Effects of Hydrocortisone on the Formation of Gap Junctions and the Abnormal Growth of Cilia Within the Rat Anterior Pituitary Gland:

Possible Role of Gap Junctions on the Regulation of Cell Development

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ABSTRACT

We investigated the effects of hydrocortisone on the formation of gap junctions in and the growth of cilia on folliculo-stellate cells. The male rats of experimental groups were given daily intraperitoneal injections of 5 mg/kg of hydrocortisone from Day 20 to 60. Five rats were killed at ages 10, 20, 30 and 40 days after initiation of injections, and the pituitary gland was removed from each rat. Then, the specimens were prepared for observation by transmission electron microscopy. A delay in the formation of gap junctions between folliculo-stellate cells was observed in hydrocortisone treated rats compared with control rats on Day 30, 40 and 50. Another finding in the present study was the increase of ciliated follicles on Day 40 and 50 in the hydrocortisone treated groups, simultaneous with the delay in gap junction formation. The results suggest that hydrocortisone has a suppressive effect on the gap junction formation between folliculo-stellate cells, and loss of intercellular communication by way of gap junctions may lead to alteration of morphological development of the cell. Anat Rec 262:169–175, 2001.

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Key words: hydrocortisone; anterior pituitary gland; rat; gap junctions; cilia; folliculo-stellate cells

Many studies have demonstrated that glucocorticoids such as hydrocortisone can modulate the development of gap junctions in myometrium and cultured hepatocytes (Klaunig and Ruch, 1990; Stutenkemper et al., 1992; Towell et al., 1992; Ren et al., 1994; Kwiatkowski et al., 1994; Kojima et al., 1995; Siddiqui et al., 1999). Within the adenohypophysis of many mammalian species, gap junctions can chiefly be found between folliculo-stellate cells that possess agranular and star-like morphological features (Soji and Herbert, 1990; Meda et al., 1993; Yamamoto et al., 1993; Morand et al., 1996). Folliculo-stellate cells show progressive maturation of their intercellular junctions and morphological features after the weaning period in Wistar-Imamichi strain male rats (Shirasawa et al., 1983; Soji et al., 1990; Soji et al., 1994). Concurrently, male rats undergo rapid sexual maturation after the weaning period, including the change of testicular sex steroids.

In the present study, we show that the formation of gap junctions between folliculo-stellate cells can be delayed by the intraperitoneal injection of hydrocortisone during these weaning and growing periods. We propose that this effect might be due to both the suppressive effect on the

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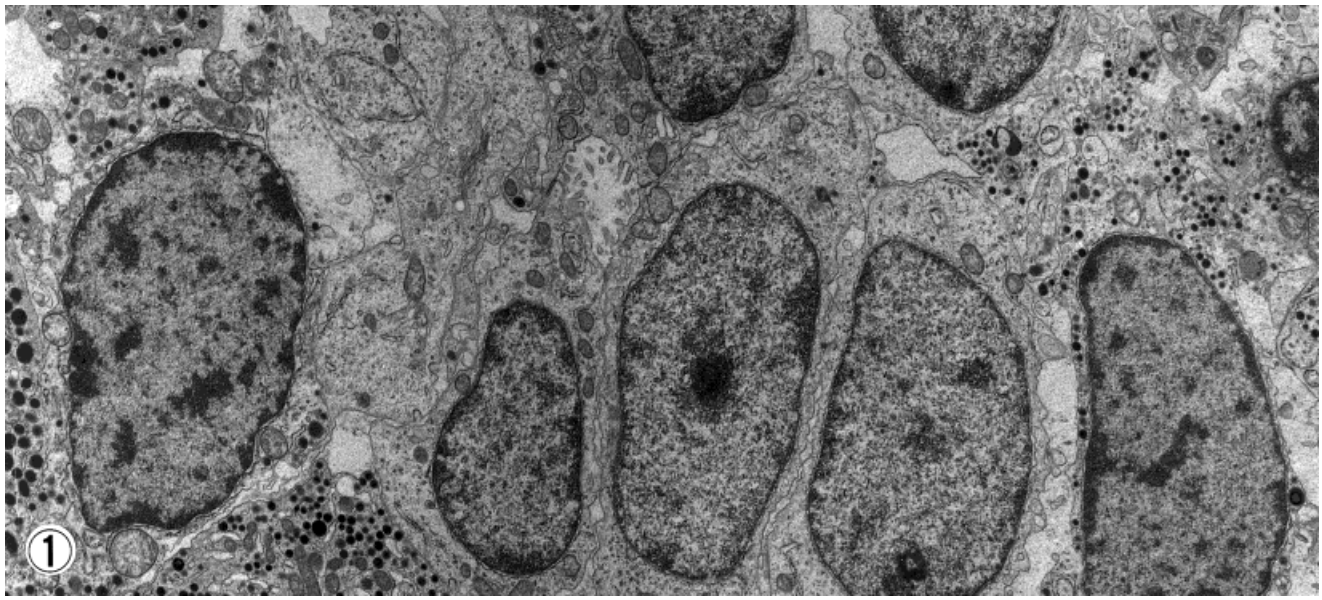

Fig. 1. In a rat anterior pituitary from a 30-day-old animal treated with hydrocortisone, columnar folliculo-stellate cells are displayed. There are no gap junctions in this picture. Magnification ×8,000.

plasma testosterone level and the direct action by hydrocortisone on folliculo-stellate cells.

Another finding in the present study was the increase of ciliated follicles on Day 40 and 50 in the hydrocortisone treated groups, simultaneous with the delay in gap junction formation.

Gap junctions are composed of transmembrane channels allowing the free transcellular exchange of small cytoplasmic molecules such as ions, amino acids, sugars and several second messengers (Loewenstein, 1981; Spray, 1994; Wolburg and Rohlmann, 1995; Kumar and Gilula, 1996). Because gap junctions are found from very early stages of embryogenesis (Sheridan, 1966; Wolpert, 1978; Chuang-Tseng et al., 1982), many investigators have suggested that the direct flow of small molecules from cell to cell through gap junctions may play a crucial role over the development in tissues of both vertebrates and non-vertebrates (Lo and Gilula, 1979; Warner and Lawrence, 1982; Guthrie, 1984; Ruangvoravat and Lo, 1992). More direct evidence for the developmental role of gap junctional communication during cell development has been provided by findings that the blockage of gap junctional communication by intercellular injection of antibodies against gap junction protein resulted in patterning defects in the amphibian embryo (Warner et al., 1984), preimplantation mouse embryos (Lee et al., 1987), chick limb morphogenesis (Allen et al., 1990) and hydra head regeneration (Fraser et al., 1987). The idea that developmental pattern may be controlled by a gradient of some growth regulatory factors that can pass from one cell to the next through gap junctions now seems to be familiar in developmental biology, although there is no precise identification for such specific growth factors.

In the present study, we propose to document that the increase of ciliated follicles that is simultaneous with the delay in gap junction formation is in part due to the disturbance of dissemination of growth-regulating information through gap junctions.

MATERIALS AND METHODS

All animal experiments were performed under the NIH Guidelines for the Care and Use of Laboratory Animals. Forty 20-day-old male rats of Wistar-Imamichi strain were placed into two groups. They were maintained under conditions of controlled temperature (22–24°C) and illumination (8:00–20:00 daily). Animals were fed a normal diet and water. The first (hydrocortisone) group was given daily intraperitoneal injections of 5 mg/kg of hydrocortisone (Sigma Chemical Co., St. Louis, MO) in the solvent (0.5 mg/1 ml distilled water containing 0.9% NaCl, 0.4% polysorbate-80, 0.5% carboxymethylcellulose, 0.9% benzylalcohol); the second (control) group with the same dose of the aforementioned solvent. Injections were continued daily up to the day before the animal was killed. Five rats from each group were killed at ages 30, 40, 50 and 60 days; or 10, 20, 30 and 40 days after initiation of injections, respectively.

On the scheduled date, the animals were anesthetized with Nembutal® (pentobarbital) and perfused with fixative for 5 min through the left ventricle of the heart. The fixative used was 2.5% glutaraldehyde and 2% sucrose in 0.05 M cacodylate buffer (pH 7.4). After the perfusion, the pituitary gland was removed from each rat, and refixed by immersion for 1 hr in the same solution used for perfusion. The sections were then postfixed for 1 hr in 1% osmium tetroxide buffered by 2% sucrose and 0.05 M sodium cacodylate (pH 7.4). After postfixation, they were rinsed in ice-cold water for 5 min and dehydrated in a graded series of ethanol. After 2 rinses in 100% ethanol for 10 min each, the specimens were immersed twice in absolute propylene oxide for 15 min each and then embedded in epoxy resin (Luft, 1961). Ultrathin sections were prepared, placed on copper grids, stained with uranyl acetate and lead citrate, and observed using a Hitachi H-7000 transmission electron microscope.

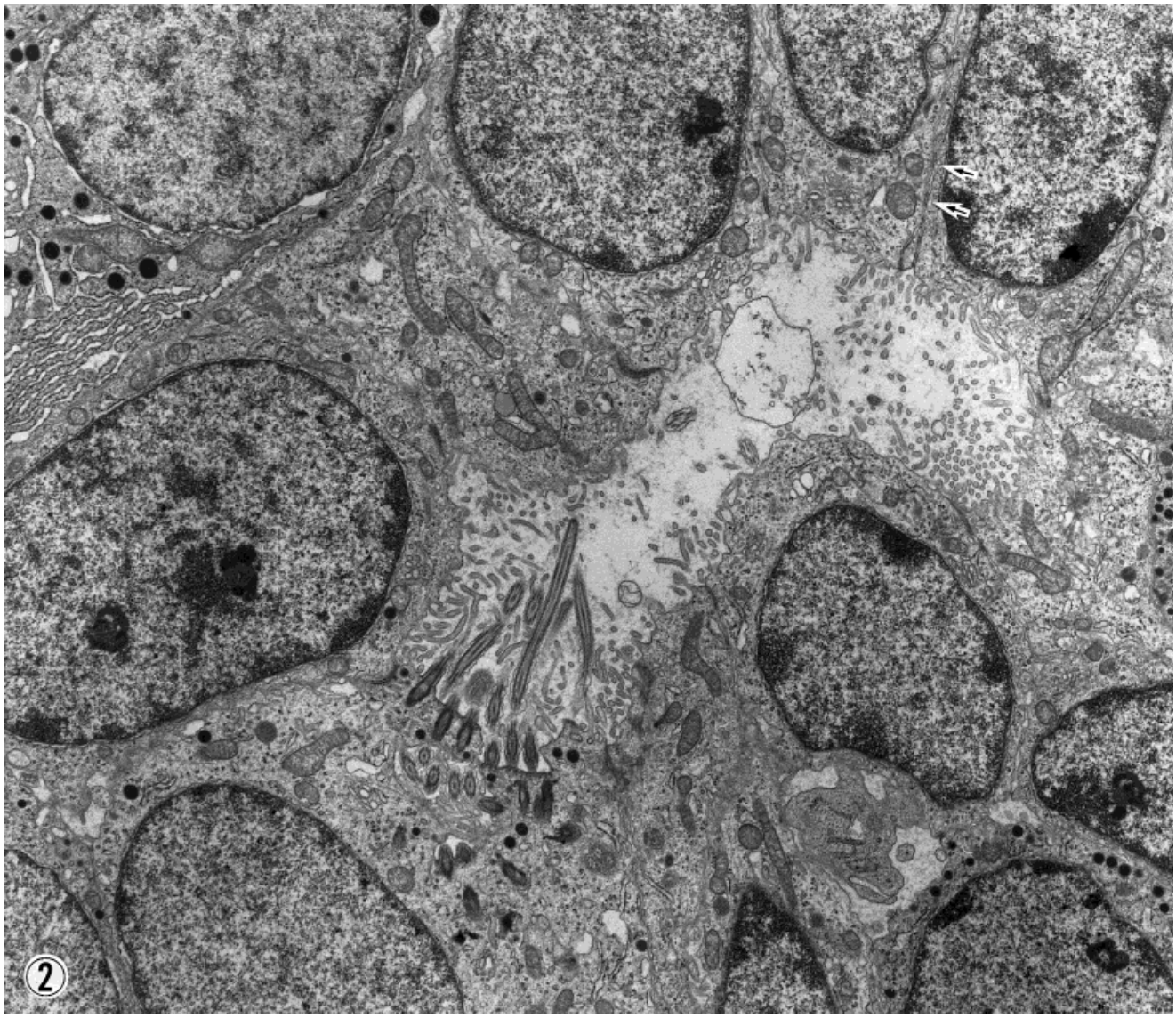

Fig. 2. Parts of many cilia and numerous microvilli are seen in the follicle from the pituitary gland of a 40-day-old hydrocortisone-treated rat. Note 2 small gap junctions (arrows). Magnification ×8,000.

The gap junctions and ciliated follicles were quantified using a total of 200 photomicrographs from each animal group. The photomicrographs were selected on the basis of each displaying a minimum of one follicle according to the identification procedures described by Soji and Herbert (1990). Distinction between the types of intercellular junctions was made based on the morphological criteria described by Staehelin (1974). We also counted the number of ciliated follicles using the criteria of Girod and Lheritier (1974). We determined the rate of ciliated folliculo-stellate cells in each follicle as the ratio of the number of ciliated cells to the total number of cells comprising a single follicle observed. The data were statistically analyzed by analysis of variance followed by the *t*-test for determining differences between means (Soji et al., 1990).

RESULTS

Controls, Solvent-Injected Animals

The development of gap junctions in the control group rats were similar to the normal rats of a previous study (Soji et al., 1990). Briefly, a few gap junctions were present on Day 30 and increased by Day 40 when they reached a level similar to that found in mature animals. The rate of ciliated folliculo-stellate cells was about 0.1 on Day 30, and remained relatively constant from Day 30 to 60.

Hydrocortisone Treatment

The folliculo-stellate cells forming the follicles in 30 day-old rats were columnar in shape (Fig. 1). Intercellular junctions, especially tight junctions, were incomplete and only a few gap junctions were observed (Fig. 5). The rate of

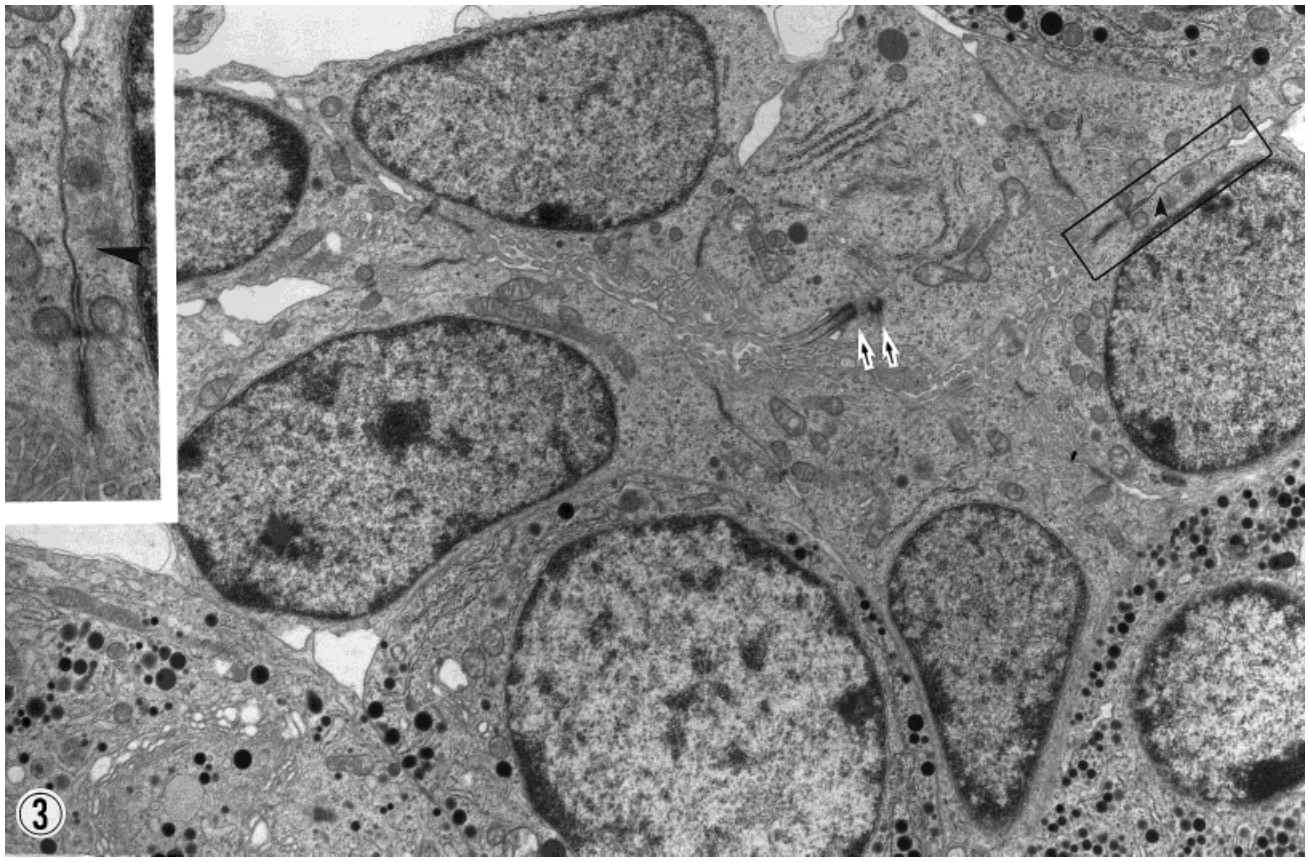

Fig. 3. A small gap junction (arrow head) and a microtubule with a basal foot (arrows) are illustrated adjoining the folliculo-stellate cells in a 50-day-old hydrocortisone treated rat. Magnification ×8,000; inset, ×16,000.

ciliated folliculo-stellate cells showed no significant difference to control group rats (Fig. 6).

In the 40 day-old rat, folliculo-stellate cells began to develop cytoplasmic processes that extended between the granular cells (Fig. 2). Gap junctions became more numerous at this age, however the level still remained lower than that of the control animals (Fig. 5). Cilia were present in approximately one-fourth of the folliculo-stellate cells observed, with several cells possessing a bundle of cilia (Fig. 2 and 6).

By Day 50, the folliculo-stellate cells were more polyhedral or cuboidal, with cytoplasmic processes extending in a number of directions (Fig. 3). There was a significant rise in the number of gap junctions present between the cytoplasmic processes of different folliculo-stellate cells (Fig. 5). A sharp increase in the number of gap junctions was found between Day 40 and 50 (Fig. 5). Ciliated folliculo-stellate cells were found more commonly in the hydrocortisone group than in control group rats (Fig. 6).

Gap junctions were frequently observed between folliculo-stellate cells at 60 days of age (Fig. 4 and 5), when their numbers reached a level similar to that seen in normal adults (Soji and Herbert, 1990). The rate of ciliated folliculo-stellate cells returned to a similar level as the control group (Fig. 5). By Day 60, there was no visible distinction between the controls and the animals that had been given hydrocortisone (Fig. 5 and 6).

In addition, gap junctions were absent along the plasma membrane of granular cells, and only motile cilia with the characteristic 9+2 arrangement of microtubules were found in folliculo-stellate cells in all samples of the present study.

DISCUSSION

Male rats undergo rapid sexual maturation between 20 and 40 days after birth. This maturation includes changes in the basal level of serum concentration of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), prolactin and the sex steroids; testosterone, estradiol and progesterone (Negro-Vilar et al., 1973; Doehler and Wuttke, 1975; Herbert, 1980). Soji et al. (1990) performed a quantitative electron microscopic study and reported that the gap junctions within the anterior pituitary gland could be first observed on Day 20, and the number approached the normal adult level by Day 40 in the case of Wistar-Imamichi strain male rats. Soji and Herbert (1990) demonstrated that the formation of gap junctions between folliculo-stellate cells was down-regulated by castration and that the slow rate in gap junction formation in the castrated rats could be compensated by testosterone injection. They reached the conclusion that the appearance of gap junctions was largely dependent on the serum concentration of testicular sex steroids.

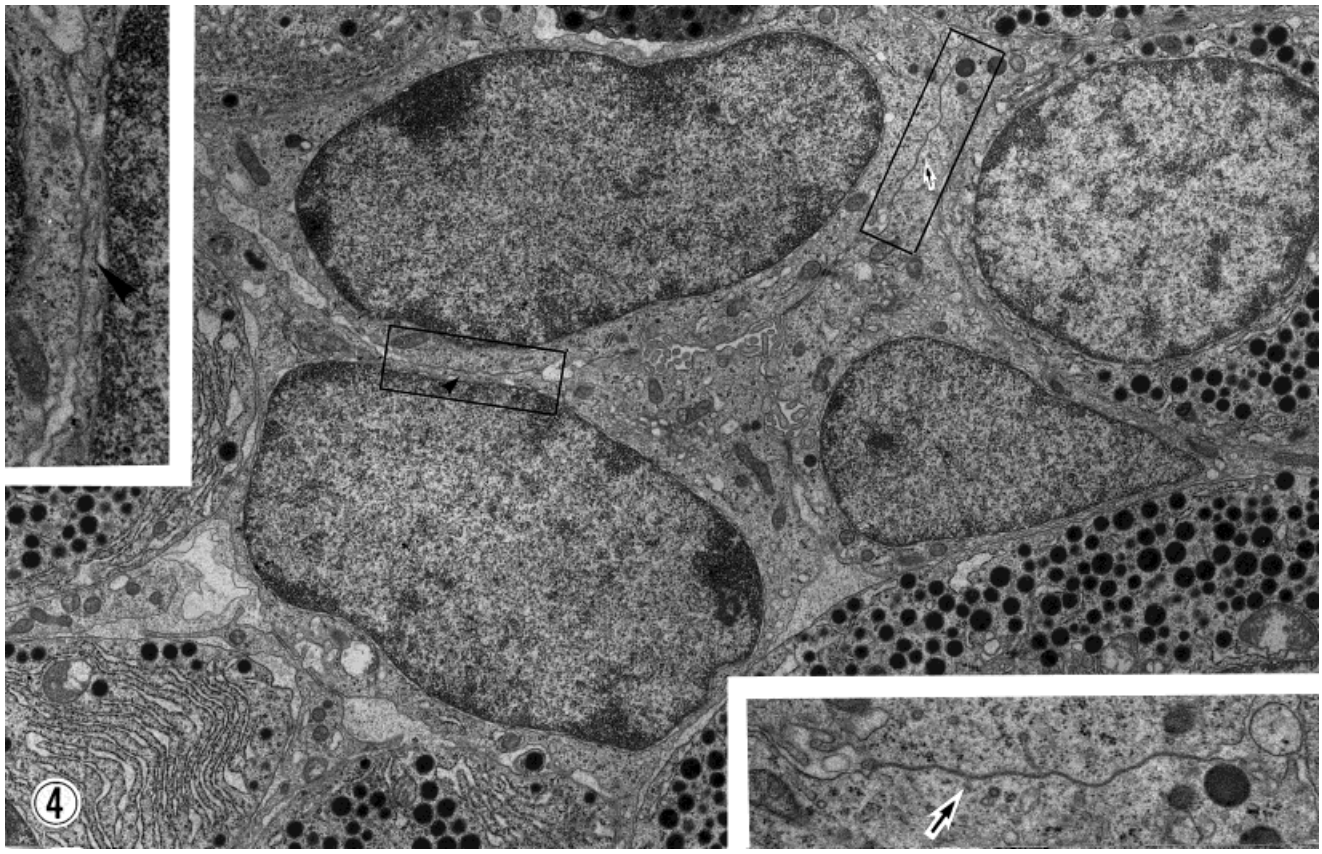

Fig. 4. By 60 days of age in a hydrocortisone treated rat, gap junctions are frequently observed (arrow and arrow head). Magnification ×8,000; insets, ×16,000

In the present study, administration of hydrocortisone induced the delay of gap junction formation between folliculo-stellate cells. We presume that hydrocortisone brought about a suppressive effect on the plasma testosterone level, and lead to the delayed gap junction formation between folliculo-stellate cells; because testosterone, the end product of the hypothalamic-pituitary-gonadal axis, can be regulated at multiple levels of this axis by the conditions that elevate the plasma concentration of glucocorticoids (Bambino and Hsueh, 1981; Padmanabhan et al., 1983; Hales and Payne, 1989; Monder et al., 1994; Briski, 1995; Fenske, 1997; Calogero et al., 1999).

Conditions that cause hyperactivity of the adrenal cortex such as Cushing syndrome are known to result in disturbances in testis function in the field of clinical medicine (Gabrilove et al., 1974; McKenna et al., 1979). Experimental evidence indicates that glucocorticoids act at the hypothalamic level to suppress luteinizing hormone releasing hormone (LH-RH) (Calogero et al., 1999), whereas they can act at the pituitary level by inhibiting the synthesis and release of luteinizing hormone (LH) (Padmanabhan et al., 1983; Briski, 1995). In addition, many investigations have also revealed that glucocorticoids have a direct suppressive influence on Leydig cells in vivo (Monder et al., 1994; Fenske, 1997), and in vitro (Bambino and Hsueh, 1981; Hales and Payne, 1989).

The major cause of the decrease in gap junction formation is the lowered concentration of serum testosterone; however, we cannot neglect the possibility of direct action by hydrocortisone on folliculo-stellate cells, because the presence of glucocorticoid receptors in the rat folliculo-stellate cells has been suggested by Shirasawa and Yamanouchi (1999). They demonstrated that pituitary folliculo-stellate cells of rats responded to hydrocortisone in vitro by increasing synthesis of glutamine synthetase and concluded that glucocorticoid receptors are present in rat folliculo-stellate cells.

The ultrastructural and immunohistochemical similarity of the folliculo-stellate cells in the pars distalis to the marginal layer cells has been reported by many investigators (Carpenter, 1971; Yoshimura et al., 1977; Cocchia and Miani, 1980; Shirasawa et al., 1983; Soji et al., 1994). The brain-specific S-100 protein can be used to demonstrate folliculo-stellate cells in light microscopic studies, and this has been considered to be one of the most reliable staining methods for folliculo-stellate cells (Nakajima et al., 1980; Cocchia and Miani, 1980; Coates and Doniach, 1988; Allaerts et al., 1999; Inoue et al., 1999). From S-100 protein immunohistochemistry studies, it is noteworthy that immunoreactivity to S-100 protein first appears in the marginal layer cells of Rathke's residual pouch before the folliculo-stellate cells within the pars anterior of rats (Shirasawa et al., 1983; Soji et al., 1994). Therefore, the possibility has been suggested that the folliculo-stellate cells of the future anterior lobe originate from the marginal layer cells (Yoshimura et al., 1977; Shirasawa et al.,

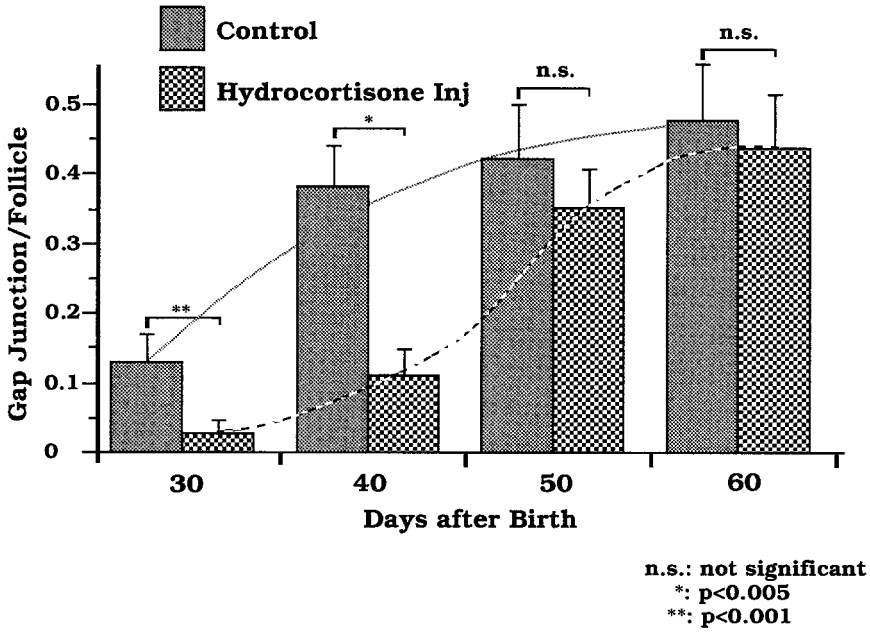

Fig. 5. Summary of the number of gap junctions present per follicle in the 2 animal groups at each of the 4 ages studied.

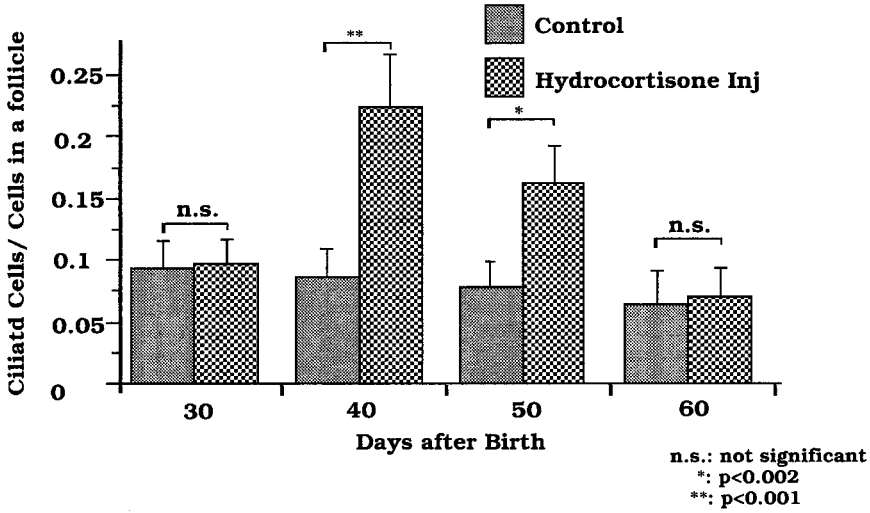

Fig. 6. Summary of the rate of ciliated cells per cells constructing a follicle in the 2 animal groups at each of the 4 ages studied.

1983; Soji et al., 1994). It is very interesting that the marginal layer cells often have many cilia on their free surface, whereas only a limited number of folliculo-stellate cells show the presence of cilia (Carpenter, 1971; Yoshimura et al., 1977). We believe that hydrocortisone in our experiments brought on the insufficient development of gap junctions between folliculo-stellate cells, that in turn led to the disturbance of cellular development as evident in altered cilia formation in follicles.

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