–
2.0	~1300	0	~4	+2.0	~10
6.0	~4400	0	~4	+2.1	–
0	~100	4	~35	0	0
2.0	~1300	4	~35	+2.0	~10

[a] Abstracted from Laidlaw *et al.* (1).

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EXPERIMENTS ON PROLIFERATION OF NORMAL HUMAN BREAST TISSUE IN NUDE MICE DO NOT SHOW THAT PROGESTERONE DOES NOT STIMULATE BREAST CELLS: REPLY

We read with interest the comments made by Professor Pike and his colleagues on our recent paper entitled "The proliferation of normal human breast tissue implanted into athymic nude mice is stimulated by estrogen but not progesterone" (1).

In reply to the first point that progesterone alone (P) would not be expected to increase proliferation because the breast epithelial cells do not express the progesterone receptor (PR), we would like to point out that these cells also do not express the estrogen receptor (ER), yet estradiol (E_2) treatment increases proliferation and causes a 15- to 20-fold increase in the number of cells expressing PR.

With regard to the second point, we agree that in the experiments where the mice were treated with a P pellet after "priming" with a 2-mg E_2 pellet, we may not have seen an effect on proliferation because the breast epithelium was already maximally stimulated. This point was made when the manuscript was reviewed before acceptance, and we have addressed it to some extent in the discussion of our results. We shall be able to comment further when the experiments to measure proliferation after P treatment has been added to a submaximally-stimulating dose of E_2 have been completed. However, we have recently used a dual-labeling technique to determine whether those cells that incorporate tritiated thymidine also express the PR. This study has shown that there are two distinct populations of breast epithelial cells that respond differently to E_2 treatment; there are those that increase their PR content and those that proliferate (3). Addition of P to E_2 treatment does not result in a recruitment of PR positive cells to the proliferating cell population. These findings suggest that proliferative breast epithelial cells do not have the capacity to respond to P, and we predict that P treatment after administration of a submaximally stimulating pellet of E_2 will have no effect.

These data together with those obtained from our original study of normal human breast tissue implanted into athymic nude mice force us to conclude that it is E_2 and not P that stimulates proliferation in the normal human breast.

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proliferation was noted. This is interpreted in the paper as implying that "P does not affect proliferation." The results, however, only support the much weaker statement that "the addition of P to a maximally stimulatory dose of E_2 does not further increase cell proliferation."

These studies of Laidlaw and colleagues are most interesting. To be able to truly draw the very strong conclusion they did in this paper, they need to repeat the E_2 + P experiment with lower doses of E_2, *i.e.* at doses lower than that producing maximum E_2 stimulated breast cell proliferation. They should also use a range of doses of E_2 and P as the binding of steroids in humans is different from that in the mouse and the "correct" physiological dose is not obvious.

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Letter 2 Continued

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