

Morphometric studies on the development of the human thyroid gland. II. The late fetal life

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Summary. Histological and morphometric studies were performed on 27 thyroid glands obtained from normal fetuses ranging between 23 to 40 weeks of intra-uterine life.

In the thyroids the volume of gland, colloid, and stroma were calculated by means of differential point-counting method and the height of the thyroid follicular cells was measured. Moreover, the epithelium/colloid ratio in the thyroid gland, a very sensitive parameter of stimulation of the glands by TSH, was calculated. Regarding the values of this ratio in human fetal thyroid gland, the intra-uterine development of the gland may be divided into three distinct stages. The first one, between weeks 10-18 is characterized by massive folliculogenesis and gradual accumulation of the colloid. The second stage, between weeks 19-29 of fetal life is characterized by rather unchanged values of epithelium/colloid ratio and the size of follicles. The third stage, after the 29th week of development is characterized by a gradual increase in the epithelium/colloid ratio and a decrease in the size of follicles.

Key words: Human fetus, Thyroid gland, Morphometry, Development

Introduction

Since the placenta is impermeable to thyroid-stimulating hormone (TSH), the thyroid structure and function in the human fetus develops autonomously of the maternal thyroid gland. Moreover, the high concentration in maternal serum of T4-binding globulin and inherent placental impermeability to the iodothyronine molecules limit the influence of maternal thyroid on fetal gland function (Fisher et al., 1964; Roti et al., 1982).

Studies on anencephalic fetuses, lacking hypothalamic structures, revealed that the differentiation and growth of pituitary thyrotropic cells is not dependent

upon the presence of hypothalamus (Hatakeyama, 1969). The placenta is permeable to thyrotropin-releasing hormone (TRH); however, TRH plays little or no part in the fetal thyroid function (for review see Roti, 1988). It remains to be established whether the maternal TRH influences thyrotropic cell differentiation in anencephalic fetuses.

In pituitary thyrotropic cells TSH-immunoreactivity is observed as early as 13-15 weeks of intra-uterine development, while by RIA the presence of TSH-immunoreactivity in fetal pituitary gland was demonstrated 3 weeks earlier (Fukuchi et al., 1970; Fisher et al., 1977; Dubois and Begeot, 1978). At the same time of intra-uterine life the first PAS-positive thyroid follicles containing colloid appear, and our earlier studies suggest that by week 17.5 of development the thyroid gland of human fetus reaches structural maturity (Bocian-Sobkowska et al., 1992).

The present study was designed to further characterize, by means of morphometric methods, the human fetal thyroid gland development during the second half of intra-uterine life.

Materials and methods

Studies were performed on 27 thyroid glands obtained from normal human fetuses, from the collection of the Department of Anatomy, University School of Medical Sciences, Poznań, Poland. Fetuses were obtained from spontaneous abortions and they had no visible developmental abnormalities.

The developmental age was estimated according to the Carnegie staging data of O'Rahilly (1975). All fetuses were weighed and their crown-rump length (C.-R.L.) was measured. Detailed data of studied fetuses are shown in Table 1. The thyroid gland was carefully excised, weighed to the nearest 0.1 mg, fixed in Bouin's solution, then embedded in paraplast and serially sectioned at 6-7 μ m. Sections were stained with haematoxylin-eosin and the PAS reaction was performed.

Stereological studies were made according to the method of Weibel (1979). Under magnification of x400, using the M49 test point system, the volume fractions of

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Table 1. Crown-rump length (C.-R.L.), body and thyroid weight, sex and age of studied fetuses.

No.	C-RL (mm)	BODY WEIGHT (g)	THYROID WEIGHT (mg)	AGE IN POSTOVULATORY WEEKS	SEX
1	220	580	380	23	M
2	220	700	570	23	F
3	230	742	690	24	F
4	231	810	770	25	F
5	235	790	690	25	M
6	238	800	740	25	M
7	235	720	640	25	M
8	242	850	830	26	F
9	251	860	900	27	M
10	252	870	900	27	F
11	264	920	980	28	F
12	266	978	1090	29	F
13	270	1050	1050	29	F
14	276	1070	1110	30	M
15	279	1110	1350	30	M
16	281	1280	1530	31	M
17	282	1320	1900	31	F
18	310	1580	1960	33	M
19	308	1850	2160	34	F
20	315	1940	2720	35	M
21	327	2660	4580	37	M
22	330	2450	3200	38	F
23	325	2720	2500	38	F
24	330	2900	3600	38	M
25	331	3100	4710	39	F
26	345	3450	3830	40	M
27	360	3980	4380	40	M

epithelium, colloid and stroma (connective tissue and blood vessels), and the number of thyroid follicles per test area, were estimated directly on the screen. Haematoxylin-eosin staining does not permit the recognition of thyroid C cells; therefore, these cells were counted as follicular cells. The area of a single analyzed field was $8.1 \times 10^{-2} \text{mm}^2$. In each fetus 5 sections of the

thyroid gland were randomly chosen and in each section 50 fields were counted along the line passing through the central part of the thyroid lobe. The specific gravity of the thyroid gland was assumed to be 1.060mg/mm^3 (Malendowicz and Bednarek, 1986). Knowing the weight of the gland and its specific gravity, the volume (in mm^3) of epithelium, colloid and stroma was calculated.

The height of the thyroid follicular cells was measured by means of a micrometer eye-piece (K-15 PZO), under magnification of $\times 450$. In each thyroid gland 10 follicles were analyzed along the line passing through the central part of the gland. In each case 4 cells located at the angle 0° , 90° , 180° , and 270° from the center of the follicles were measured.

The results were processed statistically and graphically by the Origin programme for IBM PC.

Results

As shown in Fig. 1 the thyroid gland volume of human fetuses increased proportionally with respect to their C.-R.L., with high correlation coefficient equal to 0.896. In the studied period of development the weight of the gland enlarged nearly fourfold. During the investigated period of development the relative thyroid gland weight slowly increased. However, at the end of this period individual values were more variable than in the earlier weeks of intra-uterine life (Fig. 2), and gender differences in the weight were not observed.

The number of thyroid follicles per 1mm^2 of the thyroid surface section increased gradually from 77 in the youngest fetuses studied to ca 170 at the end of intra-uterine life (Fig. 3). Typically, PAS-positive colloid containing follicles in the central part of the thyroid lobe were smaller than the peripherally located ones.

The volume (in mm^3) of thyroid epithelium,

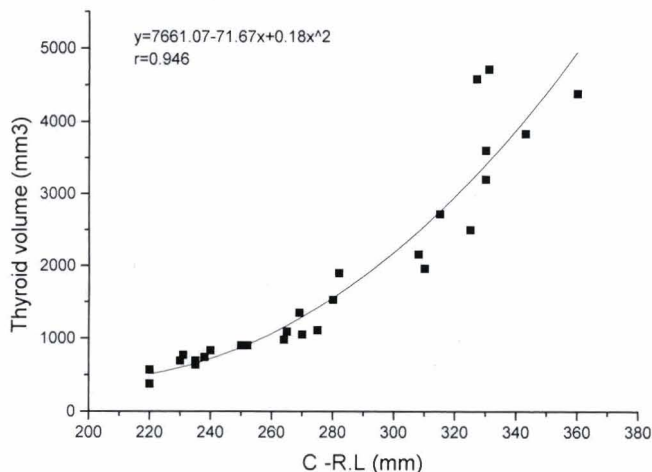


Fig. 1. Relation between thyroid gland volume and C.-R.L. Of fetuses between 23-40 weeks of intra-uterine development. Each point presents one fetus.

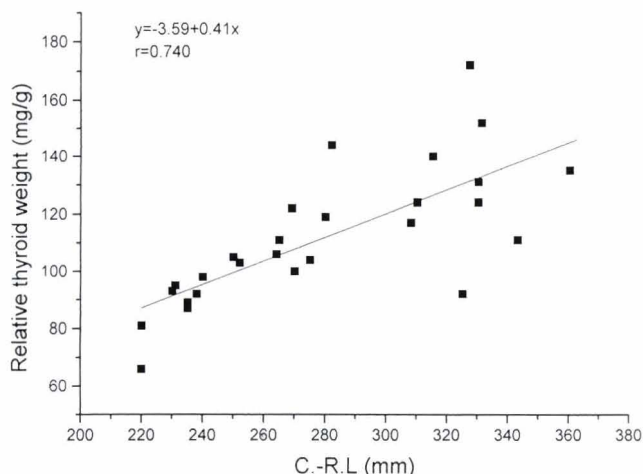


Fig. 2. Relation between relative thyroid gland weight and C.-R.L. of fetuses between 23-40 weeks of intra-uterine development. Each point presents one fetus.

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colloid and stroma increased gradually up to the 31st week and thereafter the rate of increase was notably higher (Figs. 4-6). The rate of growth was different for particular thyroid compartments. During the studied period of development the volume of colloid, epithelium and stroma compartments increased ca 5-, 10-, and 8-times, respectively. These differences in the growth of the above-mentioned compartments resulted in a notable increase in epithelium/colloid ratio (Fig. 7). This ratio reached the plateau in fetuses with C-R.L. ca 330 and longer. The height of thyroid follicle epithelial cells increased between weeks 23-31 of intra-uterine life (Fig. 8). Thereafter, until the term, the height of cells remained constant.

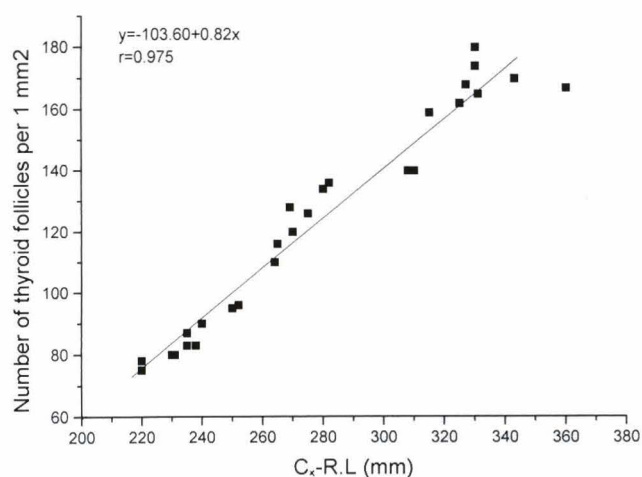


Fig. 3. Number of thyroid follicles per unit of surface of thyroid in relation to C.-R.L. of fetuses between 23-40 weeks of intra-uterine development. Each point presents one fetus.

Discussion

As far as thyroid weight of developing fetuses is concerned, the results of the present study confirm the earlier findings of Jackson (1909) and Shepard et al. (1964). The weight of the gland, as reflected by its volume is highly correlated with C.-R.L. ($r = 0.947$) of studied fetuses. Moreover, in accordance with earlier reports, no gender-related differences in the weight of the gland were observed during the late fetal life.

The present study clearly demonstrates the continuous increase in the absolute volume of thyroid colloid, epithelium and stroma during the second half of intra-uterine life of human fetuses. This growth is parallel to the increase in the weight and volume of the

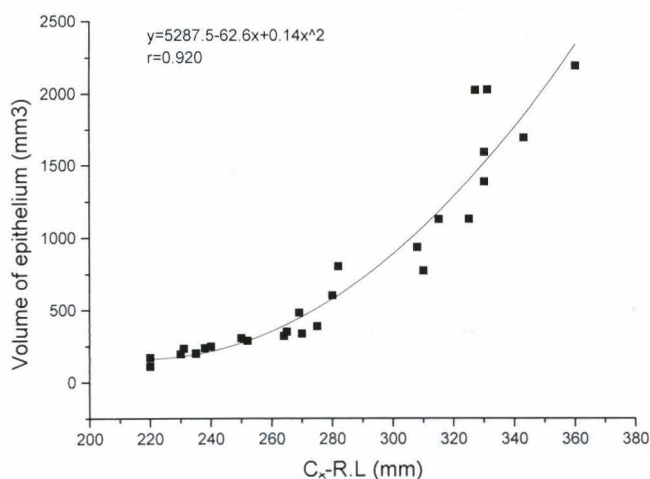


Fig. 4. Relation between the volume of thyroid epithelium and C.-R.L. of fetuses between 23-40 weeks of intra-uterine development. Each point presents one fetus.

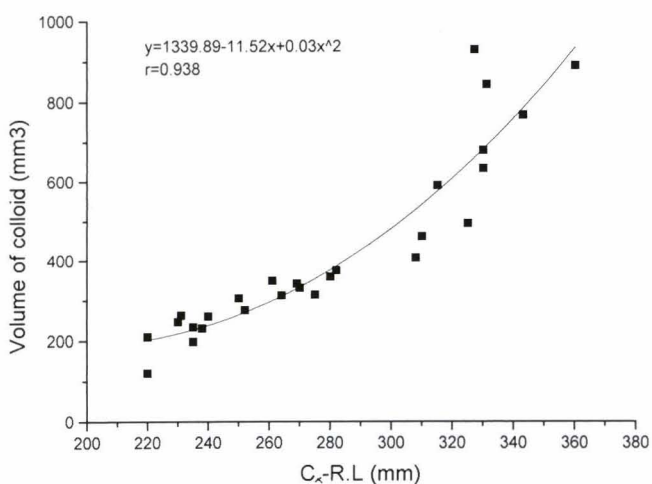


Fig. 5. Relation between volume of thyroid colloid and C.-R.L. of fetuses between 23-40 weeks of intra-uterine development. Each point presents one fetus.

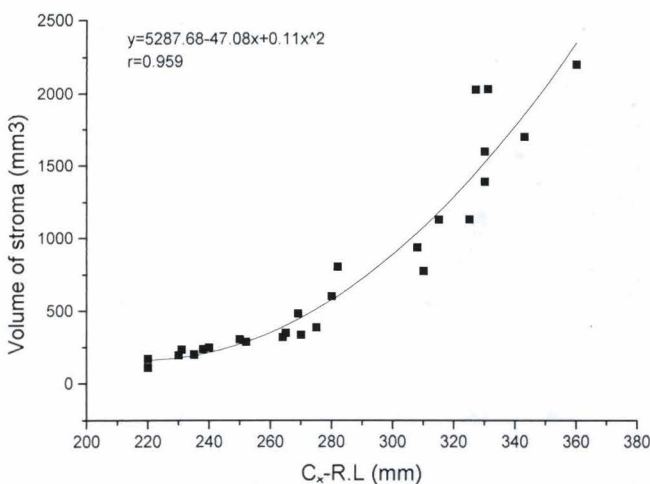


Fig. 6. Relation between volume of thyroid stroma and C.-R.L. of fetuses between 23-40 weeks of intra-uterine development. Each point presents one fetus.

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gland. However, the rate of increase differs between particular compartments of the gland. During the whole observed period the volume of the thyroid stroma increases nearly eightfold and is connected with the development of blood vessels and, to a lesser degree, connective tissue, as well.

The performed studies revealed marked differences in the rate of growth of thyroid colloid and epithelium. Analysis of obtained data shows, however, that up to the 29th week of intra-uterine life the rate of growth of both components, as evidenced by values of epithelium/colloid ratio, is similar. Thereafter, the growth of thyroid epithelium is accelerated in comparison with colloid accumulation. During the whole observed period the volume of thyroid epithelium increases from ca 250 to

3000 mm³ while the respective values for colloid are ca 200 and 900 mm³.

From a histological point of view, the most striking changes observed in the second half of intra-uterine life concern the size of thyroid follicles. In thyroid glands obtained from fetuses from the beginning of this period follicles are markedly larger and contain more colloid than those from near term fetuses. These morphological findings are reflected in epithelium/colloid ratio, which increases from ca 0.8 to 2.2 at the end of intra-uterine life.

In the human fetal thyroid gland, the first PAS-positive colloid-containing follicles may be observed at weeks 8-10 of development (Taki, 1958; Shepard, 1967; Nivelon and Tenenbaum, 1980; Dussault, 1981; Bocian-

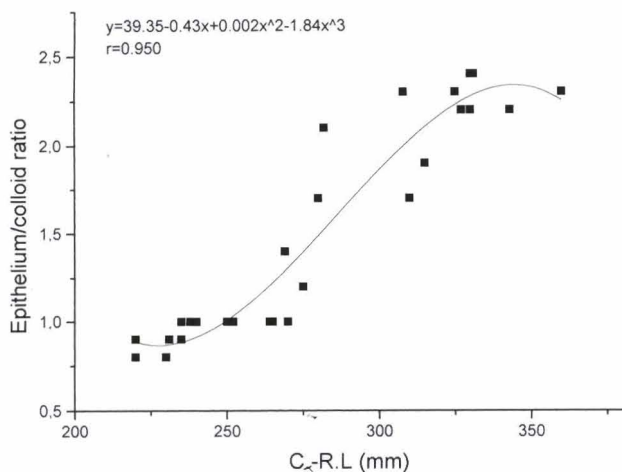


Fig. 7. Relation between epithelium/colloid ratio and C.-R.L. of fetuses between 23-40 weeks of intra-uterine development. Each point presents one fetus.

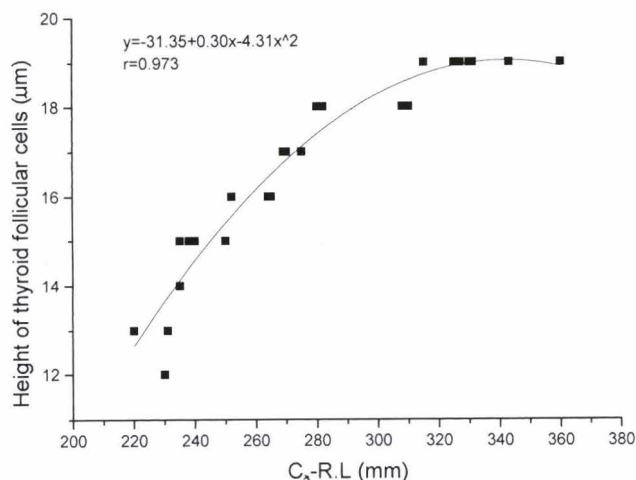


Fig. 8. Height of thyroid follicular cells in relation to C.-R.L. of fetuses between 23-40 weeks of intra-uterine life. Each point presents one fetus.

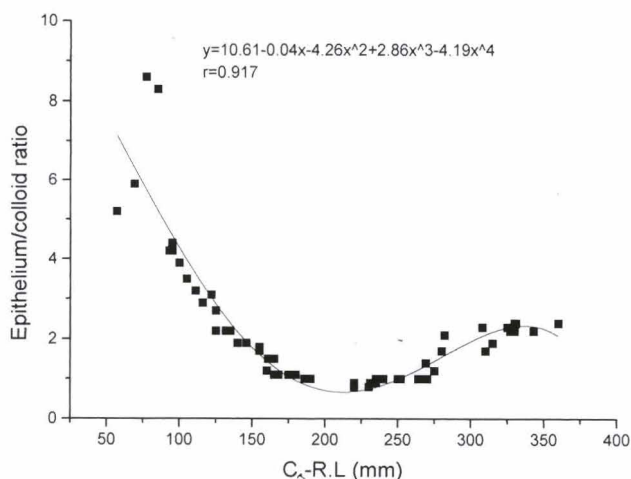


Fig. 9. Epithelium/colloid ratio in relation to C.-R.L. of fetuses between 10-40 weeks of intra-uterine development. Each point presents one fetus. Combined data from the present study and from Bocian-Sobkowska et al. (1992).

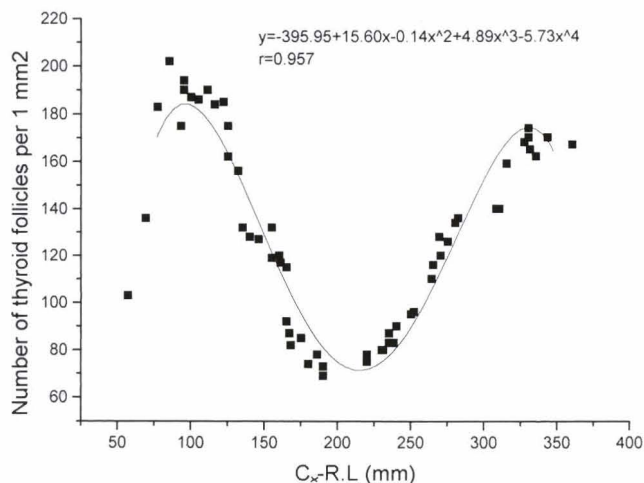


Fig. 10. Number of follicles per unit of surface of thyroid section in relation to C.-R.L. of fetuses between 10-40 weeks of intra-uterine life. Each point presents one case. Combined data from the present study and from Bocian-Sobkowska et al. (1992).

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Sobkowska et al., 1992; Bocian-Sobkowska and Woźniak, 1995). At this stage the epithelium/colloid ratio is very high (ca 8) and gradually decreases to value 1 at 17.5 weeks of intra-uterine life (Bocian-Sobkowska et al., 1992).

Graphically presented data from our earlier and present studies reveal two maximal values of epithelium/colloid ratio in human fetal thyroid gland (Fig. 9). The first peak, with value ca 8, takes place at weeks 10-11 and reflects intensive folliculogenesis in the early fetal period. These primary thyroid follicles contain a trace amount of colloid and their diameter is very small. At this stage the number of follicles per 1 mm² of thyroid surface section approximates 200 (Fig. 10). After this initial period the amount of colloid in the thyroid follicles and the size of follicles gradually increases, reaching the highest values at week 18 and remaining constant until week 29. Thereafter, the size of thyroid follicles decreases and the epithelium/colloid ratio increases, reaching the second peak (ca 2.2) in the 38th week of fetal life.

The epithelium/colloid ratio in the thyroid gland is a very sensitive parameter of stimulation of the gland by TSH. In laboratory animals, TSH administration results in a notable increase in the value of this parameter (Uotila and Kanas, 1962; Palkovits, 1963). Regarding the values of epithelium/colloid ratio in human fetal thyroid gland, the intra-uterine development of the gland may be divided into three distinct stages. The first one (weeks 10-18) is characterized by massive folliculogenesis and gradual accumulation of the colloid. In the second stage (weeks 18-29) the values of epithelium/colloid ratio and the size of follicles are stable. The third stage (weeks 29-40) is characterized by a gradual increase in the epithelium/colloid ratio and a decrease in the size of follicles. These types of change suggest the enhanced stimulation of the fetal thyroid gland by TSH in the third trimester of gestation.

Fetal serum TSH is very low at week 12 of intra-uterine life, and from that time on a progressive increase in hormone concentration occurs, with the highest values at 30-35 weeks (Fisher et al., 1970; Roti, 1988). In the fetal serum a slight concentration of T4 is first demonstrated in the 10th week and it remains rather unchanged until the 22nd week. Thereafter, the continuous increase of T4 concentration takes place, with the highest values at birth (Fisher et al., 1970; Greenberg et al., 1970; Oddie et al., 1978; Wilson et al., 1982; Roti, 1988). Considering the above-mentioned functional changes, it may be suggested that the structural changes of thyroid follicles observed in the 3rd stage of human fetal thyroid gland development (according to our division) depend upon increasing stimulation of the gland by TSH. However, we cannot exclude the suggestion of Fisher (1986) that during the third trimester of the intra-uterine life maturation of the thyroid gland responsiveness to TSH takes place and that this phenomenon could be responsible for stimulation of the gland by relatively stable serum TSH concentrations.

Our suggestion is also proved by the height of thyroid follicle epithelial cells. The height of these cells increases between weeks 21 and 31 (from 13 to 18 μ m) and afterwards it remains rather constant. It should be pointed out that the height of these cells remains constant between weeks 10 and 20 of intra-uterine life (12-13 μ m) (Bocian-Sobkowska et al., 1992).

As opposed to some literature data no gender differences in the structure of the fetal thyroid gland were observed either in the present or in the earlier studies (Albu and Georceanu, 1986).

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Accepted July 16, 1996