retic animals (6). The plasma 1,25-$(OH)_2D_3$ levels increased sharply from 50 pg/ml on the day before calving to a value of near 200 pg/ml at parturition in the paretic animals (Fig. 2). This value remained high during the remaining 2.5 days of study postpartum. In contrast, the nonparetic animals showed a much slower increase in plasma 1,25-$(OH)_2D_3$ levels beginning the day before parturition and reaching a maximum of approximately 100 pg/ml at 2 days postpartum. At each sampling, beginning on the day before calving to 2.5 days postpartum, there was a significantly higher concentration of 1,25-$(OH)_2D_3$ in the plasma of the paretic animals than in that of the nonparetic controls ($P < .01$) as determined by Student's t-test.

It is apparent, therefore, that in spite of their plasma 1,25-$(OH)_2D_3$ levels being significantly increased in response to the hypocalcemia brought about by milk formation, the paretic animals fail to adjust their plasma calcium concentration. It is well known that during this period the concentrations of circulating immunoreactive parathyroid hormone are also elevated in the paretic animals (6), a result which has been confirmed in our laboratories (7). These results demonstrate that the paretic animals are fully capable of synthesizing 1,25-$(OH)_2D_3$ in response to the hypocalcemia of milk formation, but that the target organs of this very potent hormone as well as the target organs for the parathyroid hormone are apparently resistant. Thus the search for a mechanism at least in our laboratories will be directed toward understanding the basis for end-organ resistance to these two potent calcium-mobilizing hormones.

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References and Notes

1. C. Weighton, *Vet. Rec.* **70**, 1006 (1958).
2. J. W. Hibbs, *J. Dairy Sci.* **33**, 758 (1950).
3. H. F. DeLuca, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 2211 (1974); M. Garabedian, M. F. Holick, H. F. DeLuca, I. T. Boyle, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1673 (1972).
4. J. A. Eisman, A. J. Hamstra, B. E. Kream, H. F. DeLuca, *Arch. Biochem. Biophys.* **176**, 235 (1976); *Science* **193**, 1021 (1976).
5. National Academy of Sciences–National Research Council, *Nutrient Requirements of Dairy Cattle*, (Government Printing Office, Washington, D.C., Publ. No. ISBN0-309-01916-8, rev. ed. 4, 1971).
6. G. P. Mayer, C. F. Ramberg, Jr., D. F. Kronfeld, R. M. Buckle, L. M. Sherwood, G. D. Aurbach, J. T. Potts, Jr., *Am. J. Vet. Res.* **30**, 1587 (1969).
7. N. A. Jorgensen, *J. Dairy Sci.* **57**, 933 (1974).
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Human Breast Cancer: Biologically Active Estrogen Receptor in the Absence of Estrogen?

Abstract. *The human breast cancer cell line MCF-7 does not require estrogen for growth, but paradoxically its growth is inhibited by antiestrogens. Our results show that, unlike normal target cells, MCF-7 cells carry most of their estrogen receptors in their nuclei even when these receptors are not charged with estrogens. The receptors for androgen and for progesterone, on the other hand, are localized in the cytoplasm as usual. Therefore, it is possible that the growth of these abnormal cells is stimulated by estrogen receptor in spite of the absence of the hormone and that the binding of antiestrogen molecules antagonize this stimulation.*

The MCF-7 cell line was derived from a hormone-dependent human metastatic breast cancer (1). Although these cells are not dependent on estrogen, estrogen receptor is present (2), and at least one antiestrogen (Tamoxifen) strongly inhibits growth (3). To confirm the generality of this antiestrogen action, we treated growing MCF-7 cells with the antiestrogen Nafoxidine (Upjohn U-11,100A) for 48 hours in medium with 2 percent fetal calf serum that had been stripped of steroids by treatment with charcoal. The uptake of [^{3}H]thymidine into DNA was markedly reduced by Nafoxidine (see Fig. 1). In spite of the absence of estrogen in the medium, estradiol alone showed only slight stimulation of thymidine uptake, demonstrating again that the hormone is not required for growth. Estradiol did, however, completely reverse the inhibition of thymidine uptake caused by Nafoxidine, as it had also reversed the effect of Tamoxifen. It seemed likely, therefore, that antiestrogens were acting through the estrogen receptor, thus explaining why estradiol countered their inhibitory effects. But why did the cells not appear to require estrogen otherwise?

A possible answer came from examination of the estrogen receptor distribution between nuclei and cytoplasm of MCF-7 cells. Using a new modification (4) of our ligand exchange assay for protamine-precipitated estrogen receptor (5), we found that 75 percent of the estrogen receptor in MCF-7 cells was found in the cell nuclei, even though these cells were growing in the absence of estradiol (Table 1). This receptor was not charged (bound) with estradiol, since it bound [^{3}H]estradiol in the assay at 4°C as readily as did free cytoplasmic receptor. The distribution is in striking contrast to that in normal target tissues, in which little if any uncharged estrogen receptor is ever found in nuclei. Yet this distribution does not appear to be an artifact of the preparation, since the receptors for progesterone and androgens, both of which we have shown to be present in these cells (6), are restricted to the cytoplasm, as expected in the absence

Table 1. Subcellular distribution of steroid receptor in MCF-7 cells. Cells were grown to confluence as described in Fig. 1; 3 to 5 days prior to harvest, hydrocortisone ($1 \times 10^{-9} M$) and ovine prolactin (5 μg/ml) were added to the charcoal-stripped serum medium. For progesterone receptor measurements, estradiol ($1 \times 10^{-7} M$) was also added. At harvest, cells were gently removed by a 15- to 30-minute incubation with Hanks Mg^{2+}-free, Ca^{2+}-free ethylenediaminetetraacetate solution, washed, and homogenized in 5 mM sodium phosphate (pH 7.4), 1 mM thioglycerol, and 10 percent glycerol. Free cytosol and nuclear binding sites were assayed by the protamine sulfate method as previously described (4, 5). Numerals in parentheses indicate number of experiments performed, with two to five confluent flasks per experiment.

	Receptor (pmole/mg DNA)	
	Cytosol	Nuclei
Estrogen receptor	0.47 ± 0.06 (7)	1.40 ± 0.07 (7)
Progesterone receptor	1.25	0.12
Androgen receptor	0.093 ± 0.007 (8)	0.01 (3)

Table 2. Effects of estrogen treatment on estrogen receptor distribution in MCF-7 cells. Intact MCF-7 cells grown to confluence in T-75 flasks (see Fig. 1) were treated for 1 hour with either $10^{-8} M$ nonradioactive estradiol or steroid-free vehicle. Cytoplasmic and nuclear extracts were assayed for estrogen receptor by the protamine method (4, 5); free receptor was measured by uptake of [^{3}H]estradiol at 4°C, while total receptor was determined by incubation at 30°C (cytosol) or 37°C (nuclear) for 2.5 hours. The difference in total (30°C or 37°C) and free (4°C) yielded the values for bound receptor. Abbreviations: Rc, unoccupied cytoplasmic receptor; RcE, estrogen-occupied cytoplasmic receptor; Rn, unoccupied nuclear receptor; and RnE, estrogen-occupied nuclear bound receptor.

	Receptor (pmole/mg DNA)	
	Control	Estrogen treated
Rc	0.4	0
RcE	0	0
Rn	1.2	0
RnE	0	1.5
Total	1.6	1.5

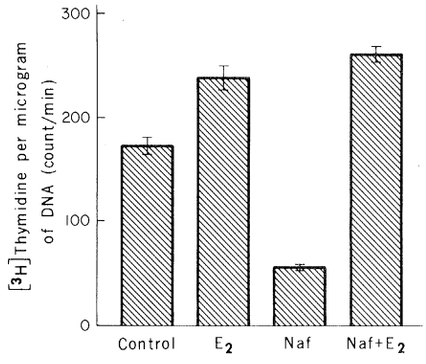

Fig. 1. Effects of estrogen (E_2) and antiestrogen (*Naf*) on DNA synthesis in MCF-7 cells. These cells were grown in 5 percent CO_2 in air at 37°C in medium consisting of Earle's basal minimal essential medium (MEM; Gibco), supplemented with nonessential amino acids (Gibco), 2 m*M* L-glutamine (Gibco), 0.006 μg of insulin (Sigma) per milliliter, 5 percent calf serum (Gibco), and 50 μg of gentamicin (Schering) per milliliter. Before confluence, the medium was changed to one containing 2 percent fetal calf serum (Gibco) which had been stripped by shaking for 30 minutes at 45°C with the pellet from two volumes of dextran-coated charcoal (*6*). Some flasks received media that also contained $10^{-8}M$ estradiol-17β or $5 \times 10^{-7}M$ Nafoxidine, or both. After 48 hours, the medium was replaced by 5 ml of MEM containing 0.5 μc of [^{3}H]thymidine and 10 m*M* Hepes, *p*H 7.4. The cells were incubated an additional 30 minutes, then rinsed with chilled MEM containing 10 μg of unlabeled thymidine per milliliter, scraped from the plastic with the use of perforated cellophane and 1*M* perchloric acid, and washed three times with chilled 0.5*M* perchloric acid. The pellet was hydrolyzed in 0.5*M* perchloric acid at 90°C for 15 minutes. Samples were counted for radioactivity and others were assayed for DNA by the method of Burton (*8*). Bars represent mean ± standard error of four culture flasks.

of their hormones. The presence of uncharged estrogen receptor in MCF-7 nuclei may explain in part the nuclear binding of estradiol seen by Brooks *et al.* (*2*), who suggested rather that cytoplasmic receptor exposed to estradiol at 0°C might have translocated into the nucleus; the interpretation of these data at the time was obscured by apparently similar observations in normal porcine uterus.

In spite of the unusual distribution of uncharged estrogen receptor which we have found in MCF-7 cells, the cytoplasmic receptor remains capable of translocating estradiol into the cell nucleus. When estradiol was added to the medium, the remaining receptor disappeared from the cytoplasm and appeared as a receptor-estradiol complex in the nucleus (Table 2). Furthermore, the previously uncharged nuclear receptor also became fully charged with estradiol.

These findings suggest the possibility that the uncharged nuclear estrogen receptor is capable of stimulating the growth of MCF-7 cells. The slight further stimulation of thymidine uptake by estradiol would then be due to the resulting small increase in nuclear receptors translocated from the cytoplasm. If this interpretation is correct, then the binding of antiestrogen molecules must inactivate the receptor, while the reversal of the antiestrogen effect by estradiol results from the blockage of antiestrogen binding and subsequent reactivation of the receptor molecule.

We have discovered at least one other cell line from another human breast cancer to have a similar distribution of estrogen receptor. Antiestrogen therapy of human breast cancer is known to cause objective tumor remission in many cases, success being mostly correlated with the presence of estrogen receptor in the patient's tumor (*7*). If the high levels of free estrogen receptor in nuclei are present in some patients with breast carcinoma, hormone-ablative therapy would probably be less successful since these nuclear receptors would be fully capable of activating cell division even in the absence of hormone. In fact, this phenomenon may explain the resistance of a number of receptor-containing breast cancers to hormone-ablative therapy. If so, antiestrogen treatment in these special cases of high estrogen receptor in nuclei may very well succeed where other endocrine therapies would fail.

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References and Notes

1. H. D. Soule, J. Vasquez, A. Long, S. Albert, M. J. Brennan, *Natl. Cancer Inst.* **51**, 1409 (1973).
2. S. C. Brooks, E. R. Locke, H. D. Soule, *J. Biol. Chem.* **248**, 6251 (1973).
3. M. E. Lippman and G. Bolan, *Nature (London)* **256**, 592 (1975).
4. D. T. Zava, N. Y. Harrington, W. L. McGuire, *Biochemistry* **15**, 4292 (1976).
5. G. C. Chamness, K. Huff, W. L. McGuire, *Steroids* **25**, 627 (1975).
6. K. B. Horwitz, M. E. Costlow, W. L. McGuire, *ibid.* **26**, 785 (1975).
7. W. L. McGuire, *Cancer* **36**, 638 (1975).
8. K. Burton, *Biochem. J.* **62**, 315 (1956).
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Cyclic GMP Accumulation Causes Degeneration of Photoreceptor Cells: Simulation of an Inherited Disease

Abstract. *Guanosine 3',5'-monophosphate (cyclic GMP) metabolism in developing eye rudiments of* Xenopus laevis *embryos in culture is disrupted by the phosphodiesterase inhibitor isobutylmethylxanthine. At low concentrations of inhibitor the rudiments develop normally, but at higher concentrations of the inhibitor, cyclic GMP accumulates in the rudiments and the retinal photoreceptor cells degenerate selectively. The isobutylmethylxanthine-induced photoreceptor degeneration is associated with an accumulation of cyclic GMP and, in this respect, it simulates an early biochemical defect in the inherited degenerative disease of* rd *mice.*

Inherited degenerative diseases of the retina cause blindness in mice, rats, dogs, and humans. About 100,000 persons are afflicted with the degenerative disease retinitis pigmentosa in the United States alone (*1*). Morphological and biochemical data from frogs, cattle, and human retinas show that the rod photoreceptor cells of vertebrate species are very similar. Human photoreceptor cells that degenerate from disease may have metabolic defects which are common to those of animal disorders, and animal studies may be helpful in understanding and developing treatment for human diseases causing blindness. Following this rationale, we are using animal models to describe and analyze the metabolic characteristics of normal visual cells and the causes of photoreceptor degeneration that result from inherited or acquired diseases.

We advanced the hypothesis that an abnormality in guanosine 3',5'-monophosphate (cyclic GMP) metabolism leads to degeneration and death of retinal photoreceptor cells (*2*). Supporting data came from studies of isolated rod outer segments from normal retinas and from our investigations of inherited retinal disorders of rats and mice. Dark-adapted rod outer segments from normal retinas contain high concentrations of cyclic GMP which are reduced by exposure to light (*3*). These observations suggest the possible involvement of cyclic GMP in dark-light adaptation or in the visual process (*4*). In the Royal College of Surgeons (RCS) strain of rats, which are afflicted