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Estrone and Estradiol Concentrations in Human Ovaries, Testes, and Adrenals During the First Two Years of Life*

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ABSTRACT. To determine the origin of estrogens in infant blood, we measured estrone (E_1) and estradiol (E_2) in the gonads of 50 girls and 64 boys who died suddenly between birth and 2 yr of age as well as in the adrenals of 18 of these infant girls and 16 of the boys. In the adrenals, E_1 [median, 2.8 ng/g (10.4 pmol/g); range, 1.1–4.8 ng/g (4.1–17.8 pmol/g)] and E_2 [median, 3.0 ng/g (10.9 pmol/g); range, 1.2–5.3 ng/g (4.4–19.5 pmol/g)] were found in similar concentrations and were independent of age and sex. In the gonads, E_2 was the major estrogen, but the concentrations differed markedly between the sexes; E_2 exceeded E_1 almost 10-fold in the ovaries and 2-fold in the testes. On the average, the gonads of the infant girls had 5 times more E_2 and 2 times more E_1 than those of the boys. As in plasma, E_2 concentrations were highest in the ovaries of 1- to 6-month-old girls [median, 10.5 ng/g (38.5 pmol/g); range, 1.1–55.1 ng/g (4.0–202.0 pmol/g)] and in testes of 1- to 3-month-old boys [median, 1.8 ng/g (6.6 pmol/g); range, 0.6–6.4 ng/g (2.3–23.5 pmol/g)]. Ovarian E_2 concentrations declined to less than 3.0 ng/g (11.0 pmol/g) by the end of the first year of life, and testicular E_2 declined to less than 1.0 ng/g (3.7 pmol/g) after only 6 months of age. Gonadal estrogen concentrations paralleled changes in gonadal morphology. Ovarian weights varied in a pattern of rise and fall similar to that of ovarian E_2 concentrations; the biggest ovaries contained multiple macroscopic cysts. Testicular E_2 closely correlated with Leydig cell development and testicular testosterone concentrations. We infer, therefore, that the surge of plasma E_2 in infant girls originates from ovarian follicles and that of boys from testicular Leydig cells, and that these both occur as a result of the postnatal surge in gonadotropin secretion. The basal plasma E_1 and E_2 pool, however, is derived from the adrenals and remains at a comparatively constant level in both sexes. (*J Clin Endocrinol Metab* **65:** 862, 1987)

DURING the first months after birth, the secretion of hypophyseal gonadotropins transiently increases in infants in a sex-related manner (1). In boys, plasma LH and FSH concentrations increase steeply a few days after birth and by 1 month of age reach peak values comparable to those in adult men. They then decline rapidly and, by 4 months, reach levels characteristic of normal childhood. The transient LH rise is followed by striking elevations of testosterone in plasma (2) and testes (3); the plasma levels reach the lower adult range during the second or third month of life. In addition, plasma estradiol (E_2) increases, although plasma estrone (E_1) changes little (4). In girls, there is a similar postnatal rise of gonadotropins. However, while LH levels return to low values in girls at the same time as in boys, the rise in FSH levels is greater and more prolonged, with the elevations lasting for 2–3 yr. Plasma E_2 concentrations parallel the rise of FSH and can reach midpubertal levels during the third or fourth month, and the period of elevated E_2 lasts at least 1 yr. As in boys, plasma E_1 is low and varies little postnatally (4, 5). To determine the estrogen content of the different steroid-producing glands in infancy and thus be able to make inferences about the source(s) of plasma E_2 and E_1, we measured E_2 and E_1 concentrations in the gonads and adrenals of boys and girls up to 2 yr of age.

Subjects and Methods

Specimens

Gonads were obtained postmortem from 50 girls and 64 boys who were previously healthy and who died of the sudden infant death syndrome or accident between birth and 2 yr of age. Premature and small-for-date infants or boys with undescended testes were excluded from the study. Both ovaries, but only 1 testis, of a subject were analyzed. Adrenals obtained from 18 female and 16 male infants were also studied. Many of the boys were identical to those from whom testes or adrenals were

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taken for previous studies on androgen production (3, 6). The time interval between death and autopsy was 4–36 h.

It is not certain whether the steroid content of gonads or adrenals removed postmortem is the same as that in normal glands *in vivo*. However, according to several reports (7, 8), changes in steroid composition in postmortem gonads or adrenals occur only very slowly. In preliminary experiments we found no change in the androgen and estrogen contents of an adrenal homogenate kept at room temperature from 12–36 h after death. Moreover, in this study we found no correlation between the steroid concentrations in glands from infants of the same age and the interval between death and autopsy, indicating that this interval did not alter the results. Thus, considering the difficulty in obtaining fresh glands from infants, the use of autopsy material seems justified.

Testes were separated from the epididymides; all glands were cleaned of adhering tissue, weighed, minced, and homogenized, as described previously (3, 6). Homogenates were stored at −80 C until analyzed. The testicular weight was doubled to obtain the total weight per pair.

Steroid determinations

E_1 and E_2 concentrations in tissue homogenates were determined in duplicate by RIA after chromatography on Sephadex LH-20 according to the method previously described for plasma (9). This method separated E_1 from E_2, estriol, and other interfering steroids. Recovery of tritiated E_1 and E_2 added to each sample was between 60–75% and was taken into account in calculation of the results. The antiserum used for both E_1 and E_2 determinations had less than 0.05% cross-reactivity with dehydroepiandrosterone, androstenedione, testosterone, and all other nonphenolic steroids tested. The standard curves for E_1 and E_2 ranged from 10–300 pg/tube (37–1100 fmol/tube). The sensitivity of the assays, defined as twice the SD of water blanks, was below 10 pg/mL (37 fmol/mL). Generally, 1 mL diluted (1:5 to 1:200) homogenate was introduced into the assay. The smallest steroid mass actually measured in the assays was 38 pg (140 fmol) and, thus, was clearly distinguishable from zero. The interassay variability for the determination of E_1 and E_2 was 12% for pooled plasma (n = 12) and 19% for pooled gonadal and adrenal homogenates (n = 18).

Statistics

The data are expressed as the median and range for each age group. Differences between age groups were compared by the Kruskal-Wallis test (10). In two-sided tests, $P < 0.05$ was considered significant.

Results

The size of gonads and adrenals changed markedly during the first 2 yr of life. Paired ovarian weight (Fig. 1) was lowest during the first month after birth (median, 0.57 g/pair; range, 0.19–1.12 g/pair). It was highest ($P < 0.01$) in 1- to 6-month-old girls (median, 1.41 g/pair; range, 0.36–4.2 g/pair), but then decreased and remained low to the end of the second year (median, 0.95 g/pair; range, 0.59–1.93 g/pair). Thus, the ovaries were largest during the period of increased gonadotropin secretion. Almost all ovaries contained visible cysts, which in some 2- to 4-month-old girls were up to 5 mm in diameter.

Testicular growth followed the pattern already described in our previous study of a smaller number of boys (3). The mean testicular weight increased steadily from birth to 5 months from 0.55 to 1.41 g/pair and changed little thereafter.

In contrast, mean adrenal weight in boys and girls decreased from 8.1 g/pair at birth to 4.3 g/pair by 5 months of age. From then on, adrenal weight increased by the end of the second year of life, but did not reach neonatal values (6).

Figure 2 shows ovarian estrogen concentrations, expressed as nanograms per g wet tissue, plotted against age. The age-related fluctuations of E_2 in the ovary resembled those in the plasma of healthy infants (4, 5). Maximum E_2 concentrations were found during the third and fourth months. In girls 1–6 months of age, ovarian E_2 concentrations [median, 10.5 ng/g (38.5 pmol/g); range, 1.1–55.1 ng/g (4.0–202.0 pmol/g)] were significantly higher ($P < 0.005$) than in girls 6–24 months old [median, 2.4 ng/g (8.6 pmol/g); range, 1.4–22.1 ng/g (5.1–81.1)]. On the average, 1 g ovarian tissue contained 400 times more E_2 than 1 mL plasma (4, 5) from healthy girls of the same age. E_1 was also detectable in all ovaries. The concentrations were significantly higher ($P < 0.01$) during the first 6 months of life [median, 1.1 ng/g (4.1 pmol/g); range, 0.3–4.4 ng/g (1.2–16.3 pmol/g)] than from 6–24 months [median, 0.6 ng/g (2.2 pmol/g); range, 0.1–1.2 ng/g (0.4–4.6 pmol/g)]. On the average, E_1 was 100 times higher in the ovaries than in the plasma (4, 5) of healthy girls of the same age. However, ovarian E_1 concentrations averaged only 1/10th the concentrations of ovarian E_2, with the exception of the first days after birth, when ovarian E_1 levels were at least half as high as E_2 levels.

As in girls, the gonadal estrogen concentrations in boys (Fig. 3) showed age-dependent fluctuations similar to those in plasma. Testicular E_2 concentrations were highest in 1- to 3-month-old boys [median, 1.8 ng/g (6.6 pmol/g); range, 0.6–6.4 ng/g (2.3–23.5 pmol/g)], decreased significantly ($P < 0.01$) in the group 3–6 months of age [median, 1.0 ng/g (3.7 pmol/g); range, 0.8–2.0 ng/g (2.8–7.2 pmol/g)], and from then to the end of the second year were even lower ($P < 0.005$) than the levels in the preceding age group [median, 0.7 ng/g (2.4 pmol/g); range, 0.4–1.4 ng/g (1.4–5.1 pmol/g)]. During the first 2 yr of life, E_2 concentrations were about 100 times higher in the testes than in the plasma of normal boys (4, 5).

E_1 concentrations in the testes averaged half of the E_2 concentrations, and in only a few testes was E_1 equal to E_2 or the predominant estrogen. Testicular E_1 levels were

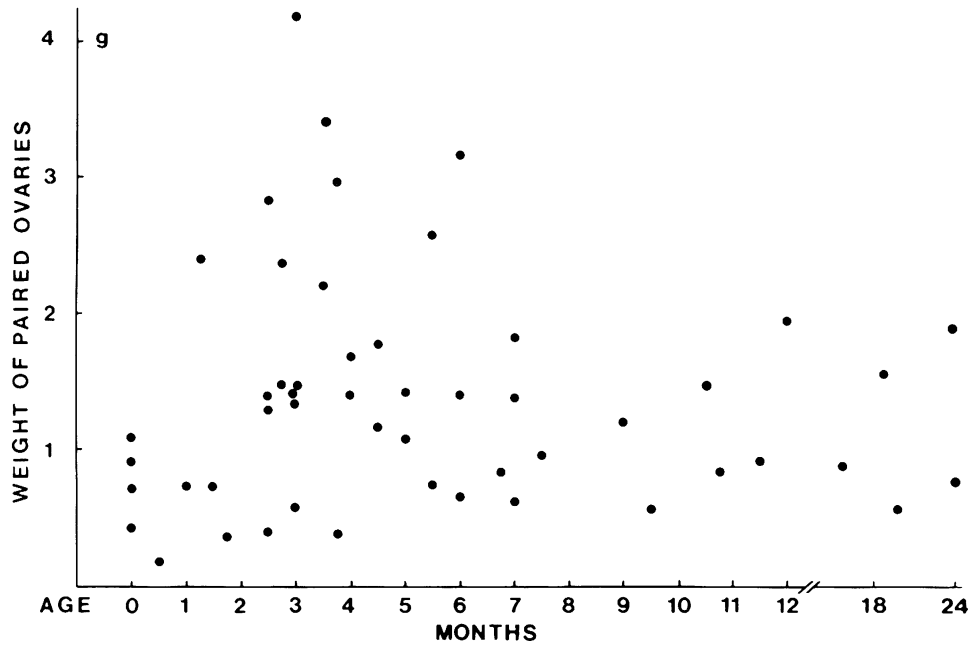

FIG. 1. Combined weight of paired ovaries plotted against age during the first 2 yr of life.

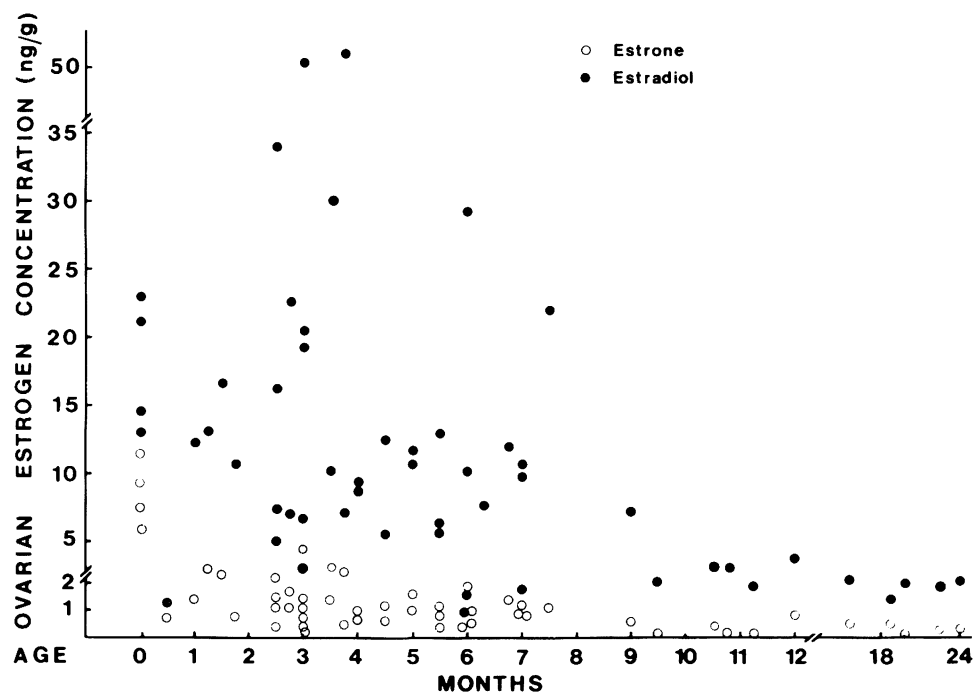

FIG. 2. Individual ovarian estrone (○) and estradiol (●) concentrations plotted against age (nanograms per g wet tissue). Conversion factors: E_1 ng/g × 3.699 = pmol/g; E_2, ng/g × 3.671 = pmol/g.

highest in the 1- to 3-month-old group [median, 0.8 ng/g (2.8 pmol/g); range, 0.3–2.1 ng/g (1.2–7.7 pmol/g)], lower, though not significantly so between months 3 and 6 [median, 0.6 ng/g (2.2 pmol/g); range, 0.2–1.3 ng/g (0.7–4.8 pmol/g)] and lowest ($P < 0.02$) after the age of 6 months to the end of the second year [median, 0.3 ng/g (1.2 pmol/g); range, 0.1–1.0 ng/g (0.3–3.7 pmol/g)]. On the average, testicular E_1 concentrations were 50 times those in plasma from normal boys (4, 5).

When we compared testicular estrogen concentrations with the respective androgen levels, determined in a previous study (3), we found a closer correlation between E_2 and testosterone ($P < 0.001$) and between E_1 and androstenedione ($P < 0.001$) than between E_1 and testosterone ($P < 0.05$) of E_2 and androstenedione ($P < 0.05$). Comparison of the estrogen concentrations in female and male gonads revealed roughly 5 times higher E_2 and 2 times higher E_1 concentrations in ovaries than in testes.

Adrenal estrogen concentrations did not significantly change with age, and no significant sex difference was found. Therefore, the results were combined. For infants from birth to 2 yr of age, the respective estrogen concentrations in the adrenals were: E_2: median, 3.0 ng/g (10.9

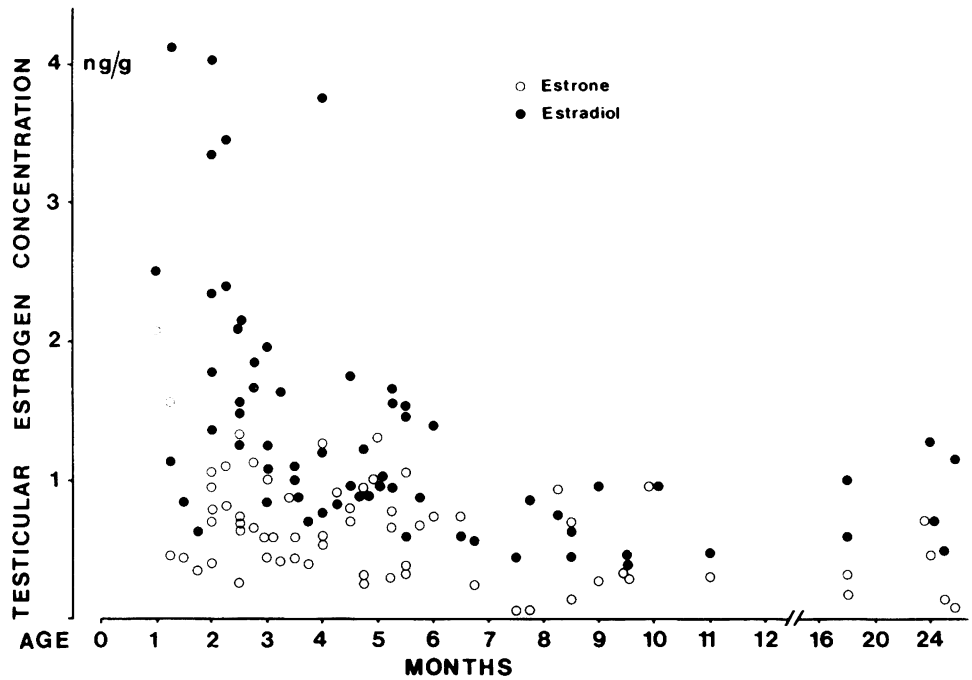

FIG. 3. Individual testicular estrone (O) and estradiol (●) concentrations in testes of infant boys plotted against age (nanograms per g wet tissue). Conversion factors: E_1, ng/g × 3.699 = pmol/g; E_2, ng/g × 3.671 = pmol/g.

pmol/g); range, 1.2–5.3 ng/g (4.4–19.5 pmol/g); and E_1: median, 2.8 ng/g (10.4 pmol/g); range, 1.1–4.8 ng/g (4.1–17.7 pmol/g; P = NS). The estrogen concentrations in the adrenals were 200–400 times higher than those in plasma and 2–3 times higher than those in testicular tissue; they were only exceeded by the E_2 concentrations in the ovaries during the first 6 months of life.

Figure 4 compares the total estrogen content of adrenals and ovaries or testes, respectively, calculated from paired weights and tissue concentrations. Because the time pattern of gonadotropin secretion differs for male and female infants, the age groups were different for each sex. While the marked fluctuations in ovarian estrogen content are due to changes in both organ weight and tissue concentrations, the fluctuations in adrenal estrogen content merely reflect the remarkable changes in adrenal weight caused by the rapid postnatal involution of the fetal zone and the slow growth of the definitive zone of the adrenal cortex.

Discussion

Although measuring the glandular hormone concentration does not necessarily indicate how much each gland contributes to hormone production, our results allow some conclusions concerning the relative importance of the gonads and adrenals to estrogen production in infant boys and girls. The adrenal estrogen content was high, and no sex difference in adrenal estrogen content was found. These results suggest that the adrenal contribution to the circulating estrogen pool is the same for both sexes, that adrenal secretion accounts for most of the basal plasma estrogen pool, and that the gonads are the source of the transient elevations in plasma E_2 and E_1 and the sex differences found in the first year of life. These differences disappeared during the second year of life, when gonadal activity becomes quiescent for the remaining prepubertal period.

It is well known that in adults the adrenals play an important part in the production of estrogens, especially E_1 (11–13), because they are the source of its major precursor, androstenedione. E_1 arises almost exclusively from extraglandular formation due principally to peripheral aromatization of adrenal androstenedione, and there seems to be no direct adrenal estrogen secretion (13). We found, in contrast, that in the adrenal tissue of infants, E_1 and E_2 were present in equal concentrations. Since the adrenal concentrations exceeded by some 100 times the plasma concentrations in normal infants, these estrogens must have been formed directly by the adrenals. Whether this is true at all stages of life or is just a peculiarity of infancy is not known, and we cannot with certainty exclude its being only a postmortem artefact.

The main gonadal estrogen in adult men and women is E_2 (13–16). Correspondingly, we found markedly more E_2 than E_1 in infant ovaries and testes. In seven adult men, Leinonen (17) found mean testicular E_2 and E_1 concentrations of 4.2 ng/g (15.4 pmol/g) and 0.5 ng/g (1.9 pmol/g), respectively. These values approximate the concentrations we found in the testes of 1- to 3-month-old boys. Comparison of the testicular estrogen concentrations with previous studies of testicular morphology (18, 19) and function (1–5) in infancy reveals a close relationship between testicular and plasma E_2 and tes-

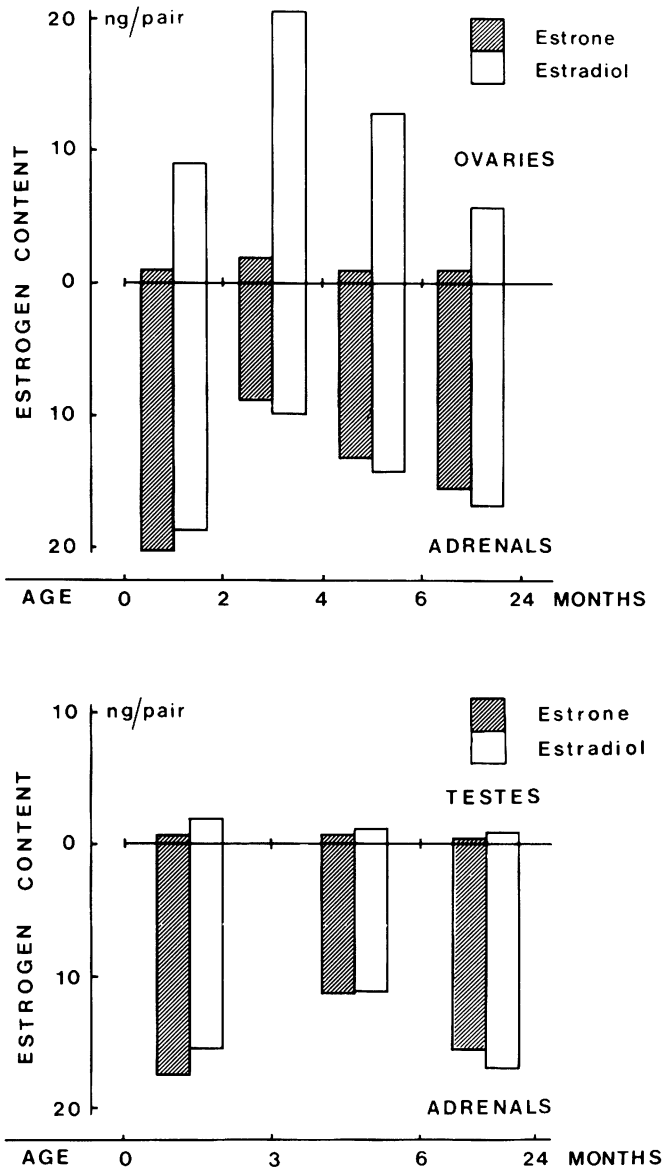

FIG. 4. Comparison of total gonadal and adrenal estrogen contents (organ pairs) in infant girls and boys during the first 2 yr of life. The *bars* represent the median values of the various age groups. Conversion factors: E_1, ng/pair × 3.699 = pmol/pair; E_2, ng/pair × 3.671 = pmol/pair.

tosterone, Leydig cell development, and, above all, plasma gonadotropins. Thus, it is reasonable to assume that Leydig cells stimulated by gonadotropins are the direct or indirect source of the E_2 surge in infant boys. The fact that testicular E_1 and E_2 concentrations were 50–100 times higher than the plasma concentrations in normal boys suggests direct E_1 and E_2 secretion by the testes. This does not exclude the possibility that, as in adults (13), an important quantity of circulating estrogens arise from peripheral aromatization of androgens. As reported previously, the testes of 1- to 3-month-old boys form and secrete large amounts of androgens (2, 3), which may serve as substrate for the aromatase enzyme in extraglandular tissue.

In the ovaries of premenopausal women, Dawood and Khan-Dawood (20) found 5 times more E_2 than E_1, and the concentrations of both estrogens varied in the different ovarian compartments, being 10 times higher in corpora lutea than in stroma. The concentrations in the whole ovaries of infants were similar to these stromal levels and, in 2- to 4-month-old girls, exceeded them. McNatty *et al.* (21) measured estrogens in follicular fluid throughout the menstrual cycle. In immature follicles smaller than 8 mm, they found estrogen concentrations similar to those we found in whole ovaries of 2- to 4-month-old girls. However, as the follicles matured, the follicular fluid concentrations increased.

In vitro, the infant ovary responds to gonadotropins by increasing steroid production (22). In patients with true precocious puberty, gonadotropins can cause a rapid increase in estrogen production, even in very young girls, In normal infant girls, the postnatal surge of plasma gonadotropins (1) obviously reaches the ovary and stimulates it, as indicated by the transient elevation of plasma E_2, described previously (4) and confirmed by the changes in ovarian morphology and estrogen content described herein. The ovarian growth curve peaked during the period of high gonadotropin levels, *i.e.* between 1 and 6 months of age, and the largest ovaries had the most advanced follicular development and, at the same time, the highest estrogen concentrations. Even before estrogens and gonadotropins could be measured in children, Kraus and Neubecker (23) concluded that pituitary gonadotropins act on the infant ovary and incite follicular development and estrogen synthesis. The occurrence of Graafian follicles in juvenile ovaries from infancy onward has been described as a normal finding (24–26). According to Polhemus (26), follicular development increases after birth and is most marked at 4 months of age, after which it declines again.

The parallels in the time sequence between hormonal and morphological changes in the hypophyseal-ovarian system suggest that the changes in ovarian morphology and function during infancy are strongly dependent on gonadotropin secretion. It has been suggested that in infant boys, transient activation of the testes is important in promoting tubular growth and germ cell maturation (3, 27). Likewise, it is conceivable that in infant girls, transient activation of the ovaries plays a role in the maturation of primordial follicles and their reduction in number.

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