Thyroid Morphological and Functional Heterogeneity: Impact on Iodine Secretion

C. PENEL, J. B. ROGNONI and P. BASTIANI

Groupe Endocrinologie Cellulaire, LA 202 Institut de Biochimie, Université de Provence, 13331 Marseille Cedex 03, France

Abstract. Thyroid iodine turnover heterogeneity includes morphological (cellular and colloidal distribution space for iodide) and functional heterogeneity (hormone synthesis in the colloid). In 'normal' rats, both iodide actively trapped by the epithelial cell and that coming from deiodination of iodotyrosines present the same probability for thyroglobulin (Tg) iodination (Tg iodination flux: 4.0 ± 0.3 μgI/day). A portion of the thyroid iodide is sequestered in the colloid lumen and is inoperative in the Tg iodination mechanisms. The masses of cell and colloid compartments are equivalent (0.018 ± 0.002 μgI) while colloid iodide concentration is twice that of the cell (0.11 and 0.06, respectively). The turnover of about 3 μgI of colloid iodine (Tg) is follicle diameter-dependent (inter-follicular heterogeneity) and it is mainly characterized by 2 different half lives of 8 and 16 hours, respectively. Ninety percent of the thyroid iodine (hormone) secretion (1.10 ± 0.11 μgI/day) is provided by this compartment rich in iodotyrosine residues (70 %). The remaining 10 % of iodine secretion is provided by a Tg pool (7 μgI) characterized by 2 compartments (intra-follicular heterogeneity) with slow and very slow turnovers. The longer the transit time of Tg molecules in the colloid, the higher their iodothyronine content.

Key words: Thyroid-iodine (Tg) metabolism — Follicle size distribution — Structure-function correlation

Introduction

The thyroid gland is composed of numerous follicles of different sizes which represent the functional units of the gland. Each follicle has the same basic structure with a single layer of cells surrounding a lumen which contains colloid (Wollman 1980). The epithelial cell fixes plasma iodide (trapped iodide) for hormone synthesis. The luminal colloid is composed of different iodinated thyro-

This work is dedicated to the memory of Professor C. Simon

globulin (Tg) molecules which contain the thyroid hormones: triiodothyronine (T3) and Thyroxine (T4). Tg molecules enter the cell by endocytosis (Ekholm 1977) and they are degraded by intracellular hydrolysis (Van Denhove-Van Denbrouke 1980). Hormones T3 and T4, monoiodotyrosine (MIT) and diiodotyrosine (DIT) residues are then released. The iodotyrosine residues are deiodinated and the discharged iodide (internal iodide) is recycled inside the cell (Hildebrandt and Halmi 1981).

It has been shown that the turnover of only a fraction of the Tg molecules stored in the follicle lumen is correlated to the follicle diameter and to the position of each follicle in the gland: inter-follicular heterogeneity (Nadler et al. 1954; Triantaphyllidis and Verne 1963; Huber et al. 1969; Riviere et al. 1971; Loewenstein and Wollman 1973; Kobayashi and Greer 1975; Miloni and Studer 1980; Bazin et al. 1981). On the other hand, different iodinated Tg molecules with different turnover rates have been characterized inside each follicle: intra-follicular heterogeneity (Schneider 1964; Lissitzky et al. 1966; Nunez et al. 1966; Robbins et al. 1966; Van Middlesworth et al. 1970; Berg 1973; Kobayashi et al. 1974; Cortese et al. 1976; Vignal et al. 1978; Burkhardt et al. 1979; Smeds et al. 1979; Heaberli et al. 1981).

While there is voluminous literature on inter and intra-follicular heterogeneity, to our knowledge no attempt has been made to investigate the effective role of each of these mechanisms in the process of thyroid hormone synthesis and secretion. The aim of this work was thus to contribute to better understanding of the intrathyroid iodine metabolism discriminating the inter-follicular from the intra-follicular heterogeneity.

Materials and Methods

Animals: Two month-old male Wistar rats weighing about 200 g were kept under strictly controlled conditions of temperature (23 ± 1 °C) with constant air humidity (55 ± 5 %) and regular lighting (light from 8.00 to 20.00). Eighty rats were adapted to receive 5 μg of iodide daily as a drinking solution during two months to establish a steady state in their iodine metabolism.

Inter-follicular heterogeneity: (I) Modeling: in each thyroid follicle, the turnover of a fraction of the iodine (Tg) pool was correlated to the diameter of the follicle and to the position of the follicle in the gland (Loewenstein and Wollman 1973). These authors have shown under similar experimental conditions that the transfer rate k of this fraction of iodine, the turnover of which is follicle diameter (D) dependent, may be expressed as $k(D) = aD + b$, where $a = -0.015$ and $b = 3.138$ for central follicles, and $a = -0.009$ and $b = 1.945$ for the peripheral ones. The global iodine turnover, $y_e(t)$, was the sum of the contributions of all the follicles acting in parallel:

$$y_e(t)/y_e(\infty) = 1 - \int_0^\infty f(D).D^3.\exp(-k(D).t).\mathrm{d}D \Big/ \int_0^\infty f(D).D^3.\mathrm{d}D$$

where $f(D)$ was the follicle size distribution. This relation was used to integrate the contributions of

individual follicles to the global thyroid iodine turnover; the follicle size distribution of central and peripheral follicles was determined by morphometric analysis.

(II) Morphometric analysis: thyroids of 4 rats were placed at 4 °C in 2.5 % glutaraldehyde, 0.177 mol/l phosphate buffer. The osmolality was 604 mOs/kg. To reduce the bias induced by the fixation procedure, the osmolality of our fixative and rinsing buffer was chosen between the osmolality used by Denef et al. (1979) and that used by Wyndford Thomas et al. (1982). After 24 hours fixation, the glands were rinsed in the same buffer the osmolality of which was adjusted with sucrose. The tissue was then dehydrated and embedded in paraffin. In order to study the average follicle structure with minimum variance, 5 μm thick slices were cut perpendicular to the greatest axis of each lobe (Miles and Davy 1976; 1977). Then for each lobe, a central slice (about 400 follicle sections) was chosen at random since a central slice through a lobe represents the whole lobe of the gland (Denef et al. 1979). The area of about 2000 central and 1000 peripheral follicle sections were measured with a Hewlett Packard digitiser. Follicles in contact with the surface of the thyroid lobes and isthmic follicles are referred to as peripheral follicles, other follicles are referred to as central follicles (Loewenstein and Wollman 1973). From the distributions of follicle sections (apparent diameters) the equivalent sphere model (Weibel 1979) was chosen to determine the follicle size distributions (true equatorial diameters) (Penel and Simon 1976) following a procedure as described previously (Penel et al. 1981).

Intra-follicular heterogeneity: (I) Biochemical study: at time zero, the drinking solution was labelled with ^{125}I (specific radioactivity 4.44 kBq/μg) and given to the rats without any modification on 120 consecutive days. At different time intervals, the rats were sacrificed and the glands excised and homogenized in a Tris/HCl (pH7) sucrose 0.15 mol/l medium (Simon et al. 1971). After centrifugation of the homogenate at 600 g for 10 min and subsequently at 34,000 g for 15 min, thyroglobulin (Tg) was purified from the 34,000 g supernatant by centrifugation on a 5—20 % sucrose gradient. Thyroid iodine was separated from iodinated Tg by paper electrophoresis of the 34,000 g supernatant. Plasma iodine was extracted by chromatography on Sephadex G25 (Simon and Peyron 1970). Iodine in each of the fractions was determined by chemical analysis (Martin and Ames 1961). It was shown that all the iodine pools were in a steady state during the 120 days of the experiment. Under our experimental conditions, the precursor iodine (ingested iodine) was labelled at a constant specific radioactivity (SRA = $^{125}I/^{127}I$); isotopic decay excepted. Thus, at each time interval, SRA of the renewed iodine inside each pool was equal to SRA of the drinking solution (the same isotopic decay). At each time interval, SRA of the drinking solution was measured and for each iodine pool, the renewed iodine was determined by dividing the ^{125}I of the sample by the SRA value of the iodide in the drinking solution. For a given pool, the renewed iodine fraction was calculated by dividing the amount of renewed iodine by the total iodine pool content determined by chemical analysis.

(II) Modeling: this long term kinetic study provided us with informations on the quantities of renewed iodine at each time interval which were used for compartmental analysis of the thyroid iodine system. The maximum likelihood estimation of the parameters of the minimal model (Bergman and Cobelli 1980) was performed by minimizing the objective function:

$$F = \det([\bar{y}_i - y_i] \times [\bar{y}_i - y_i]^T)$$

where det symbolized the determinant of the matrix; $\bar{y}_i$ were the experimental values of each pool **i** (vectorial notation); and y_i was the corresponding model response (Box and Draper 1965). To avoid relative minima, the minimum of F was obtained in two steps: the first approximation of the parameters was obtained by a random search method (Bazin 1970), then the absolute minimum was obtained by a simplex algorithm (Olsson and Nelson 1975). An estimate of the variance-covariance matrix of the parameters was obtained by the inverse of the Hessian matrix (Southwell 1975). A priori identifiability (Cobelli and Distefano III 1980) was tested by the computer program of Vajda and Varkonyi (1982).

A posteriori identifiability was checked by the covariance matrix of the parameters; as a rule, a model was unacceptable if one parameter had fractional standard deviation in excess of 100 % (Carson et al. 1983).

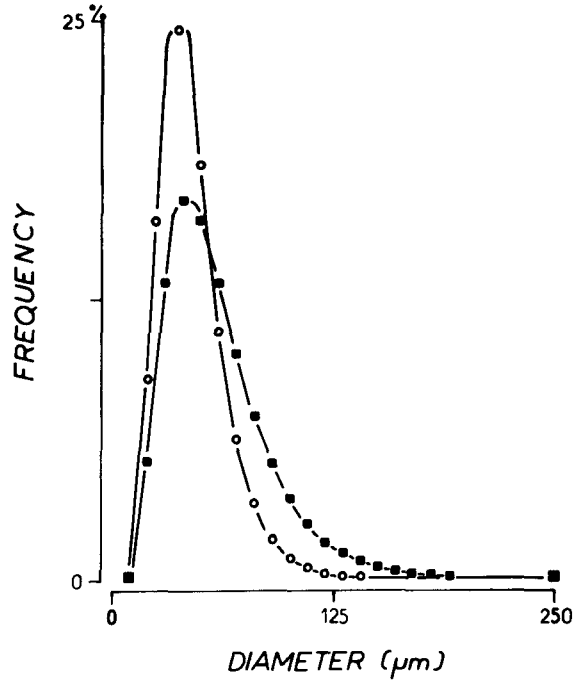

Fig. 1. Rat thyroid follicle size distribution ■—■ peripheral follicles, O—O central follicles (mean value obtained from 4 animals).

Results

Inter-follicular heterogeneity: Peripheral and central follicle size distributions (Fig. 1) were described by a lognormal law:

$$f(D) = (D.\, s.\sqrt{2\pi})^{-1}.\exp -\frac{1}{2}\left(\frac{\log (D-m)}{s}\right)^2$$

Its characteristic parameters are reported in Table 1. If on an average, peripheral follicles were greater than the central ones, a maximum size for small diameters (40 μm) was observed for both distributions. The evolution with time of the renewed fraction of iodine (Tg), the turnover of which was follicle diameter--dependent is presented in Fig. 2. Ninety-six and eighty per cent of iodine contained in central and peripheral follicles, respectively were renewed within 2 days. The curves in Fig. 2 were fitted by a biexponential function:

Table 1. Typical parameters of the thyroid follicle size distribution. *A* is the relative proportion (in size) of peripheral and central follicles in the gland.

	A (%)	Mean (μm)	Standard deviation (μm)	Mode (μm)
Central follicles	78	46	19	40
Peripheral follicles	22	60	31	40

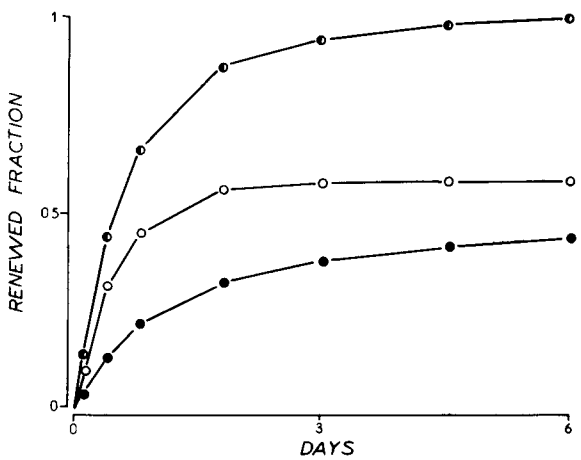

Fig. 2. Evolution with time of the fraction of the thyroglobulin (iodine) pool the turnover of which is follicle diameter dependent. ●—● contribution of peripheral follicles acting in parallel, ○—○ contribution of central follicles, ◐—◐ global response of the gland (peripheral and central follicles).

$$f(t) = 1 - A1.\exp(-K1.t) - A2.\exp(-K2.t)$$

Its parameters are shown in Table 2. The inter-follicular heterogeneity for central and peripheral follicles can thus be characterized by only two half lives (T1 and T2). Since T2 was similar for both groups of follicles, the thyroid inter-follicular heterogeneity which was the sum of the contributions of central and peripheral follicles was defined by only 3 half lives of 8h, 16h, and 3 days for 76 %, 15 %, and 9 % of this iodine pool, respectively.

Intra-follicular heterogeneity: Long term kinetic studies were performed on plasma iodide, thyroid iodide and Tg pool (Fig. 3). Plasma iodide was rapidly renewed, but total turnover for thyroid iodide was observed after 60 days, while the

Table 2. Kinetic parameters describing the thyroid inter-follicular heterogeneity. A, K (day^{-1}), T (day) are the amplitude, the rate constant, and the half life of both exponential models respectively.

	$A1$	$K1$	$T1$	$A2$	$K2$	$T2$
Central follicles	0.98	1.93	0.34	0.02	0.25	2.77
Peripheral follicles	0.67	1.12	0.60	0.33	0.25	2.77

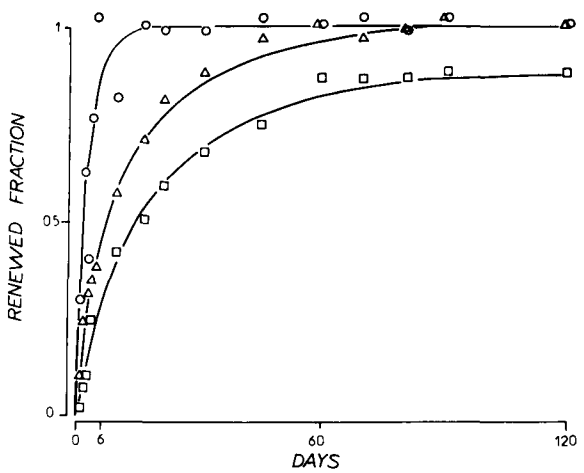

Fig. 3. Evolution with time of the renewed fraction of plasma iodide (○—○), thyroid iodide (△—△), and thyroglobulin (iodine) (□—□) for the entire population of follicles (peripheral and central). Each point represents the mean value obtained from 4 animals.

turnover of the Tg pool was only 85% after 120 days (Fig. 3). These experimental data were analysed by the compartmental model presented in Fig. 4. Since data were obtained after homogenization of the gland, fluxes (μgI/day) and masses (μgI) represented the sum of the contributions of all the follicles acting in parallel. The thyroid iodide pool was described by two compartments (C3 and C4) which represented the cellular and colloidal space of distribution for iodide, respectively. The masses of these compartments were similar (0.018 ± 0.002 μgI). Trapped and internal iodide rapidly mixed inside the cell and, under our experimental conditions, they presented identical probabilities for Tg iodination (equivalent iodination rate constants were obtained for these latter iodide pools). Consequently, both trapped and internal iodide were lumped together to give compartment C3. Colloid

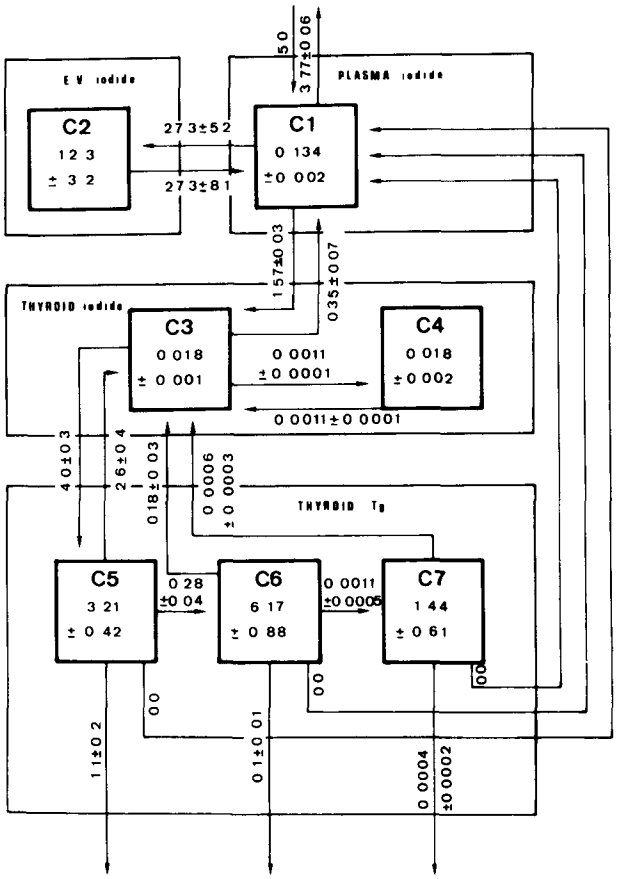

Fig. 4. Compartmental model depicting long term iodine metabolism. Iodine compartments (C) are given with their mass (μgI). Values over arrows represent iodine fluxes ($F_{i,j}$ = flux from compartment Cj to compartment Ci in μgI/day; hormone secretion fluxes are represented by $F_{x,x}$ with x = 5; 6; 7). Plasma iodide (Cl) is distributed in the extravascular space (C2) and actively trapped ($F_{3,1}$) by the thyroid epithelial cell (C3). A portion of iodide is organified at the apical pole of the cell ($F_{5,3}$) to give the thyroglobulin (Tg) pool (C5, C6, C7). By a shunt mechanism ($F_{4,3}$), a portion of thyroid iodide is sequestered in the colloid (C4). After endocytosis, Tg molecules are hydrolysed and the hormones are secreted ($F_{5,5}$, $F_{6,6}$, $F_{7,7}$). Iodide coming from iodotyrosine residues deiodination is recycled ($F_{3,5}$,$F_{3,6}$,$F_{3,7}$).

iodide was inoperative in the Tg iodination mechanisms since its Tg iodination flux ranged from 0 to 10^{-3} μgI/day, i.e. from 0 to 4 10^{-4}% of the total Tg iodination flux. The thyroid intra-follicular heterogeneity for the Tg pool was characterized by two compartments with a slow (C6) and a very slow (C7) turnover. In this long term kinetic study, the rapidly renewing compartment C5 mainly represents Tg

Table 3. Iodotyrosine (MIT + DIT) and iodothyronine (T3 + T4) residues expressed as iodine or in percent of the whole iodine pool for each thyroglobulin compartment.

	C5		C6		C7	
	MIT + DIT	T3 + T4	MIT + DIT	T3 + T4	MIT + DIT	T3 + T4
μgI	2.25 ± 0.36	0.96 ± 0.22	3.96 ± 0.57	2.20 ± 0.33	0.86 ± 0.38	0.57 ± 0.28
%	70 ± 11	30 ± 7	64 ± 9	36 ± 5	60 ± 26	40 ± 19

molecules the turnover of which was follicle diameter dependent. A secretory pathway was associated with each Tg compartment. Ninety per cent of the iodine (hormones T3 and T4) were provided by compartment C5 which represented only 30% of the Tg (iodine) pool.

For a given compartment, Tg molecules can be considered as being homogeneous as for their composition in iodotyrosine (MIT and DIT) and iodothyronine (T3 and T4) residues. The ratio of the intracellular iodide recycling flux to the iodine endocytotic flux reflects the mean proportion of iodotyrosine residues (expressed as iodine) in the corresponding Tg compartment. Then, for each Tg compartment Cx,

$$\text{MIT} + \text{DIT} = \frac{F_{3,x}}{(F_{3,x} + F_{x,x})} . Mx \text{ and } \text{T3} + \text{T4} = \frac{F_{x,x}}{F_{3,x} + F_{x,x})} . Mx$$

where $F_{3,x}$ was the iodide recycling flux, $F_{x,x}$ the iodine (hormones) secretion flux, and Mx the mass of compartment Cx. The corresponding values are given in Table 3; the proportion of iodothyronine residues in each Tg compartment was correlated to the transit time of the Tg molecules in the colloid, i.e. the higher the Tg turnover, the lower the iodothyronine content of Tg.

Discussion

Inter-follicular heterogeneity: As shown by Loewenstein and Wollman (1973), the mathematical analysis of Tg turnover (follicle diameter dependent) is only valid if the radioiodide pool (plasma and thyroid iodide) remains constant throughout the experiment. The renewed fraction of plasma iodide rapidly reaches a plateau; on the contrary, thyroid iodide is completely renewed after more than 2 months (Fig. 3). In fact, as shown by compartmental analysis, the cell iodide (C3) which is the precursor of the Tg pool is rapidly renewed (the kinetics is similar to that in the plasma), while the colloid iodide (C4) is slowly renewed but inoperative in the Tg iodination mechanisms.

Peripheral and central follicle size distributions exhibit maximum values for small diameters. This is noteworthy since it is commonly accepted (from observa-

tions on thyroid slices) that large follicles are in the periphery of the gland, while the smallest ones are in the center (for a review see Nadler 1974). A similar shape of follicle size distribution curves minimizes the thyroid inter-follicular heterogeneity which is mainly characterized by two half lifes (8 and 16 hours). Consequently, every bimodality in Tg turnover observed (at least in the rat) during a pulse labelling experiment (Miquelis and Simon 1980) cannot be interpreted only in term of intra-follicular heterogeneity. On the other hand, it must be emphasized that thyroid inter-follicular heterogeneity can be preponderant in some pathophysiological states, such as thyroid nodular goitres (Miloni and Studer 1980), thyroid carcinoma (Bazin et. al. 1981) etc.

The problem of follicular heterogeneity in thyroid iodine turnover can be generalysed to kinetic experiments with heterogeneous tissues. For example, interpretation of tracer washout curves from a population of muscle fibers has been performed (Zierler 1966) using a cylindrical model, where individual fiber kinetics were inversely proportional to the fiber diameters. In this case, despite a wide range of fiber diameters, the global washout curve was described by a single exponential.

Intra-follicular heterogeneity: The thyroid intrafollicular heterogeneity was investigated by compartmental analysis of long term kinetic studies of plasma and thyroid iodide and Tg pools. Following this procedure (i) very slow iodine compartments the presence of which had been suspected (Van Middlesworth and Murphy 1970) were detected, and (ii) Tg diffusion phenomena inside the colloid did not significantly change the Tg pool mixture (an essential limitation to compartmental analysis); under similar experimental conditions, Loewenstein and Wollman (1967) have shown that only 3% of follicles presented an heterogeneous distribution of Tg after 1 day of labelling.

The compartmental model shown in Fig. 4 is the simplest (minimal model) compatible with both the experimental data and the physiological limitations. Some fundamental parameters of thyroid iodine metabolism are structure invariants (Berman and Schoenfeld 1956): the thyroid iodide and Tg pools which are described by 2 and 3 compartments respectively, the mass (μgI) of these compartments, the net thyroid iodide intake, the Tg iodination flux and the thyroid iodine secretion fluxes. It is noteworthy that Kohler et al. (1971), possessing only a global information on thyroid iodine pool (external neck counting), have used 3 different models for Tg subsystem analysis. In spite of a very different experimental and analytical procedure, we have found the same number of compartments with the same relative iodine mass for the Tg pool. It must be outlined that the daily urinary iodide excretion was not checked, thus it was impossible to quantify the fraction of plasma iodide coming from peripheral hormone degration. However, estimation of the thyroid parameters gave similar

results whether the peripheral deiodination of hormones was considered as being total or zero.

The thyroid intra-follicular heterogeneity includes a morphological heterogeneity (cellular and colloidal space of distribution of thyroid iodide) and a functional heterogeneity (hormone synthesis in the colloid).

a) Morphological heterogeneity: Two compartments are necessary to describe the thyroid iodide turnover which have been identified (Wollman and Reed 1959; Rocmans et al. 1977; Hays 1978) as cell (C3) and colloid (C4) iodide. In this study, their respective role in the Tg iodination process has been investigated (i) to validate the model of Loewenstein and Wollman (1973), describing iodine turnover in individual follicles and (ii) to improve the speculative models recently proposed by Hildebrandt and Halmi (1981), describing the thyroid iodide metabolism. Iodide actively trapped by the epithelial cell and that coming from intracellular deiodination of iodotyrosine residues both are rapidly mixed and, under our experimental conditions, had the same probability for Tg iodination. A portion of thyroid iodide which is not organified at the apical pole of the cell is sequestered in the colloid; it is noteworthy that this colloid iodide is inoperative in the Tg iodination mechanisms. Knowing the cell and colloid volumes in rats maintained under similar experimental conditions (Penel et al. 1982), it is possible to transform the mass (μgI) of the iodide compartments into concentration (fgI/μm^3). The cell and colloid iodide concentrations are Icell = 0.06 and Icoll = 0.11 respectively. Thus, under our experimental conditions, the colloid iodide concentration is about twice that of the cell iodide, this is in agreement with autoradiographic observations on thyroid slices of rats (Doniach and Logothetopoulos 1955; Andros and Wollman 1967; Pitt-Rivers and Trotter 1953). It should be emphasized that the negative charge of Tg molecules which could have decreased the concentration of diffusible anions in the colloid has been neglected, since the Donnan distribution ratio for sulfate between the colloid and the interstitial fluid is 1.1 in the rat (Chow and Woodbury 1965).

b) Functional heterogeneity: In normal rats, the thyroid functional heterogeneity is characterized by two Tg compartments with a slow (C6) and a very slow (C7) turnover. The "young mature" Tg molecules of C5 have the greatest proportion of iodotyrosine residues; the "maturation" of Tg molecules in the colloid lumen is characterized by an increasing proportion of iodothyronine residues (compartments C6 and C7). This model prediction is in good agreement with biochemical data obtained from a gland homogenate (Robbins et al. 1966; Lissitzky et al. 1966). The total flux of hormonal iodine secretion is mainly (90%) provided by C5, while this compartment is amounting only 30% of the total Tg pool. Thus, from an operational point of view, thyroid handles iodine in a "last come, first served" manner (Schneider 1964).

Since Tg molecules are homogeneously distributed in the colloid lumen, the ratio of iodine secretion flux versus iodine mass (transfer rate) of each compartment would inform us about the probability of Tg endocytosis, provided that the Tg iodination level is constant. Unfortunately, iodination levels are correlated to the "maturation" of Tg molecules (Cortese et al. 1976) and thus the mean iodination level of C5 Tg molecules must be lower than that of C6 and C7 molecules. Since in the rat under similar experimental conditions, the iodination levels of the most and least iodinated Tg differ by a factor of 2 (Cortese et al. 1976), the ratio r of the mean iodination level of C5 versus C6 Tg will be between the limits $0.5 \leqq r \leqq 1$. Thus:

$$P = \frac{(F_{3,5} + F_{5,5})}{M_5} \cdot \frac{M_6}{(F_{3,6} + F_{6,6})} \cdot r$$

will represent the ratio of endocytotic probalities of C5 versus C6 Tg. Since $17 \leqq P \leqq 34$, C5 Tg molecules have a greater endocytotic probability than C6 Tg molecules (the same applies to C7 molecules). Two complementary mechanisms can be postulated to explain this result: (i) a fluid endocytosis but a different accessibility of Tg molecules to the sites of endocytosis, since it has been shown that Tg molecules can form aggregates during maturation (Berg and Dahlgren 1974), and/or (ii) a receptor mediated endocytosis. In the latter case, in addition to a selective endocytosis between newly iodinated and mature Tg molecules (Cortese et al. 1976; Van Denhove et al. 1981), a competition would exist between "young mature" (C5) and "old mature" (C6 and C7) Tg molecules. In both cases, it is reasonable to suppose the turnover of C5 Tg molecules to be correlated to the number of endocytotic sites on the apical membrane of each follicle and thus to be roughly correlated to the follicle diameter (Fan et al. 1982). The sequestered C6 and C7 Tg likelihood represent a potential reservoir to maintain the concentration of thyroid hormones in the extrathyroidal space constant during periods of severe and uninterrupted lack of iodine in the diet (Studer et al. 1974).

In conclusion, the follicle size dependent turnover of 30% of the Tg (iodine) pool (inter-follicular heterogeneity) in rat is characterized by two half lives of 8 and 16 hours, respectively. Ninety percent of the thyroid iodine (hormone) secretion are provided by these rapidly renewed Tg molecules with a greater iodotyrosine residues content. The Tg intra-follicular heterogeneity is characterized by two compartments with a slow and a very slow turnover. This Tg "maturation" is correlated to an increase in their iodothyronine content. These slowly renewed Tg molecules account only for 10% of the thyroid hormone secretion. Thus, despite a great heterogeneity in iodine metabolism, from an operational point of view, thyroid handles iodine in a simple catenary compartmental model (C1 – C3 – C5) which is the mathematical equivalence of the still up to date "last come, first served" concept.

Acknowledgments. The authors wish to thank Prof. Vajda (Lab. of Chemical Cybernetics, Budapest) for the model structural identifiability analysis. This work was supported by grants from the CNRS (LA 202) and from the INSERM (CRL 79—4—1803).

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Received December 8, 1983 / Accepted July 6, 1984