

CORRIGENDUM

Volume **232**, Number 2 (1997), in Article No. RC976283, "Estrogen Affects Development of Alveolar Structures in Whole-Organ Culture of Mouse Mammary Glands," by John Barlow, Theresa Casey, Jen-Fu Chiu, and Karen Plaut, pages 340–344: In experiments conducted following this report the authors identified occasional treatments in which the reported effect of estrogen on mammary development was not observed using the methods described in the article.

In a series of four independent experiments, serial dilutions of various estrogenic compounds, of antiestrogens, or of nonestrogenic steroids were examined for their effect on alveolar development *in vitro*. In each experiment mammary glands were cultured in positive (phenol red free Waymouth's medium containing lactogenic growth factors insulin, aldosterone, hydrocortisone, prolactin, and epidermal growth factor (IAHPE) and 8 ng/ml estradiol), and negative (phenol red free Waymouth's medium containing IAHPE with and without 1% ethanol vehicle) control medium. Stock solutions of 17- β estradiol in ethanol were prepared and stored at -20°C for use in positive control medium, and a single stock source of ethanol vehicle was used. During these experiments the authors obtained inconsistent results for glands cultured in control medium. After eliminating the possibility of estradiol contamination of negative controls, or errors in the estradiol stock solutions, they examined methods of media preparation as a potential source of error. They found lack of alveolar development was possibly associated with aliquots of media which had been first in a series of small aliquots of media being filter sterilized.

Based on this finding, the authors conducted experiments to examine the effect of filtration of small aliquots of media on lobulo-alveolar development. They cultured mammary glands in paired treatments of filtered or unfiltered 30-ml aliquots of media. Lobulo-alveolar development occurred in all unfiltered treatments, but not in the filtered treatments. Lobulo-alveolar development was not affected by estrogen or phenol red concentration in filtered or unfiltered treatments. Further, they used cellulose nitrate membrane filters which have significant protein binding affinities, also supporting the possibility that filtering reduced the concentrations of the required lactogenic growth factors (IAHPE) for initial aliquots of media. They also measured insulin levels by radioimmunoassay (RIA; Insulin Coat-A-Count kit, Diagnostic Products Corp, Los Angeles, CA) in stored aliquots of medium from the experiments. Reduced insulin concentrations in filtered media were associated with a lack of lobulo-alveolar development in these experiments. It is also possible that other hormones and growth factors were being removed by filtration during media preparation. The interactions of hormones and growth factors in the regulation of mammary gland development and lactation are incompletely understood, and further study of hormonal effects during development *in vitro* is warranted.

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