B. R. Cooper, I. C. Wilson, M. P. Plotnikoff, *ibid.*, 447; L. Schenkel-Hulliger, W. P. Koella, A. Hartmann, L. Maitre, *Experientia* **30**, 1168 (1974); A. J. Prange, Jr., *et al.*, *Life Sci.* **16**, 1907 (1975).
4. I. M. D. Jackson and S. Reichlin, *Life Sci.* **14**, 2259 (1974); M. J. Brownstein, M. Palkovits, J. M. Saavedra, R. M. Bassiri, R. D. Utiger, *Science* **185**, 267 (1974).
5. S. Almqvist, *Front. Horm. Res.* **1**, 38 (1972).
6. M. B. Rosenthal, *New Eng. J. Med.* **291**, 1308 (1974).
7. J. Jacob, J. M. Girault, R. Peindares, *Neuropharmacology* **11**, 1 (1972). The injection coordinates used in our experiment were modified somewhat from the original paper (A = 1 mm; L = 1 mm; DV = 12 mm).
8. By "pressure patterns" we are referring to the different forms or types of activity that are recorded by intraluminal pressure devices in the large intestine. Classically there are waves of types I, II, and III; however, this system of classification has proved to be oversimplified and has gradually fallen into disuse.
9. B. J. Ormston, *Front. Horm. Res.* **1**, 42 (1972).
10. J. F. Wilber, *Annu. Rev. Med.* **24**, 353 (1973).
11. E. Gellhorn, *Autonomic Imbalance and the Hypothalamus* (Univ. of Minnesota, Minneapolis, 1957), pp. 147–158.
12. S. C. Truelove, *Physiol. Rev.* **46**, 457 (1966).
13. S. C. Wang, G. Clark, F. L. Dey, S. W. Ranson, *Am. J. Physiol.* **130**, 81 (1940); G. Strom and B. Uvnas, *Acta Physiol. Scand.* **21**, 90 (1950).
14. L. Demling, *Digestion* **2**, 362 (1969); E. D. Williams, *Proc. R. Soc. Med.* **59**, 602 (1966); J. J. Bernier, J. C. Savoie, H. Garnier, D. Cattan, M. Boury, J. C. Rambaud, C. Bognel, C. Prost, *Gastroenterology* **54**, 469 (1968).
15. A typical polygraph recording apparatus was used to accumulate all tracings. The area under the curve was shaded to enhance contrast, which facilitated photographic duplication. The base line for each figure was arbitrarily chosen and remained constant within each figure.
16. This work was supported by DHEW Training Grant GM-00109 and HL-15426. The TRH was generously supplied by Abbott Laboratories, North Chicago, Ill.

20 September 1976; revised 19 November 1976

Adequate Response of Plasma 1,25-Dihydroxyvitamin D to Parturition in Paretic (Milk Fever) Dairy Cows

Abstract. *The concentration of 1,25-dihydroxyvitamin D was measured by means of a radioactive receptor assay in the plasma of cows during the period immediately prior to, during, and following parturition. Nonparetic cows showed initially a slight decrease in plasma 1,25-dihydroxyvitamin D which was followed by a significant increase during parturition and 2 days postpartum. The highest concentration achieved in the control or nonparetic cows was 100 picograms per milliliter. In the paretic animals the plasma 1,25-dihydroxyvitamin D concentration increased sharply during the day preceding calving and reached a maximum of 200 picograms per milliliter at parturition. This level was maintained during the ensuing 2.5 days. These results demonstrate that parturient paresis cannot be the result of insufficient synthesis or secretion of 1,25-dihydroxyvitamin D.*

Parturient paresis (milk fever) is a metabolic disease occurring at or near parturition, especially in high-producing dairy cows. The onset is associated with the initiation of lactation and is characterized by a rapid decline in serum calcium and phosphorous concentrations. Ultimately, the animal suffers from a low-calcium tetanic state which is usually rapidly corrected by the infusion of calcium gluconate. If the disease is left untreated, approximately 60 to 70 percent of the animals will succumb to this condition (*1*).

The underlying cause of parturient paresis remains unknown despite many years of investigation. Several hypotheses have appeared; one of the more popular is the idea that bone and intestine are resistant to the parathyroid hormone which is secreted in response to the hypocalcemia brought about by milk formation (*2*). Because parathyroid hormone also stimulates production of 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], a major calcium-mobilizing hormone (*3*), it is possible that parturient paresis results from a failure to produce 1,25-$(OH)_2D_2$ in response to hypocalcemia and parathyroid hormone. The absence of adequate 1,25-$(OH)_2D_3$ would then cause skeletal resistance to parathyroid hormone and inadequate calcium absorption, giving rise to parturient paresis.

Recently we developed an assay for 1,25-$(OH)_2D_3$ which is highly specific and which can be carried out on reasonably small amounts of plasma (*4*). By using this assay we have measured the plasma concentrations of 1,25-$(OH)_2D_3$ in paretic and nonparetic animals prior to, during, and immediately following parturition. The results clearly demonstrate that paretic animals are fully capable of increasing their plasma 1,25-$(OH)_2D_3$ levels in response to the hypocalcemia brought about by parturition and milk formation.

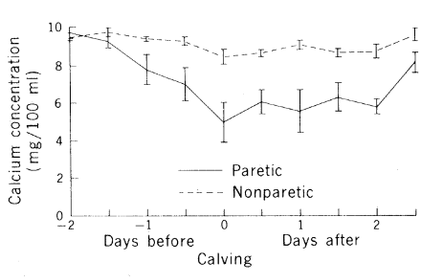
Eight Holstein cows (5 years or older) were fed a basal diet of low-moisture silage made from alfalfa, plus 1.8 kg of grain. This diet was adequate in energy and protein according to National Research Council requirements for maintenance and pregnancy (*5*). The diet supplied 80 to 85 g of calcium and 30 to 35 g of phosphorus daily from 35 days prepartum to 2.5 days postpartum.

Blood (50 ml) was collected from the jugular vein every 12 hours from 2 days before to 2.5 days after calving in heparinized tubes (5 unit/ml) packed in ice. In the case of parturient paretic animals, blood was always taken just prior to calcium infusions (500 ml of 25 percent calcium borogluconate). The plasma was separated immediately in a refrigerated centrifuge at 4°C and divided into 5-ml portions which were stored at −10°C until assayed.

The assay for 1,25-$(OH)_2D_3$ was carried out according to the method of Eisman *et al.* (*4*). Calcium was measured in the presence of 0.1 percent $LaCl_3$ by atomic absorption spectrophotometry with a Perkin-Elmer Model 403 instrument.

Four of the eight cows showed clinical signs of parturient paresis, that is, cold extremities, lateral recumbency, and hypocalcemia. These animals responded to infusions of calcium borogluconate and were, therefore, diagnosed as having parturient paresis.

Plasma calcium levels in the paretic cows decreased from 9.5 mg/100 ml at 2 days before calving to 5 mg/100 ml at calving (Fig. 1). On the other hand, the nonparetic animals showed only a slight decrease in serum calcium concentration during this period. Although the calcium infusions relieved the paretic symptoms, an elevated level of calcium in the serum was not sustained since the values were not appreciably higher at 0.5 days after the infusion. These results are consistent with previous reports for parturient pa-



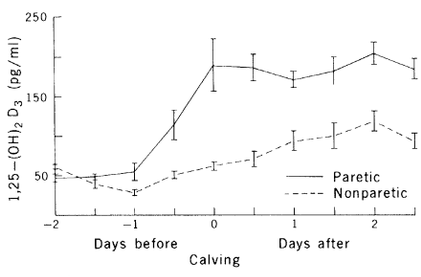


Fig. 1 (left). The concentration of calcium in the plasma of paretic and nonparetic cows at parturition. Day 0 represents parturition (calving), while negative figures represent days prior to calving. The data are presented as the mean ± standard error (vertical bars) for four animals in each group. Fig. 2 (right). The concentration of 1,25-$(OH)_2D_3$ in the plasma of paretic and nonparetic cows at parturition. Designations are as described in Fig. 1. Vertical bars represent standard errors, and there were four values for each point on the curve.

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.

R. L. HORST, J. A. EISMAN
N. A. JORGENSEN, H. F. DELUCA
Departments of Biochemistry and *Dairy Science, College of Agricultural and Life Sciences, University of Wisconsin–Madison, Madison 53706*

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 (19 → *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).
8. Supported by Hatch Act Funds No. 5051 of the College of Agricultural and Life Sciences of the University of Wisconsin–Madison, PHS grant AM-14881, and the Harry Steenbock Research Fund.

30 August 1976; revised 26 November 1976

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