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Cytological aspects of the human adrenal cortex development in the course of intra-uterine life

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Summary. Stereological studies were performed on 20 pairs of adrenal glands of human foetuses. The volume of particular adrenocortical zones, average cell volume and number of parenchymal cells were calculated.

In 50 to 320 mm crown-rump length (C.-R.L.) foetuses (9-38 weeks of the intra-uterine life) an exponential increase in adrenal gland weight was found. If compared with the earlier period, the rate of increase was evidently higher beginning from the 20th week and depended mainly upon enlargement of the foetal zone, with less marked changes in the glomerulosa-fasciculata zone.

The zona glomerulosa (ZG) and the outer zona fasciculata (ZF) began to delineate by the 20th week of gestation. Zona reticularis was not observed in our material. From the beginning of development, fasciculata cell volume was markedly higher than that of ZG cells and lower than foetal zona (ZX) cells.

Until the 20th week of intra-uterine life the volume of glomerulosa-fasciculata zone (G-FZ) increased at a slow rate, while the rate of increase in volume of ZX was higher. After 20 weeks the rate of increase in volume of all adrenocortical zones was markedly higher (the same as that of the stroma). The volume of stroma in ZX markedly exceeded that in the remaining parts of the cortex.

The average cell volume of ZG and ZF remained constant during the foetal period, while the average cell volume of ZX cells increased gradually from the 9th to the 20th foetal week and afterwards remained unchanged.

Of interest is the fact that the percentage of ZX cells in the gland remained rather constant during the whole period of observation (about 35-40% for ZX and 60-65% for ZG and ZF).

Key words: Adrenal gland, Human foetus, Foetal zone, Definitive zones, Cytology, Cell volume, Cell number, Stereology

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Introduction

Growth and differentiation of the human foetal adrenal gland has been the subject of numerous reports and investigations. Starklówna and Wegrzynowski (1910) first described a thick inner layer of cortical cells which is doomed to rapid involution upon birth - the foetal cortex. From this time onwards a lot of work has been devoted to this region of the foetal adrenal gland (Keene and Hewer, 1927; Benner, 1940; Uotila, 1940; Gruenwald, 1946; Velikan, 1948; Bachmann, 1954; Benirschke et al., 1956; Wolyńska, 1956, Lanman, 1960; Johannisson, 1968; Sucheston and Cannon, 1968) to determine the developmental, physiological and histological sequences that occur.

Crowder (1957) called attention to the origin and ultimate location of cell types lending support to the cell migration theory of cortical development. Subsequent studies, to establish a hormonal function of the foetal adrenals, were undertaken by Bloch et al. (1955, 1956); Lanman and Silverman (1957); Solomon et al. (1958); Ville et al. (1959); Bloch and Benirschke (1962); Longchamp and Axelrod (1964); Pasqualini et al. (1966); Johannisson (1968); Seron-Ferre et al. (1978).

The foetal adrenal gland is adapted in utero to function in collaboration with the placenta to produce steroid hormones. This system, the so-called foetoplacental unit, has been reviewed in several recent publications (Jaffe, 1983; Fisher, 1986).

In such abundant literature we have noticed a lack of quantitative data on the development of the human foetal adrenal cortex. Therefore, the purpose of the present investigation was to describe the volume of particular adrenocortical zones, average cell volume and the number of parenchymal cells in the developing human adrenal glands.

Materials and methods

Stereological studies were performed on 20 pairs of adrenal glands of human foetuses. Adrenal glands were obtained from the collection of the Department of

Anatomy and from the Department of Clinical Pathomorphology, School of Medicine, Poznań. The collection consists of normal foetuses obtained from spontaneous abortions.

The age of foetuses was estimated by C.-R.L., foot length and body weight, according to Carnegie staging data of O'Rahilly (1975). Detailed data of studied foetuses are shown in Table 1. Adrenals were carefully excised under a dissecting microscope and weighed to the nearest 0.1 mg. After fixation in 10% formalin or Bouin's solution, and embedding in paraffin, glands were serially sectioned at 5-6 μm, and sections were stained with haematoxylin and eosin.

Stereological studies were performed as described by Weibel (1979). Using a magnification of about 100 and a square lattice test system of type A (Weibel, 1979), the volume densities of the adrenocortical zones, medulla and capsule were evaluated. In two glands of the youngest foetuses all sections of the gland were analyzed. In the older ones the measurements were made on every fifth paraffin section of the gland. The volume of the adrenal gland was calculated from its weight, by assuming that the average specific gravity of the gland was 1.039 mg/mm^3 (Swinyard, 1938).

The volume densities of nuclei and cytoplasm of parenchymal cells were estimated on a screen at x3,000, using the multipurpose test system M42 (Weibel, 1979). For each adrenal gland a single paraffin section containing zona medullaris was chosen, and in this section 40 test areas of the ZG, ZF and ZX were counted. On the basis of earlier karyometric studies (Malendowicz, 1974), the shape coefficient beta, which relates N_v to N_a and V_v and depends on axial ratio of estimated nuclei, was assumed to be 1.382 for the ZF and ZX, and 1.500 for the ZG. The number of nuclei of adrenocortical cells per mm^3 was calculated according to Weibel and Gomez (Weibel, 1979). Since human adrenocortical cells are mononucleated, the numerical density of the nuclei corresponds to the number of parenchymal cells per mm^3. Subsequently, the average volume and the number of parenchymal cells in each adrenocortical zone were computed. Results were expressed per adrenal pair.

The results were processed statistically and graphically by Sigmaplot vs.4.10. programme for IBM PC AT.

Table 1. Crown-rump (C.-R.L) and foot length (mm), body (g) and adrenals (mg) weight, and age (in postovulatory weeks) of studied fetuses.

No.	C.-R.L (mm)	Length of foot (mm)	Body weight (g)	Adrenal weight (mg)	Weight of gestation
1	47	5	11	63	9,0
2	69	10	23	127	10.0
3	90	14	70	556	14.0
4	122	20	120	764	14.5
5	135	28	219	1248	15.0
6	155	31	310	1430	17.0
7	155	31	344	1680	17.0
8	161	34	390	1957	17.5
9	170	34	400	2166	18.0
10	170	35	340	1821	18.0
11	175	35	450	2422	18.5
12	180	36	540	2642	19.0
13	190	36	526	2514	20.0
14	220	41	700	3172	22.0
15	231	43	810	3602	24.0
16	230	44	850	3737	25.0
17	251	46	978	4160	29.0
18	282	49	1320	7030	31.0
19	307	53	2260	10130	37.0
20	320	64	2900	13352	38.0

Results

In foetuses of 50 to 320 mm C.-R.L. (9-38 week of intra-uterine life) an exponential increase in adrenal gland weight was found (Fig. 1). If compared with earlier periods the rate of increase was evidently higher from the 20th week on and depended mainly on

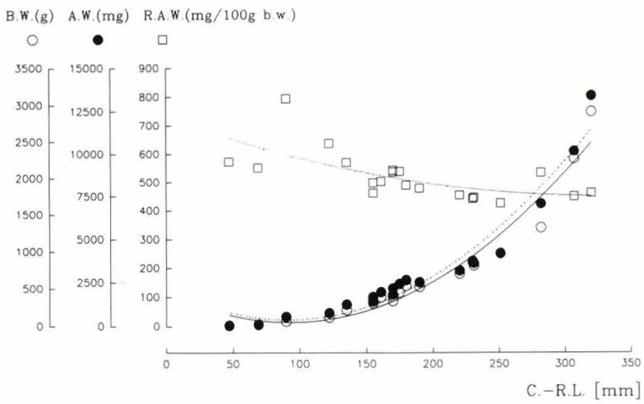

Fig. 1. Body weight, absolute and relative adrenal gland weight in the course of human intra-uterine development as plotted against the crown-rump length (C.-R.L. in mm). Each point presents one case.

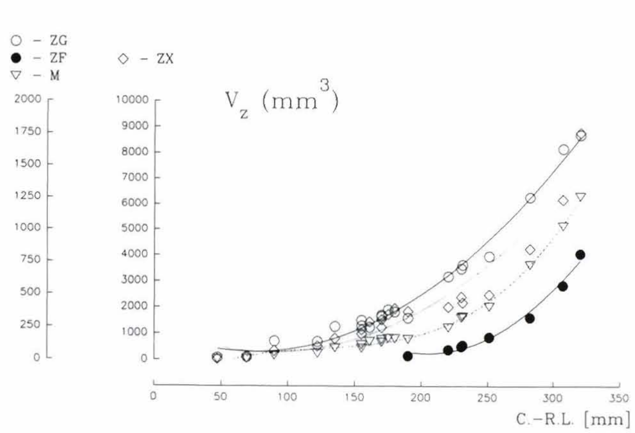

Fig. 2. Volume of adrenocortical zones and medulla in the course of intra-uterine development of human adrenal glands as plotted against the crown-rump length (C.-R.L. in mm). Each point presents one case. ZG - zona glomerulosa, ZF - zona fasciculata, M - medulla, ZX - foetal zone.

enlargement of the ZX (with less marked changes in G-FZ). On the other hand, the relative adrenal gland weight insignificantly decreased to the end of foetal period. There was no difference between the adrenal weights of male and female foetuses.

The ZG and the outer ZF began to delineate by the 20th week of intrauterine life. By the end of foetal life the outer ZF appears as distinct cell columns while the zona reticularis was not observed in our material. From the beginning of ZF development, fasciculata cell volume was markedly higher than that of ZG and lower than that of ZX cells.

Until the 20th week of foetal period, volume of G-FZ increased at a slow rate while the rate of increase in the volume of ZX was higher. After that time the rate of increase in volume of all adrenocortical zones was

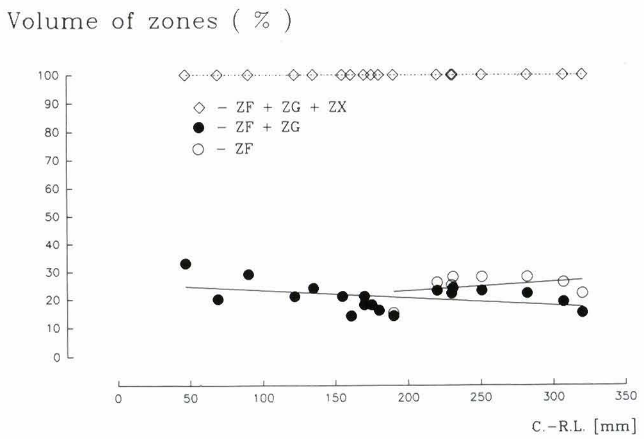

Fig. 3. Relative volume (%) of adrenocortical zones in developing human adrenal gland as plotted against the crown-rump length (C.-R.L. in mm). Volume of the cortex assumed as 100%. Portions of the gland occupied by particular adrenocortical zones are shown. Other explanations as in Fig. 1. and Fig. 2.

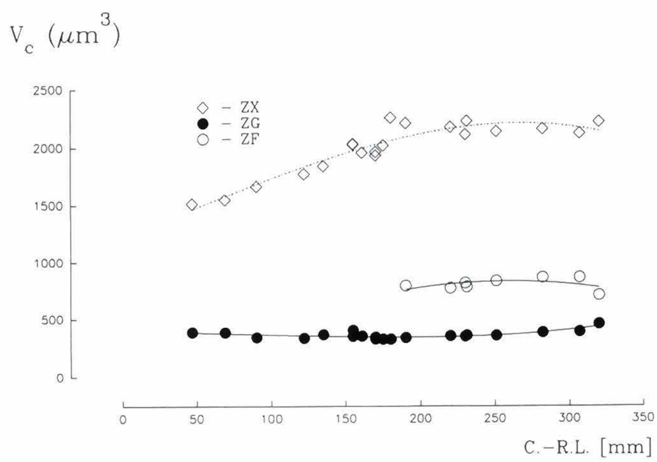

Fig. 5. Average volume of adrenocortical cells in the course of intra-uterine development of human adrenal gland. Explanations as in Fig. 1 and Fig. 2.

markedly higher. It can be seen that the increase in volume of adrenal gland was mainly due to enlargement of the ZX (Fig. 2). Of interest is the fact that in the whole period of observation the ZX comprised about 70% of total adrenal volume (Fig. 3). The fraction of the gland occupied by ZG gradually decreased toward the end of the foetal period, an effect accompanied by an increase in volume fraction of developing ZF.

The volume of stroma (connective tissue and blood vessels) significantly increased in the course of intra-uterine development of the gland (Fig. 4). The rate of increase seemed to be similar in all adrenocortical zones. However, the volume of stroma in the ZX markedly exceeded that of the remaining parts of the cortex.

In the period studied the average cell volume of ZG and ZF remained rather constant (the former 321 to 385 μm^3, the latter 704 to 861 μm^3), while the average volume of the ZX cell increased gradually from the 9th week of intra-uterine life (1511 μm^3) to the 20th week (2205 μm^3) and afterwards remained unchanged

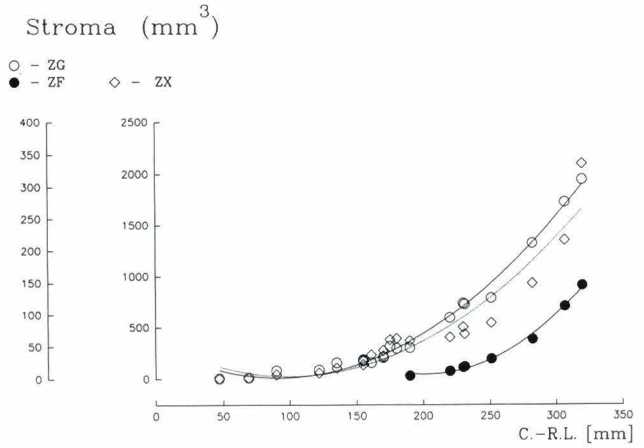

Fig. 4. Volume of stroma in the developing adrenal zones in the course of intra-uterine life. Explanations as in Fig. 1 and Fig. 2.

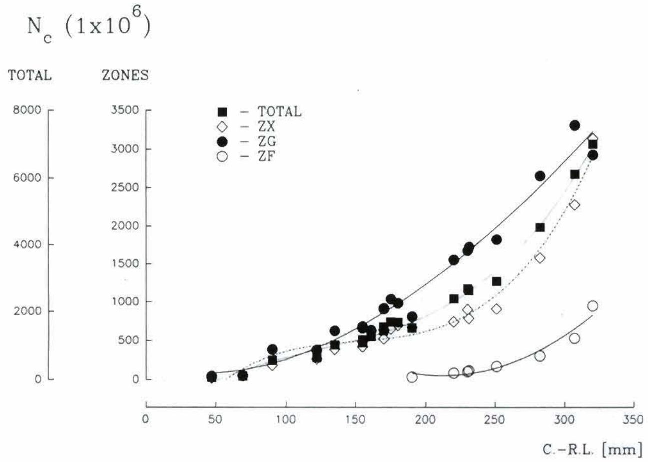

Fig. 6. Number of parenchymal cells in particular adrenocortical zones and in the entire cortex of developing human adrenal cortex in the course of gestation. Explanations as in Fig. 1 and Fig. 2.

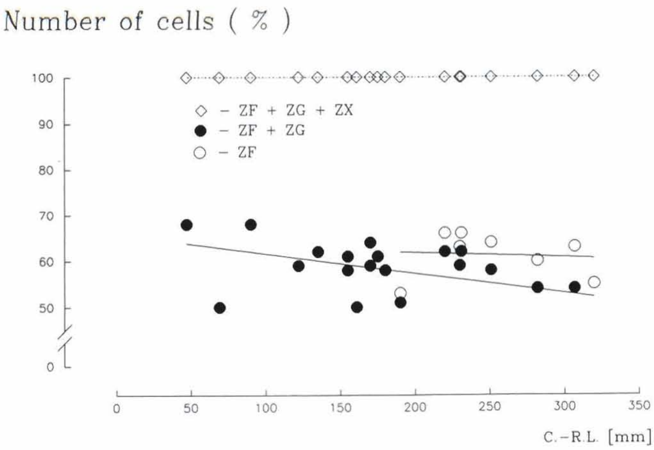

Fig. 7. The distribution of parenchymal cells between particular adrenocortical zones of developing human adrenal cortex in the course of intra-uterine life. Total number of cells in adrenal cortex set at 100%. Explanations as in Fig. 1. and Fig. 2.

(Fig. 5).

A notable increase in the number of parenchymal cells of the developing adrenals began from the 20th week. The similar rate of proliferation was seen in the foetal and glomerulosa-fasciculata zones (Fig. 6). It has to be pointed out that the percentage of ZX cells in the gland remained quite constant during the whole period of observation. On the other hand, the percentage of ZG cells declined toward the end of the foetal period and this decline was compensated for by an increase in the number of ZF cells. Due to these changes the distribution of parenchymal cells between particular adrenocortical zones remained constant with ZX cells comprising about 35-40% of parenchymal cells of the developing adrenal cortex (Fig. 7).

Discussion

Data presented above on the absolute and relative adrenal gland weight in the course of intra-uterine life confirm earlier reports (Jackson, 1909; Bachmann, 1954). It is well known that the relative adrenal gland weight of human foetuses is 10 to 20 times larger than that of adults and this value declines toward the end of foetal life. On the contrary, absolute adrenal gland weight steadily increases in the studied period of development.

As far as hypothalamo-pituitary control of adrenal gland growth in human foetuses is concerned, available data suggest that this axis exerts a regulatory role well before the 20th week of intra-uterine period (Gray and Abramovich, 1980). However, at that earlier time the main regulatory factor seems to be the placental chorionic gonadotropin (HCG) and placental ACTH while foetal pituitary adrenocorticotropin (ACTH) is essential for maintenance of the gland from the 20th week onward (Fisher, 1986). This concept is supported by the finding that, from week 20 of gestation, adrenal gland weight in anencephalic foetuses is lower than that in normal human foetuses (Meyer, 1912; Bernirschke, 1956; Gray and Abramovich, 1980).

It is generally accepted that the foetal and permanent areas of the human adrenal gland originate at the beginning of the 5th week of embryonic period and give rise to cortical zones of the adult adrenal gland (Uotila, 1940; Gruenwald, 1946; Velikan, 1948; Bachmann, 1954; Crowder, 1957; Lanman, 1960; Sucheston and Cannon, 1968). As observed by Sucheston and Cannon (1968), the ZG, outer ZF, ZX, and medulla begin to delineate by the 28th to 30th week of foetal life. Other authors avoid distinguishing between the foetal ZG and ZF. Usually, recognizable ZF has been described at the end of the foetal period (Benirschke et al., 1956; Wolyńska, 1956).

Our quantitative studies revealed the presence of a narrow rim of cell islands between glomerulosa and foetal zones in foetuses at the 20th week of intra-uterine life. These cells were larger than those of the ZG and smaller than ZX cells. On the basis of quantitative parameters we may assume that the ZF cells begin to appear in human foetuses about the 20th week of gestation. Foetal ACTH is responsible for differentiation, growth and function of fasciculata cells (Johannisson, 1968; Seron-Ferre et al., 1978; Gray and Abramovich, 1980). Thus, the appearance of ZF cells in the foetal adrenal gland accompanies the beginning of foetal ACTH secretion (Jaffe, 1983). It should be emphasized that Gray and Abramovich (1980) in 17-18-week-old foetuses described the «indeterminate» zone, formed by the rim of cells lying between the foetal zone and definitive zone, and showing some morphological features of both.

As far as the volume of adrenocortical zones is concerned, Swinyard (1943) demonstrated a steady increase in the volume of foetal zone and permanent cortex up to the end of the foetal period, with the highest value about 4000 mm^3. In the present study, however, for both adrenal glands the appropriate volume was notably higher (amounting to over 12000 mm^3).

Data on the mean percentage volume of adreno-cortical zones in the developing human foetal gland are incomplete and differ markedly between particular authors. Lanman (1961) and Johannisson (1968) estimated the foetal zone volume as 80% of the gland. In observations of Gray and Abramovich (1980), the volume of definitive zone, foetal zone and «indeterminate» zone were calculated to be 18.8%, 76.5% and 3.8% respectively. As demonstrated in the present study, in the course of intra-uterine development the volume of ZG notably declined toward the end of the foetal period, while that of the ZX remains rather constant, amounting to 70-80% of the volume of the glands. Moreover, the steady increase in the volume of the ZF was observed from the 20th week of gestation (1%) to the end of intra-uterine life (7%).

The available literature contains a wealth of information on the structure and ultrastructure of the developing human adrenal cortex (Bachmann, 1954; Johannisson, 1968; Nussdorfer, 1986). To our knowledge, the present study provides, for the first time, quantitative data on cell size and number in such glands. As expected, definitive zone cells (ZG and ZF) are markedly smaller than those of foetal zone in the course of foetal development; no significant changes in their volumes were observed. On the contrary, the number of parenchymal cells in the foetal adrenal cortex increased toward the end of foetal period. Of interest is the fact that during the whole period of observation the number of ZX cells was nearly the same as that of the permanent cortex.

Analysis of parenchymal cell distribution in the human foetal adrenal gland has shown that, in the whole period of intra-uterine life, foetal zone cells comprise about 40-45% of all adrenocortical cells. On the contrary, the percentage of ZG cells notably declined toward the end of intra-uterine life while that of the ZF increases, reaching 9-13% at the end of the observation period.

As far as involution of the foetal zone is concerned, some authors observed this process during the last month of the foetal period (Benirschke, 1956; Wolyńska, 1956; Johannisson, 1968). Foetal zone involution is usually associated with the development of dilated blood vessels. In agreement with these observations, we noticed a marked increase in the volume of stroma (blood vessels and connective tissue) in adrenals of 38-week-old foetus, which could be related to the onset of involution of this gland.

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