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Stereology of human fetal adrenal medulla

J. Bocian-Sobkowska[1], W. Woźniak[1], L.K. Malendowicz[2] and W. Ginda[2]

[1]Department of Anatomy and [2]Department of Histology and Embryology, University School of Medical Sciences, Poznan, Poland

Summary. Stereological studies were performed on 27 pairs of adrenal glands of human fetuses (9-38 weeks of intra-uterine development).

Medullary chromaffin cells were identified by immunostaining for chromogranin-A. The volume of adrenal medulla, average cell volume, and the number of chromaffin cells were calculated.

The volume of adrenal medulla increased slowly up to the 20th week and afterwards it enlarged rapidly to the 31st week of the fetal period.

A gradual, linear increase in the number of chromaffin cells of developing adrenal medulla was observed during the studied period. On the contrary, the average volume of the adrenal medullary cells remained quite constant until the 17th week of the development. Afterwards, a gradual, linear increase in the cell volume was observed until the 31 st week, reaching a plateau by the end of intra-uterine development.

Key words: Adrenal medulla, Immunocytochemistry, Human fetus, Chromogranin A, Cytology, Cell number, Stereology

Introduction

The first descriptions of the human fetal chromaffin tissue were made by Zuckerkandl (1901, 1912), Wiesel (1902), and Kohn (1903). In their opinion the adrenal medulla is not predominant among the chromaffin cells, and the extra-adrenal tissue is the main source of catecholamines during fetal life. This suggestion has been confirmed in studies by Sheperd and West (1952), Niemineva and Pekkarinen (1952, 1953), Coupland (1952), West et al. (1953) and Hervonen (1971).

It is generally accepted that the chromaffin cells and sympathetic neurons originate from the neural crest (Yntema and Hammond, 1947; Coupland, 1965). The neurogenic progenitor cells and the sympathoadrenal progenitors have been studied by Patterson (1990). These cells are perhaps the best-studied case of determination of neural crest derivatives by environmental factors. Experiments with trans-differentiation of chromaffin cells to nerve cells support the hypothesis that the adrenal chromaffin cells and sympathetic noradrenergic neurons originate from the same precursor cells in the neural crest (Landis and Patterson).

At least two different polypeptide growth factors influence the development of sympatho-adrenal progenitors along the neuronal pathway of differentiation. One is the fibroblast growth factor (FGF) and the other, the nerve growth factor (NGF). FGF (or related molecule) promotes proliferation and initial neuronal differentiation of sympathoadrenal progenitors within embryonic ganglia (Anderson and Axel, 1986; Birren and Anderson, 1990; Carnahan and Patterson, 1991; Anderson, 1992).

Other cells migrate to the adrenal gland, where they differentiate to chromaffin cells under the influence of adrenal glucocorticoid hormones (Unsicker et al., 1978; Doupe et al., 1985; Anderson and Axel, 1986; Seidl and Unsicker, 1989; Carnahan and Patterson, 1991). Initially, the neural crest cells form a mass on the medial side of the fetal cortex. The cells that form the fetal cortex are derived from the mesothelium lining the posterior abdominal wall and form an oval anlage in 5 week-old embryos. At the end of the embryonic period the adrenal cortex is composed of two zones: the outer permanent cortex; and the inner fetal zone. Differentiation of the cortical zones also begins during the fetal period and cells migrate from the outer cortex. The «cell migration theory» has been proved in recent experimental studies (Stachowiak et al., 1990; Sarria et al., 1995).

The conversion of sympathoadrenal progenitors to chromaffin cells of adrenal medulla and expression of the epinephrine-synthesizing enzyme appears to be mediated by the type II glucocorticoid receptor (Michelsohn and Anderson, 1992). This receptor both induces chromaffin cell-specific genes and represses neuron-specific genes (Anderson, 1992).

There are no quantitative investigations on the development of the human adrenal medulla during intra-uterine period. Therefore, the aim of present study was to investigate the volume of adrenal medulla, average cell volume, and the number of chromaffin cells in the

Offprint requests to: Dr. J. Bocian-Sobkoska, Department of Anatomy, University School of Medical Sciences, 6 Swieciski str., P 1-60-781 Poznań, Poland

developing human adrenal glands.

Materials and methods

Stereological studies were performed on 27 pairs of adrenal glands of human fetuses from the collection of the Department of Anatomy, University School of Medical Sciences in Poznań. The collection consisted of normal fetuses obtained from spontaneous abortions. The permission of the University Ethic Committee was obtained.

The age of fetuses was estimated by C-R length, foot length, and body weight, according to Carnegie staging data of O'Rahilly (1975). Detailed data of studied fetuses are shown in Table 1. Adrenal glands 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 paraplast, the glands were serially sectioned at 5-6 µm, and sections were stained with hematoxylin and eosin.

For immunocytochemistry (ABC method; Hsu et al., 1981), sections were deparaffinized and permeabilized for 5 min with Triton X-100 in PBS-buffered saline followed by incubation (2 x 5 min) with 0.03% H_2O_2 in 10% methanol in PBS to block endogenous peroxidase activity. Sections were preincubated with 2% rabbit serum in PBS for 1 h and subsequently incubated with mouse prediluted antiserum directed against human chromogranin A (CGR-A Dianova, Germany). Tissue sections were incubated overnight at 4 °C. As secondary antibody, biotylinated anti-mouse antiserum obtained from rabbit (1:500, DAKO, Denmark) was used, followed by incubation with a commercial ABC reagent (Vectastain kit, Camon). The immunoreaction was visualized by using 3,3' diaminobenzidine-hydrochloride (Aldrich, Milwaukee, WI, USA) and 0.01% H_2O_2 in tris-HCl. As a control we used mouse serum in the same dilution as the first antibody, or omitted the first antibody.

Stereological studies were performed according to Weibel's description (1979). Using a magnification of about 100 and a square lattice test system of type A (Weibel, 1979), the volume densities of adrenal medulla were evaluated. In two glands of the youngest investigated fetuses all sections of the glands were analyzed. In the older fetuses the measurements were made on every fifth section of the glands. The volume of adrenal glands was calculated from its weight, by assuming that the average specific gravity of the gland was 1.039 mg/mm^3 (Swinyard, 1943).

Results

In fetus at the 9th week the medullary chromaffin cells identified by immunostaining for CGR-A were present within the adrenal gland. Small islands of cells were dispersed throughout gland, being more numerous in the central part and adjacent to the medial border. They consisted of 5-14 cells with dark, pycnotic nucleus and scarce cytoplasm. Single chromaffin cells were also scattered in the transitional zone of the gland. Medullary blood vessels (capillary sinusoids) were also observed. These sinusoids were larger in the central part of the gland. Until the 20th week of intra-uterine development the volume of sinusoids was low (they occupied about 7% of the volume of the adrenal medulla), while in the oldest fetus they were wider and more numerous, and

Table 1. Crow-rump (CRL) and foot length (mm), body (g) and adrenal (mg) weight, sex and age (in postovulatory weeks) of studied fetuses.

No	CRL (mm)	FOOT LENGTH (mm)	BODY WEIGHT (g)	ADRENAL WEIGHT (mg)	SEX	AGE (weeks)
1	47	5	11	63	M	9.0
2	69	10	23	127	F	10.0
3	90	14	70	556	M	14.0
4	122	20	120	764	M	14.5
5	135	28	219	1248	M	15.0
6	155	31	310	1430	F	17.0
7	155	31	344	1680	F	17.0
8	161	34	390	1957	F	17.5
9	170	34	400	2166	M	18.0
10	170	35	340	1821	M	18.0
11	175	35	450	2422	M	18.5
12	180	36	540	2642	M	19.0
13	190	36	526	2514	M	20.0
14	220	41	700	3172	M	22.0
15	222	42	756	3280	F	23.0
16	231	43	810	3602	M	24.0
17	230	43	816	3572	F	24.0
18	230	44	850	3737	M	25.0
19	236	46	900	3810	F	26.0
20	237	46	911	3800	F	26.0
21	247	46	950	3710	M	27.0
22	251	46	978	4160	M	29.0
23	282	49	1320	7030	M	31.0
24	293	49	2420	8745	M	35.0
25	296	49	2860	8920	M	36.0
26	307	53	2260	10130	F	37.0
27	320	64	2900	13352	F	38.0

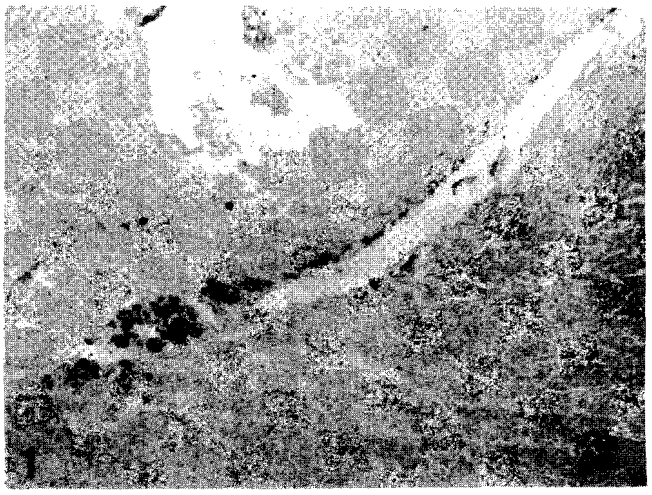

Fig. 1. Adrenal gland of 10-week-old fetus. Chromogranin A-immunopositive medullary cells in the central region of developing adrenal gland. x 80

occupied about 36% of the medullary volume.

The developing sinusoids were surrounded by chromaffin cells, which formed easily visible cellular columns between adjacent sinusoids. With increasing age these columns underwent enlargement due to proliferation of cells and were arranged in characteristic whorls. Other chromaffin cells were arranged into round and ovoid groups. Each chromaffin cell was in close contact with the capillary wall. Whorls of chromaffin cells were often observed in intimate contact with adrenal sympathetic cells (Figs. 1, 2).

Quantitative data describing the development of the adrenal gland are presented in Table 2 and Figs. 3-5. In fetuses from 47 to 320 mm C-R length (9-38 weeks of intra-uterine life) a marked increase in adrenal gland weight and adrenal volume was found. As compared with the earlier periods, the increase rate was notably higher from the 20th week on (Fig. 3). Similar rate of growth was observed for adrenal medulla (Figs. 3, 4).

The volume of adrenal medulla increased slowly up to the 20th week (173 mm^3) and afterwards it enlarged rapidly, reaching 698 mm^3 at the 31st week (Fig. 4). Enlargement of the adrenal medulla was caused mainly by the developing capillary sinusoids and proliferation of the medullary cells.

A gradual linear increase in the number of chromaffin cells of developing adrenal medulla was observed during the studied period (Fig. 5). On the contrary, the average volume of the adrenal medullary cells remained quite constant until the 17th week of development. Afterwards, a gradual linear increase in the cell volume was observed until the 31st week, reaching a plateau by the end of intra-uterine development (Fig. 5).

Discussion

While fetal adrenal cortical formation and regulation

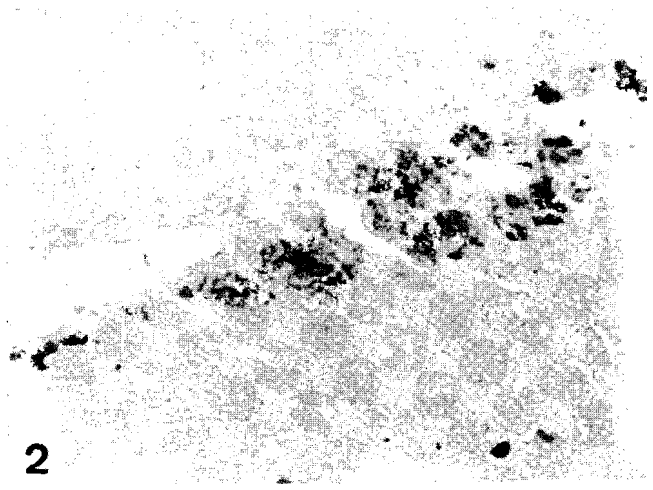

Fig. 2. Adrenal gland of 14-week-old fetus. Chromogranin A-immunopositive medullary cells in vicinity of blood sinusoid. x 80

Table 2. Volume of adrenals (V_{adr}), volume of adrenal medulla (V_{med}) and volume of chromaffin cells ($V_{chrom\ cells}$) (mm^3), average volume of chromaffin cells (V_{cell}) (μm^3) and total number of chromaffin cells (N_{cells}) ($1x10^6$) of studied fetuses.

No	V_{adr} (mm^3)	V_{med} (mm^3)	$V_{chrom\ cells}$ (mm^3)	V_{cell} (μm^3)	N_{cell} ($1x10^6$)
1	61	4	4	810	5
2	122	8	8	840	8
3	535	34	30	818	34
4	735	43	45	890	46
5	1201	92	83	920	86
6	1376	91	80	900	106
7	1617	116	180	820	147
8	1884	141	122	980	114
9	2085	156	143	1120	122
10	1753	142	132	1220	120
11	2331	186	165	1430	114
12	2543	183	170	1316	119
13	2420	172	160	1411	140
14	3053	298	248	1620	165
15	3157	384	320	1511	205
16	3467	463	338	1616	200
17	3438	643	353	1517	230
18	3597	752	392	1910	205
19	3667	664	480	2110	217
20	3657	758	521	2110	235
21	3571	978	533	2120	241
22	4004	1083	720	2210	310
23	6766	1092	698	2010	254
24	8417	1106	732	2050	341
25	8585	1200	774	2310	330
26	9750	1201	790	2240	342
27	12851	1259	800	2220	365

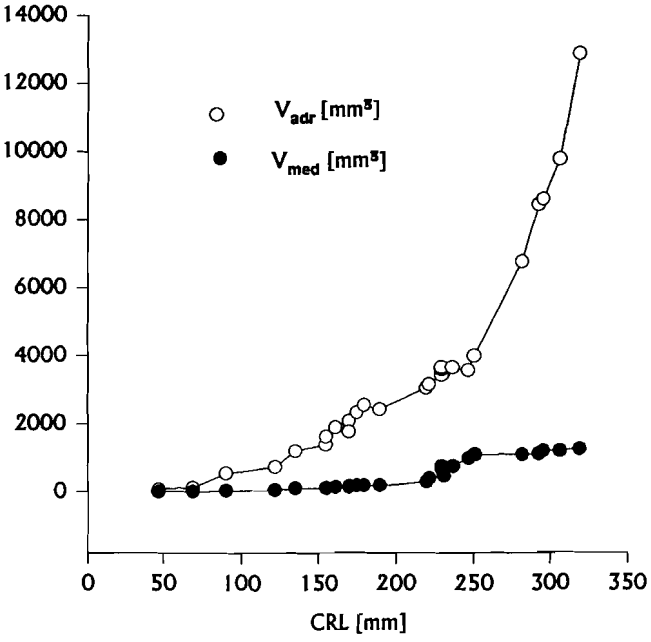

Fig. 3. Volume of adrenal glands and medulla in the course of intra-uterine development as plotted against the crown-rump length in mm. Each point presents one case.

are relatively mature at term, the adrenal medulla develops much more slowly and medullary maturation continues during postnatal life (Coupland, 1965; Hervonen, 1971; Seron-Ferre and Jaffe, 1981; Neville and O'Hare, 1982).

The beginning of encapsulation of the medulla by the cortex in developing human adrenal glands is observed at the end of the embryonic period (8th week), and the medulla is well formed in the human fetus by 10-12 weeks (Bachman, 1954; Crowder, 1957; Coupland, 1965; Bloch, 1967; Johannisson, 1968; Hervonen, 1971; Ville, 1972). This was also confirmed in the present study. In 9 week-old fetus the chromaffin cells are present in the central part and adjacent to the medial border of the gland as small cellular islands.

The envelopment of the medulla by the cortex ensures a high corticosteroid environment, facilitating chromaffin cell development. Catecholamines can be detected in the human fetal adrenal medulla by 10-15 weeks of gestation (Niemineva and Pekkarinen, 1952; Greenberg and Lind, 1961). In contrast, catecholamine content predominates in the paraganglial tissue which shows nearly the same developmental features as the adrenal medulla during the fetal period (Iwanow, 1927; Coupland, 1965; Hervonen, 1971). After the 12th-15th weeks the great majority of granule-containing cells in both paraganglia and adrenal medulla have assumed certain stabile characteristics and the cells are supposed to be morphologically mature (Hervonen, 1971).

In the present study it has been shown that the volume of the adrenal medulla increases slowly up to the 20th week and that then the more rapid growth of the medulla is observed.

Sucheston and Cannon (1968) have studied human adrenal glands from 58 autopsy specimens ranging from one month gestation to 69 years postnatally. Their microscopic examinations revealed a pertinent developmental pattern in the establishment of definitive zonation. According to them, in fetuses between 8.5 and 18 weeks of gestation, the ratio of the fetal cortex to permanent cortex to medulla was 75/20/5, while after the 30th week of gestation it was 60/25/15. Our results are similar to their observations. However, the authors did not investigate fetuses between 18 and 30 weeks. During this period we have observed that the volume of adrenal medulla was above 20%.

The adrenal glands of 12 anencephalic fetuses and 17 normal fetuses of 11 to 21 weeks gestation were examined histometrically by Gray and Abramovich (1980). According to them the volume of neuroblasts in the normal adrenal glands was 0.77-0.36% in the studied period. In our material in this period of development the volume of chromaffin cells was 6.3-0.65%. The authors used the term «neuroblasts» for chromaffin cells of the adrenal medulla.

According to Hervonen (1971) the final organization of the fluorescing cells in the adrenal medulla is the following: a) cells form round or ovoid clusters or are organized in whorls; b) cells surround the wide capillary sinusoids and show close contact with the capillary wall; c) medullary cells of fetuses older than 14 weeks are frequently gathered around the collection of the primitive sympathetic cells. Our observations on chromaffin cells identified by immunostaining for CGR-A are in agreement with results of Hervonen.

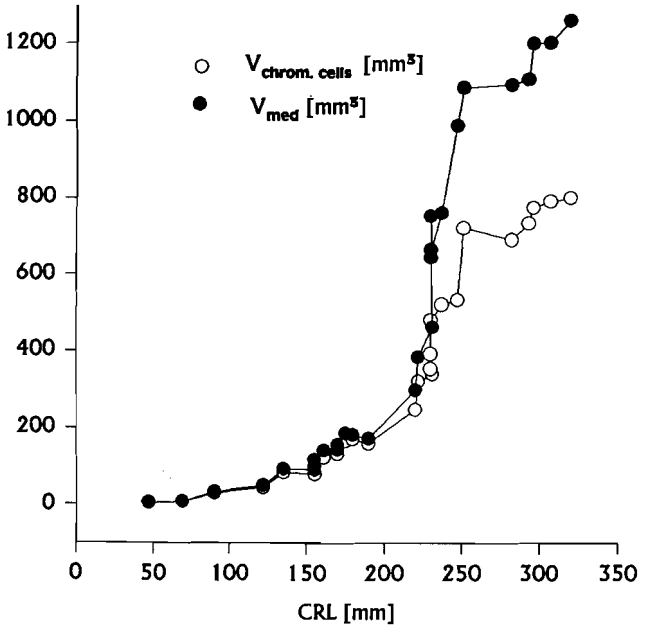

Fig. 4. Volume of adrenal medulla and volume of chromaffin cells in the course of intra-uterine development as plotted against the crown-rump length. Each point represents one case.

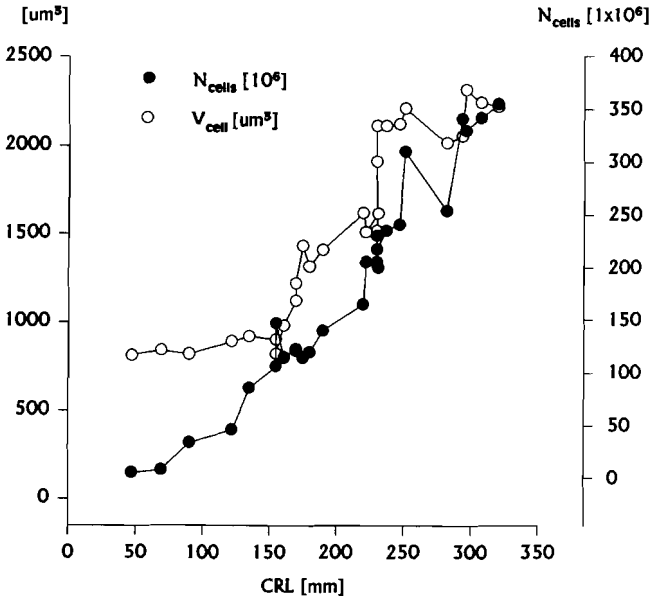

Fig. 5. Average volume and number of chromaffin cells in adrenal medulla during intra-uterine development as plotted against the crown-rump length. Each case represents one case.

The present study provides the first quantitative data on cell size and number in human fetal adrenal medulla. It should be pointed out that during the whole period of observation the number of chromaffin cells increases gradually to the end of fetal period, while the volume of chromaffin cells increases between the 17th and 31st week of intra-uterine development and then is constant to the end of the fetal life.

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