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# Dietary Zinc and Parturition in the Rat

## II. Myometrial Gap Junctions

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## ABSTRACT

The number and appearance of uterine myometrial gap junctions in spontaneously delivering rats was evaluated as a function of dietary zinc. Based on quantitative electron microscopy, the number of gap junctions detected in the tissue from zinc-deficient rats was only 49 or 39% of *ad-libitum*-fed controls, dependent on the basis of comparison (per cell or per 1000 μm membrane). Moderate food restriction (pair-feeding to the voluntary intake of the zinc-deficient rats) reduced the number to 70% of the *ad-libitum*-fed control. The results are interpreted as contributory to the irregular and poorly synchronized uterine pressure cycle patterns previously detected in zinc-deficient female rats and may indicate poor compliance to estrogen-controlled gene expression.

**Index entries:** Zinc; zinc deficiency; parturition; gap junctions; uterus; estrogen; gap junctions, number and appearance of in spontaneously delivering rats as a function of dietary zinc; estrogen-controlled gene expression.

## INTRODUCTION

The female rat fed a diet low (<3 ppm) in zinc during gestation suffers a difficult labor, which may be delayed in onset, prolonged, and

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accompanied by excessive bleeding (*1*). Pups born alive are usually abandoned or cannabilized by the dam. These difficulties can be generated by offering the low-zinc diet as late as d 18 of gestation or prevented by zinc injections beginning on d 19 (*2*). The effects of zinc appear to be relatively specific in that the syndrome is not produced by restriction of total food intake (pair-feeding) or by consumption of diets deficient in protein, thiamin, copper, or manganese (*3*, *4*).

O'Dell et al. (*5*) drew attention to the similarities between zinc deficiency and aspirin toxicity in pregnant rats and proposed that zinc was necessary for synthesis or release of eicosanoids, especially prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Bunce et al. (*6*) observed that appearance of the luteolytic marker enzyme, 20α-hydroxysteroid dehydrogenase, was delayed by about 8 h in zinc-deficient pregnant rats and that $PGF_{2\alpha}$ injection was successful in forcing the appearance of the enzyme in such animals, but efforts to demonstrate consistent alterations in eicosanoid synthesis in zinc-deficient rats have been unsuccessful (*7–10*).

Lytton and Bunce (*11*) observed that the uterine pressure cycle pattern was abnormal in zinc-deficient pregnant rats during oxytocin-induced labor. Both contractile synchrony and propagation appeared to be diminished. In particular, the birth of individual pups was prolonged and accompanied by intense abdominal straining. The coordination and propagation of contractile activity of the uterus during labor depends upon functional cell-to-cell communication through the gap junction network (*12*). The number and size of myometrial gap junctions increases enormously during the last 48 h predelivery, as the uterus comes under estrogen dominance (*13–15*).

We have sought to determine the status of the uterine gap junction network during or immediately after spontaneous labor in zinc-deficient, as opposed to zinc-normal, rats, using quantitative electron microscopy. The number of myometrial gap junctions was diminished by 30% by food restriction and by >50% by consumption of a low-zinc diet.

## MATERIALS AND METHODS

Eighteen mature virgin female Sprague–Dawley rats (VPI & SU vivarium), weighing 250–300 g, were paired with males and checked daily for the presence of vaginal plugs or sperm in a vaginal lavage. Upon evidence of mating (designated d 1), they were placed in individual polypropylene cages (27 × 21 × 15 cm), containing a floor layer of chopped corn-cob absorbent. The animal room was maintained at 21–23°C temperature and 65% relative humidity. Air was exchanged and filtered continuously. Lighting was regulated automatically to provide a schedule of 12 h on and 12 h off. For the first 9 d of pregnancy, all rats were allowed free access to Purina lab chow and distilled water. On d 10, they were randomly assigned to either a low-zinc diet (<3 ppm) or a control diet (40-ppm zinc), offered either *ad libitum* or pair-fed by group

mean to the voluntary intake of the low-zinc group. The diet has been described in detail in a previous publication (*6*).

Each animal was allowed to begin delivery spontaneously, which in our colony may occur on either the afternoon of d 22 or the morning of d 23. During or immediately at the conclusion of parturition, animals were sacrificed by cervical dislocation. Plasma was collected by cardiac puncture and analyzed for zinc by conventional atomic absorption spectrophotometry (Table 1). Samples of myometrium (approximately 1 × 2 cm × 1 mm) were removed, pinned out on dental wax in a Petri dish, and fixed by one of the following methods. (A) Sample was immersed in a 1:1 mixture of a 2%-solution of osmium tetroxide and a 4%-solution of glutaraldehyde in cacodylate buffer (0.1*M*, pH 7.4) for 1.5 h, rinsed briefly in cacodylate buffer, and then postosmicated in 2% osmium tetroxide in 0.1*M* cacodylate buffer for an additional 2 h. The fixing procedure was performed at 4°C (*16*). (B) Sample was immersed in 2.5% glutaraldehyde in 0.1*M* cacodylate buffer, pH 7.4, at 4°C for 1.5 h, then washed in cold cacodylate buffer, and postfixed for an additional 2 h at 4°C in 2% osmium tetroxide dissolved in 0.1*M* cacodylate buffer (pH 7.4).

After fixation, specimens were cut into blocks of approximately 1 $mm^3$, washed in cold distilled water, soaked for 10 h in aqueous 1% uranyl acetate at 4°C, dehydrated in an ethanol and acetone series, and embedded in a mixture of Epon and Araldite (*17*). Transverse sections of myometrial cells were obtained by gluing tissue blocks onto wooden dowels in such a way that the long axes of the cells were perpendicular to the plane of section. Thin sections were cut with a diamond knife on a Sorvall-Blum MT-2B ultramicrotome, retrieved on 100-mesh Formvar-coated copper grids, and stained in aqueous uranyl acetate (*18*) and lead citrate (*19*). Sections were examined and photographed with a Zeiss EM 10 CA electron microscope, operated at an accelerating potential of 60 kV.

TABLE 1
Weight Gain, Litter Size, and Plasma Zinc

| Treatment | | Total weight gain during gestation, g | Total pups in litter | Plasma Zn, μg/mL |
|---|---|---|---|---|
| *Ad-libitum*-fed | Range | 75 – 170 | 8 – 13 | 0.86 – 1.15 |
| | Mean ± SEM ($n$ = 6) | 128 ± 20 | 10.8 ± 1.0 | 0.98 ± 0.07 |
| Pair-fed | Range | 55 – 78 | 6 – 14 | 0.52 – 0.86 |
| | Mean ± SEM ($n$ = 6) | 67 ± 4 | 11.0 ± 1.3 | 0.63 ± 0.08 |
| Low Zn, 3 ppm | Range | 50 – 107 | 10 – 17 | 0.35 – 0.69 |
| | Mean ± SEM ($n$ = 6) | 70 ± 10 | 12.6 ± 1.2 | 0.49 ± 0.07 |

The length of lateral plasma membrane surveyed for each tissue sample was determined by the method of Weibel (*20*). Each tissue sample was photographed 10–15 times at a primary magnification of 12,000× and printed at 35,000× on 20 × 25 cm paper. A transparent grid (multipurpose coherent test grid) was superimposed over each photo. The total length of membrane was determined by counting the intersections of the membrane with the grid and using the following formula:

$$B = \pi/2 \times I/L \times A \times CF$$

where $B$ = length of membrane; $I$ = average number of intersects determined from two estimates taken at right angles to one aother; $L$ = total length of probe lines (138.6 cm or 84 lines of 1.65 cm length); $A$ = area of the photograph examined in the grid system (384 $cm^2$); and $CF$ = magnification correction factor.

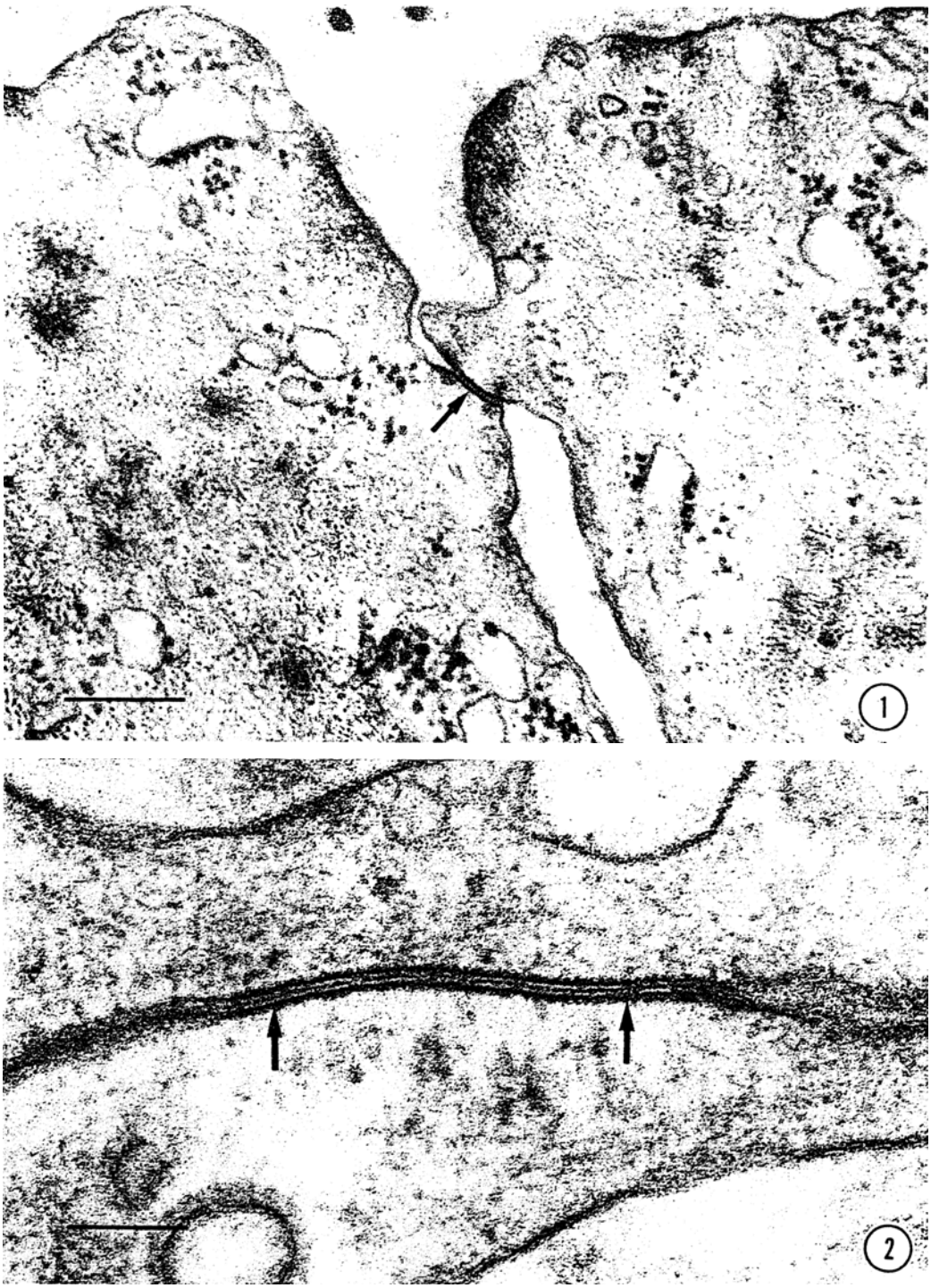
## RESULTS

Figures 1 and 2 show electron micrographs of gap junctions found in the *ad-libitum*-fed control rat myometrium at parturition. Junction complexes were usually present on cytoplasmic extensions. In transverse section, each complex was either 5- or 7-layered and clearly defined. There was no discernible difference in appearance when comparing simultaneously vs sequentially fixed tissues. Examination of tissue from pair-fed control (Figs. 3–6) and zinc-deficient (Figs. 7–9) rats revealed an increased number of regions that appeared to be incomplete or, perhaps, nascent, gap junctions. Apposing membranes sometimes displayed differential deposition of osmium along the cytoplasmic surface, but were separated by a gap of 30–40 nm, compared to the space of only 2–3 nm in complete gap junctions. Micrographs from zinc-deficient rats in particular showed numerous regions of cytoplasmic extensions and near contact without clearly visible formation of gap junctions.

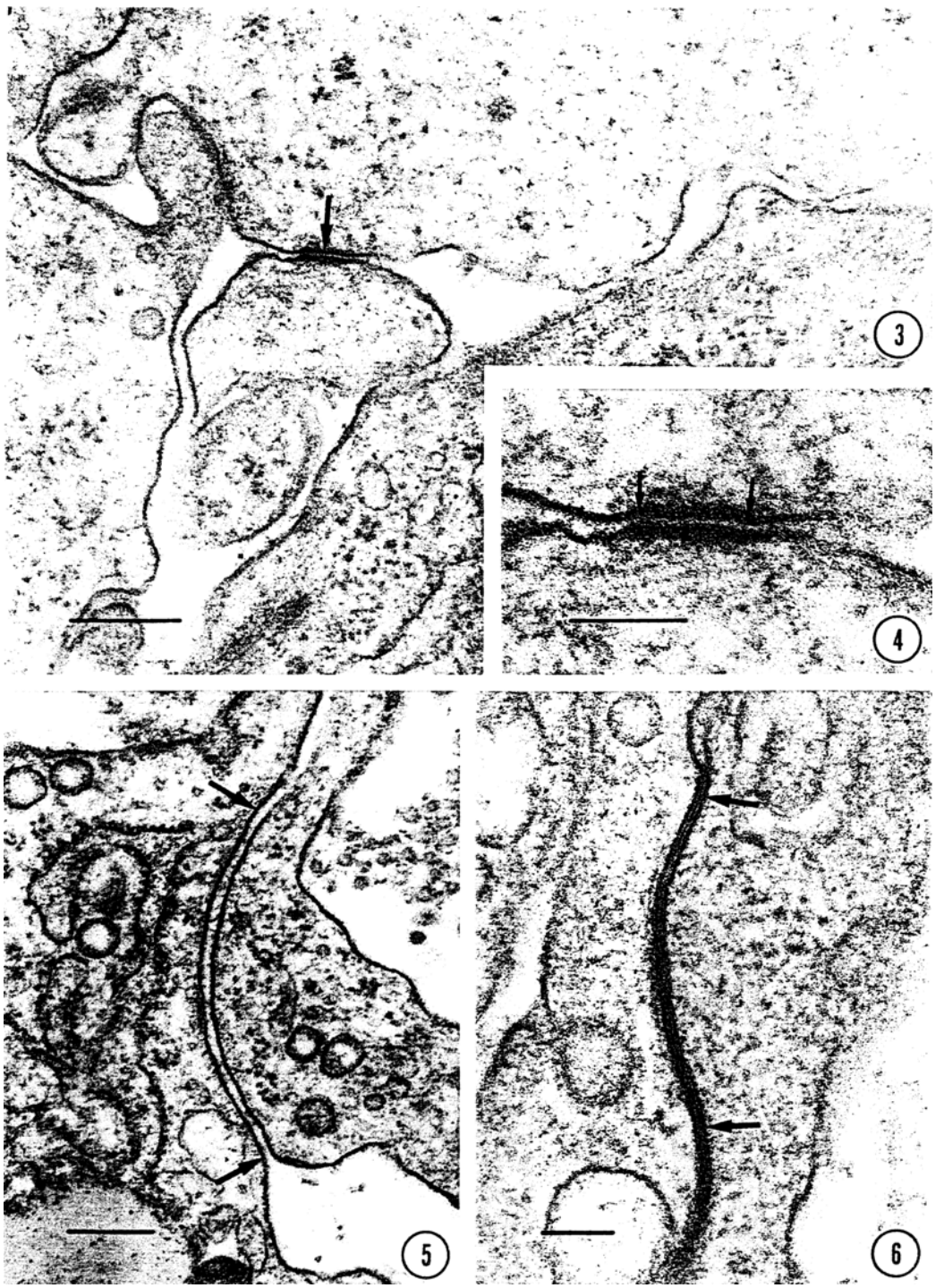
Fully formed, definitive gap junctions were counted in each of the three groups, either on the basis of gap junctions per cell or gap junctions per 1000 μm of membrane length (Table 2). Food restriction alone as associated with a 30% reduction in gap junctions per cell ($24.0 \times 10^{-3}$ vs $16.7 \times 10^{-3}$), which was significant at the 5% level of probability. The zinc-deficient rats showed a further reduction to $11.7 \times 10^{-3}$ gap junctions per cell, which was significantly less than either the pair-fed group ($P < 0.05$) or the *ad-libitum*-fed group ($P < 0.05$) when tested by Duncan's Multiple Range Test. The same trend was observed when the complexes were counted per length of membrane.

## DISCUSSION

Garfield et al. (*13–15*) have used quantitative thin-section and freeze-fracture microscopy to demonstrate that gap junctions are found be-




Figs. 1 and 2. Myometrial gap junctions at parturition from *ad-libitum*-fed animals. (1) Survey micrograph of gap junction (arrow) between smooth muscle cells. Scale bar = 250 nm. (2) High-magnification micrograph of gap junction (arrows), viewed in transverse section, showing a five-layered structure formed from the lateral plasma membranes of adjacent cells. Scale bar = 100 nm.

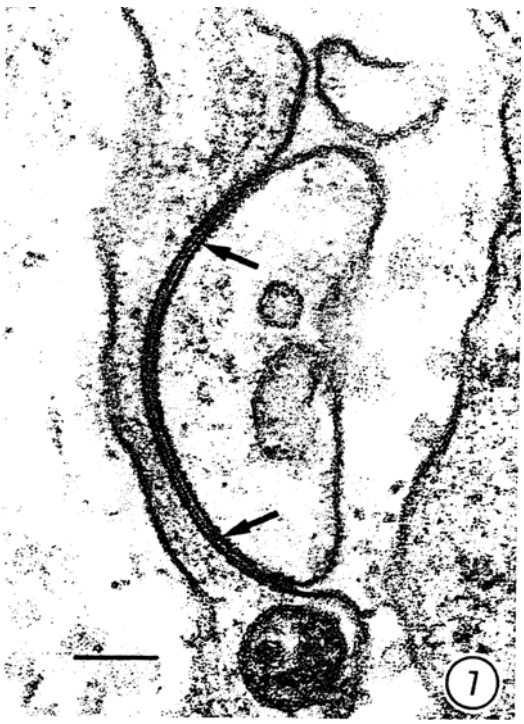



Figs. 3–6. Modifications of the lateral plasma membranes of adjacent myometrial cells at parturition from pair-fed animals. (3) Survey micrograph of

tween smooth muscle cells of the myometrium of pregnant rats only immediately prior to, during, and immediately after, parturition. The gap junctions were principally present on cytoplasmic extensions between cells. The rapid formation and appearance of gap junctions is believed to be a significant factor in the initiation and progress of parturition. By allowing the spread of electrical information over large areas of low resistance, uterine contractions are synchronized during delivery (*12*). The appearance of gap junctions at term has been linked to the withdrawal of the inhibitory effect of progesterone and the increase in the stimulatory impact of estrogen, possibly modulated by eicosanoids (*15*). Thus, progesterone both inhibits delivery and significantly lowers the number of uterine gap junctions in animals treated at the end of gestation, whereas estrogen promotes abortion and the appearance of abundant numbers of gap junctions.

The aberrant uterine pressure cycle patterns detected in our earlier study of zinc deficiency (*11*) suggested a defect in the efficient flow of electrical information through the uterus and prompted this evaluation of gap junction status. In the present study, zinc-deficient rats displayed a frequency of gap junctions per cell or per 1000 μm of membrane length, which was only 49 or 39%, respectively, of the *ad-libitum*-fed control, whereas the pair-fed controls achieved a level of 70% of the maximum observed value on either basis of comparison. One may ask what is the minimal number of gap junctions required for normal contractile strength and synchrony at delivery. Puri and Garfield (*21*) performed sequential measurements of myometrial gap junction frequency and hormone levels in rats from d 15 of pregnancy through spontaneous delivery on d 22. On d 15–20, gap junction frequency was about 0.5/1000 μm of membrane length. On d 21 the observed number was 1.7/1000 μm or 42.5% of the peak value of 4.0/1000 μm observed at birth. Since the peak value is likely to contain some excess and since normal labor does not begin until d 22, it seems a reasonable extrapolation that the threshold value is not less than 40–45% of the maximum value observed at birth. We propose that the clinical syndrome of delayed, prolonged, and exhausting delivery is likely to arise from poorly coordinated uterine involuntary contractile activity, which, in turn, is a consequence of an incomplete or marginally adequate gap junction network. This does not, of

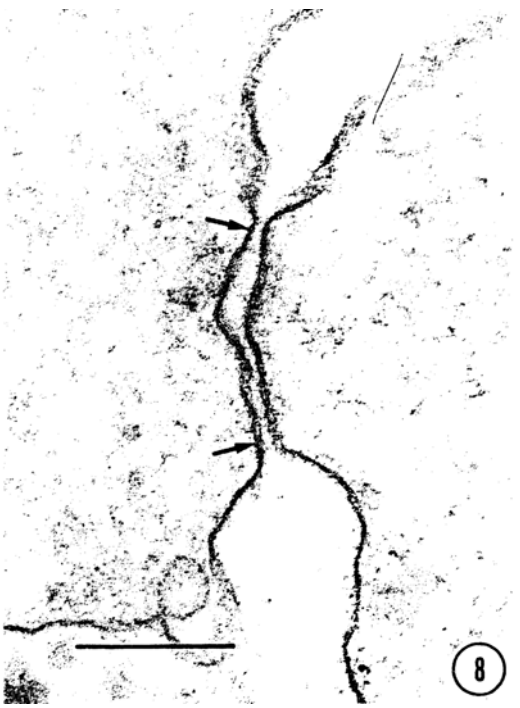
several neighboring cells showing what appears to be a differential deposition of osmium along the cytoplasmic surfaces of the membranes in one region (arrow). Scale bar = 250 nm. (4) High-magnification micrograph of modified membranes (arrows) observed in Fig. 3. Scale bar = 100 nm. (5) Portions of two adjacent smooth muscle cells showing region of close membrane association (arrows). Note that a space of 30–40 nm exists between the apposing membrane surfaces. Scale bar = 250 nm. (6) "Typical" gap junction (arrows) viewed in nearly transverse section. Scale bar = 100 nm.




Figs. 7–9. Modifications of the lateral plasma membranes of adjacent myometrial cells at parturition from low zinc fed animals. (7) "Typical" gap junction (arrows) viewed in nearly transverse section. Scale bar = 250 nm. (8) Portions of two adjacent cells showing region of close membrane association (arrows). Note the similarity of this structure and that observed in pair-fed animals (*see* Fig. 5). Scale bar = 250 nm. (9) Portions of two adjacent cells showing numerous areas of near contact (arrows) between apposing plasma membranes. Each region of membrane interaction involves a small amount of membrane surface area when compared to that of a gap junction. Scale bar = 250 nm.

course, rule out a contribution from additional factors, such as cervical stretching or oxytocin sensitivity.

The precise site(s) where zinc operates to permit normal development of the gap junction network remains to be defined. Possibilities include a role for zinc as a component of gap junction protein structure or assembly, as a catalytic agent in eicosanoid synthesis or action, or as a factor in the process of estrogen-directed gene expression. We are especially attracted to the latter possibility for the following reasons. First, one of the well-recognized outcomes of zinc deficiency in experimental animals is functional hypogonadism (*22,23*). Second, several authors have proposed that steroid receptors are metalloproteins (*24,25*) or that zinc enhances binding of the steroid-receptor complex to chromatin (*26*). Third, Chesters has proposed that the effects of zinc upon cell growth and duplication can best be explained by a freely exchangeable form of





this element, being involved in the unmasking of eukaryotic genes (*27*). Fourth, the elegant studies of Vallee and Falchuk (*28*) with *Euglena gracilis* have shown that zinc deficiency has its most profound impact in this organism at the point of gene expression. Definition of the roles of zinc in gene expression, especially in processes under steroid control, would provide important new insights into the influence of this element upon animal health and disease.

## ACKNOWLEDGMENTS

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TABLE 2
Myometrial Gap Junctions at Parturition as a Function of Dietary Zinc

| Treatment | No. of cells examined[a] | No. of gap junctions | Gap junctions per cell, $\times 10^b$ | Length of membrane surveyed, μm | Mean No. of gap junctions per 1000, μm |
|---|---|---|---|---|---|
| *Ad-libitum*-fed | 823 | 21 | 26 | | |
| | 1025 | 21 | 21 | | |
| | 927 | 23 | 25 | | |
| | 980 | 22 | 22 | | |
| | 895 | 19 | 21 | | |
| | 961 | 28 | 29 | | |
| $\bar{x}$ ± SEM | 935 ± 29 | 22.3 ± 1.3 | 24.0 − 1.3 | 3726 | 2.15 |
| Pair-fed | 970 | 14 | 14 | | |
| | 846 | 12 | 14 | | |
| | 964 | 24 | 25 | | |
| | 950 | 15 | 16 | | |
| | 890 | 11 | 12 | | |
| | 944 | 18 | 19 | | |
| $\bar{x}$ ± SEM | 927 ± 20 | 15.7 ± 1.9 | 16.7 ± 1.9 | 3263 | 1.53 |
| Low Zn, 3 ppm | 870 | 10 | 11 | | |
| | 935 | 11 | 12 | | |
| | 942 | 10 | 11 | | |
| | 892 | 12 | 13 | | |
| | 915 | 9 | 10 | | |
| | 980 | 13 | 13 | | |
| $\bar{x}$ ± SEM | 922 ± 16 | 10.8 ± 0.6 | 11.7 ± 0.5 | 3624 | 0.83 |

[a]Six animals/treatment, five blocks/animal.
[b]Each mean different from the other two ($P < 0.05$) when evaluated by Duncan's Multiple Range Test.

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