Abstract. Immunohistochemical detection of T₄, T₃ and thyroglobulin (Tg) was undertaken in foetal and neonatal rat thyroid glands in an attempt to elucidate the functional development and maturation of the gland during these periods. Immunoreactive Tg first appeared in the cytoplasm of the immature thyroid epithelium on the 15th day of gestation, followed by the occurrence of T₄ and T₃ in the lumen of primitive follicles of the thyroid gland 2 days later. The stainability of Tg, T₄ and T₃ increased as the follicular structure became mature; however, no significant difference in staining patterns was observed during the perinatal periods.

Thyroxine (T₄) and triiodothyronine (T₃) are important hormones, especially for the growth of the neurons and skeletal systems of the foetus (Eayrs 1971), and are considered to be supplied from the mother through the placenta (Knobil & Josimovich 1959). Prenatal maturation of thyroid function with establishment of the pituitary-thyroid system is a problem of considerable interest, since maternal supply is suddenly terminated at birth and newborns need to depend on their own hormones thereafter.

Although several studies have examined the foetal development of the thyroid gland through various approaches (Feldman et al. 1961; Shepard 1967; Rémy et al. 1980a,b), there is still a paucity of direct evidence for the production of these hormones in early foetal life.

Recently, we demonstrated that the immunohistochemical method is applicable for demonstrating thyroxine and triiodothyronine in tissue sections of the human and rat thyroid glands (Kawai et al. 1981), and that the pattern of immunostaining reflects fairly well the functional state of the thyroid glands under various experimental conditions (Kawai et al. 1983).

In this paper, we describe results for the immunohistochemical detection of T₄, T₃ and thyroglobulin in developing foetal and newborn rat thyroid glands in an attempt to elucidate the functional aspects of these organs.

Materials and Methods

Foetuses of Wistar rats from the 14th to 21st day of gestation, and newborns from the 1st to 7th day after birth were examined. The day following overnight mating was regarded as the 1st day of gestation. Each age group consisted of 3 to 5 animals. The animals were sacrificed by bleeding, and the thyroid glands which were removed with the head and neck in the case of the foetuses and separately in the newborns, were fixed immediately in Zamboni's solution overnight at 4°C.

The fixed tissue blocks were rinsed in cold 0.01 M phosphate buffered saline, pH 7.2 (PBS), and washed thoroughly. After dehydration through an ethanol series, they were embedded in paraffin as usual. Serial or semi-serial section of 3-4 µm in thickness were cut and stained with haematoxylin and eosin, the PAS reaction and the immunoperoxidase technique for T₄, T₃ and thyroglobulin (Tg).

An indirect immunoperoxidase method was employed, using anti-T₄ and anti-T₃ rabbit antisera provided for radioimmunoassay of 500 tubes per ml (Cappel Laboratories, Cochraneville, PA; and E.Y. Laboratories, Inc., San Mateo, CA, USA), anti-rat thyroglobulin rabbit antisera
which were prepared in our laboratory according to the
procedure by Chopra et al. (1971) and were absorbed of
cross-reactivity to T₄ and T₃ by affinity column chroma-
tography (Pensky & Marshall 1969), and horseradish
peroxidase labelled anti-rabbit IgG goat immunoglobulin
prepared in our laboratory according to the method of
Nakane & Kawaoi (1974). The deparaffinized tissue
sections were incubated with the anti-T₄ or anti-T₃ anti-
sera (diluted to 1:100 in PBS containing 1% bovine
serum albumin) or anti-Tg (diluted to 1:1000 in PBS) and
with the labelled antibody for 60 min at room tempera-
ture, followed by visualization of the peroxidase activity
by incubation in Graham & Karnovsky's solution (Gra-
ham & Karnovsky 1966) for 5–10 min. As negative
controls, the first antisera were replaced by either the
antisera previously absorbed with the corresponding
antigens (Kawaoi et al. 1981) or the non-immune rabbit
serum, and confirmation was obtained that no significant
staining occurred in either experiment. Furthermore,
icubation with the substrate alone showed that no endo-
genous thyroid peroxidase activity (Strum & Karnovsky
1970; Tice & Wollman 1974) was detectable in the tissue
sections.

Results

Thyroid glands at the 14th day of gestation
A trabecular or solid mass of immature thyroid
tissue without follicular structures was formed
along the bilateral sides of the trachea. At this
stage, Tg, T₄ and T₃ staining was completely nega-
tive. No PAS reaction was obtained.

Thyroid glands from the 15th to 16th day of gestation
The general histological features were almost the
same as those on the 14th day. Tg positive cells
occurred for the first time at this stage, although
they were quite few in number. The Tg immuno-
reactivity was located in the cytoplasm of the imma-
ture epithelium. Both T₄ and T₃ remained nega-
tive.

On the 16th day the thyroid glands revealed
lobulation, and interlobular growth of blood capil-
laries became remarkable. However, no clear folic-
ular structure was yet seen. Epithelial cells with
cytoplasmic staining for Tg were increased in
number, with almost half of the cells being positive
(Fig. 1), but T₄ and T₃ were still negative at this
stage.

Thyroid glands at the 17th day of gestation
A few, primitive follicular structures appeared in
the thyroid lobules between the epithelial cells. The
cells with cytoplasmic Tg were further increased
(Fig. 2a). Together with the Tg immunoreactivity,
both T₄ and T₃ were demonstrated in the lumina of
primitive follicles (Fig. 2b). The positive follicles
for T₄ and T₃ were rather small in number and
were scattered in the thyroid glands. Examination
of serial sections demonstrated simultaneous locali-
ation of T₄, T₃ and Tg in the same follicles,
although the stainability was variable among them.
In general, T₄ was more marked than T₃ in its
staining intensity. PAS reactivity remained nega-
tive.

Thyroid glands from the 18th to 21st day of gestation
On the 18th day of gestation the follicular struc-
tures, although small in size, were found to be
more well developed than at the earlier stage with
remarkable intrafollicular stainability for T₄, T₃
and Tg (Figs. 3a,b,c).

PAS reactive colloid material was demonstrated
on the 19th day, and its stainability increased until
the day just before birth.

There were some follicles with well-expanded
lumina on the 20th day, and the neonatal type of

---

Fig. 1.
Foetal thyroid at the 16th day of gestation,
stained for Tg. × 400.
Figs. 2a–c.
Serial sections of foetal thyroid at the 17th day of gestation. Both cytoplasm of epithelium and primitive follicles are stained for Tg (2a), whereas T₄ localization is limited to the lumens of follicles (2b). No significant staining occurred by incubation with non-immune rabbit serum (2c). × 400.

Figs. 3a–c.
Serial sections of foetal thyroid at the 18th day of gestation, stained for Tg (3a), T₄ (3b), and T₃ (3c), respectively. Intrafollicular staining of T₄ and T₃ is evident. × 400.
Figs. 4a–c.
Serial sections of foetal thyroid at the 21st day of gestation, stained for Tg (4a), T₄ (4b), and T₃ (4c), respectively. Intraluminal staining of T₄ and T₃ is evident. × 400.

Figs. 5a–c.
Serial sections of newborn thyroid at the day of birth, stained for Tg (5a), T₄ (5b), and T₃ (5c), respectively. The majority of follicles shows positive for each of them. × 400.
The thyroid glands from the 1st to 7th day after birth
On the day of birth the thyroid glands revealed no significant differences in general histology compared to that just before birth, except that the contours of each follicle became more regular and rounded. The luminal content of most follicles, especially the marginal border of colloid, was strongly positive for T₄, T₃ and Tg throughout these periods (Figs. 5a,b,c). As compared to late foetal life, the Tg immunoreactivity of the cytoplasm of the follicular epithelium became irregular among the follicles and less marked. Cytoplasmic staining for T₄ and T₃ was substantially negative during the foetal and neonatal life. The colloid showed strongly positive staining for PAS, although no prominent colloid droplets in the cytoplasm were observed.

Discussion
The appearance of the primordium of the rat thyroid gland in the pharyngeal wall occurs at the 11th day of gestation, when 25 somites are formed. While descending along the anterior neck, the primitive thyroid tissue continues to proliferate and is located at the anterior portion of the trachea at the 15th day.

It has been established by various experimental techniques that the thyroid glands thereafter show a rapid development in both structural and functional characteristics until the end of foetal life. Ultrastructural studies have demonstrated remarkable development of rough surfaced endoplasmic reticulum, mitochondria, and Golgi apparatus, and follicular spaces which, especially from the 17th to 18th day, appear as a primitive follicular structure formed between neighbouring epithelia (Feldman et al. 1961; Ishikawa 1965; Rémy et al. 1980b). Autoradiographic experiments have indicated that the beginning of organization of iodine by the follicular epithelia occurs at the 17th day (Carpenter 1959; Rémy et al. 1980b), and in vitro analysis has demonstrated the production of moniodotyrosine (MIT) and diiodotyrosine (DIT) by cultured thyroid tissues from the rat foetus at the 17th day of gestation (Nataf et al. 1965; Nataf 1968).

It was also found by enzyme histochemical techniques that the thyroperoxidase which is essential to iodine organification in the colloid spaces was detected first on the 17th day in the nuclear envelope and rough surfaced endoplasmic reticulum, and was finally transferred to the apical surface of the epithelial cells where iodination of thyroglobulin is thought to take place (Rémy et al. 1980a).

According to the immunofluorescence observations of Feldman et al. (1961), Tg immunoreactivity first appeared in the follicular spaces of the foetal rat thyroid on the 20th day of gestation. In the present study, however, a highly sensitive immuno-peroxidase technique succeeded in demonstrating Tg in the cytoplasm of the immature thyroid epithelium on as early as the 15th day when no follicular structure had developed, and both T₄ and T₃ were still negative, providing evidence that the production of Tg precedes the synthesis of T₄ and T₃ in the foetal thyroid gland.

To our knowledge, this is the first report to demonstrate immunohistochemically the localization of T₄ and T₃ in the foetal rat thyroid. Both hormones were found to occur on the 17th day of gestation, i.e., 2 days after the detection of Tg, when primitive follicles were observed by electron microscopic studies. The localization of the thyroid hormones was limited only to the follicular lumen throughout foetal life, and failed to be demonstrated in the epithelial cytoplasm like Tg which was detected in the follicular space as well.

Among the factors which control or influence the development and functional differentiation of the thyroid glands of foetuses, the thyrotrophic hormone (TSH) from their own anterior pituitary glands appears to be important, since transplacental action of maternal TSH is considered unlikely (Knobil & Josimovich 1959). Immunohistochemical studies have shown that TSH cells appeared on the 16th or 17th day of gestation in the anterior pituitary gland of the rat foetus (Watanabe & Daikoku 1979; Begeot et al. 1981), suggesting onset of TSH secretion at around these periods. Thereafter, the pituitary-thyroid system develops rapidly and is established before birth (Noumura 1959; Eguchi et al. 1980).

A good coincidence in the appearance of T₄ and
T3 in the thyroid follicles and that of thyrotrophs in the anterior pituitary of the foetal rat, appears to suggest that foetal TSH affects the initiation of the thyroid hormone synthesis in the foetal thyroid. However, the occurrence of immunoreactive Tg production would not depend on the foetal TSH, since Tg is detected in the thyroid epithelium prior to the appearance of thyrotrphs.

In a previous study, we reported the simultaneous localization of T4 and T3 in the same follicles of adult rats in various functional states (Kawaoi et al. 1983). A similar result was obtained in the present study, suggesting a synchronized synthesis of both hormones by the foetal rat thyroid involving the Tg molecule in the follicular lumen (Ekholm & Wollman 1975).

PAS stainability, which is characteristic of thyroid colloid, became detectable lastly on the 19th day and rapidly increased towards the end of gestation. Although the PAS-positive carbohydrate component of the colloid substance has not yet been fully described, the above findings seem to support the view that the Tg immunoreactivity is not necessarily linked to the PAS reactivity of the colloid substance, and that maturation of the colloid in terms of PAS stainability occurs not earlier than on the 19th day of gestation.

Initially, it was expected to find differences in the patterns or intensity of immunostaining for Tg and the thyroid hormones as well between the thyroid gland of the latest gestational stage and those of the early postnatal period, being reflected probably by a rapid withdrawal of maternal hormones, acute and transient elevation of TSH in the serum which is known in the human neonate (Hirayu et al. 1983), and other environmental factors to which the newborn rats are exposed. However, when the postnatal thyroid glands were compared with those in the latest gestational stage, there was no significant difference in general histology as well as in the immunostainability for Tg and thyroid hormones. Further examination of the ultrastructural features is required in order to obtain a final conclusion on this point.

In summary, the present study demonstrates that the occurrence of immunoreactive T4 and T3 in the foetal rat thyroid is closely correlated to the appearance of primitive follicles in the immature thyroid glands, the onset of iodine metabolism, the induction of thyropheroxidase activity in the follicular epithelium and the appearance of thyrotrphs in the anterior pituitary glands, being preceded 2 days by that of thyroglobulin. These results offer useful information for further analysis of the development of thyroid function in foetal life.

Acknowledgments
The authors wish to thank Miss I. Saito and Mrs. M. Yoda for their technical assistance and Miss M. Gomi for helping to prepare the manuscript.

References

Downloaded from Bioscientifica.com at 11/29/2018 01:58:25PM via free access.


Received on September 11th, 1984.