FRACTIONATION OF THYROID CELLS

QUANTITATIVE DISTRIBUTION OF THYROXINE, DIIODOTYROSINE AND NUCLEIC ACIDS IN ISOLATED CELL FRACTIONS

by

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The manner in which thyroid cell elaborates the hormone has been investigated until now by cytomorphological methods consisting only of microscopic observations of the stained tissue sections. In that way, the two main manifestations of the secretory process have been described: the one concerning the cell nucleus, the other the chondriome.

The nucleus of a secretory cell shows, according to the FEULGEN's reaction, a decrease in the content of desoxypentose nucleic acid, whereas the nucleolus appears to be poor in pentose nucleic acid (CRAMER AND LUDFORD[3], UOTILA[20]). On the contrary, during the preliminary phase of the thyroid activity, *i.e.*, the assimilation of the glandular colloid, the staining characteristics demonstrate that both nuclei and nucleoli are rich in nucleic acids, and simultaneously the follicular colloid shows a rise in the amount of desoxypentose nucleic acid.

Thus, it has been deduced that the cellular elaboration of the hormone is closely related to the metabolism of nucleic acids, and that in the process of secretion the cell nucleus takes part in the sense of CLAUDE BERNARD's "nuclear theory". However, no direct chemical findings concerning the thyroid nuclei have been brought to support this view. In the course of the investigations on 4-methyl-thiouracil, RERABEK AND RERABEK[13] observed a decrease of the desoxypentose nucleic acid in the thyroid which was connected with the goitrogenic action of the drug.

Concerning the chondriome, a secretory cell shows an increase in quantity of the mitochondria which are accumulated in the apical cytoplasm, where the resorption of the colloid takes place (OKKELS[10], TAKAGI[19], UOTILA[20], PONSE AND ALTSCHULER[11,12]). After staining, fuchsinophilic plasmas in the centres of many mitochondria can be found, which often seem to penetrate into surrounding cytoplasm. These particles bear resemblance to the secretion granules to such a degree that the so-called "chondriosome theory" was proposed, according to which the chondriosomal substance should be transformed in the product of thyroid secretion. This "chondriosome theory", however, has not been supported by chemical investigations up to now.

At the present time, the older methods for the isolation of cell organoids were improved for the purpose of liver fractionation. CLAUDE[2] studied in 1943 the filamentous and granular bodies of the liver cell chondriome during fractional centrifugation, and with the aid of his method, HOGEBOOM *et al.*[8], like SCHNEIDER[15], isolated fractions of

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liver cells for investigations of enzymatic activity. They found the whole content of the cytochrome oxidase present in the "large" granules of a size 0.5–2.0 μ approximately, and SCHNEIDER *et al.*[16] had a similar result in respect of the succinic oxidase. The presence of both enzymes in granular fractions demonstrates the possibility of isolating cellular components without an essential alteration in their quality. The small losses in enzyme content, owing to the prolonged suspension of the granules in water, made HOGEBOOM, SCHNEIDER, AND PALLADE[9] carry out the isolation in a medium of 0.884 *M* sucrose; the mitochondria obtained in this way should be morphologically intact, they stain well with Janus green B, and consist of 19% of the whole content of the liver homogenate in pentose nucleic acid.

The isolation of liver cell fractions suggested an idea of investigating the components of the thyroid cell in respect of the above-mentioned problems, concerning the secretory mechanism and the rôle of the cellular organoids. Experiments performed in that way are the subject of the present communication.

EXPERIMENTAL

Isolation of thyroid cell nuclei. Fresh swine thyroids from the slaughterhouse were used. The isolation was performed according to the method of DOUNCE AND BEYER[6] for fractionation of liver cells. This method was modified as follows: 100 g of cleaned thyroids were minced, frozen and than minced anew, 200 ml of ice-cold distilled water was added, and the mixture homogenized with the aid of a high-speed stirrer (5000 r.p.m.) for 1 minute. In the course of further homogenization during the next 10 minutes, portions of citric acid solution were added up to the final 0.3 molarity; the temperature of the mixture was maintained at 0–5° C by ice additions. The homogenizate was then filtered through a cheese-cloth, and centrifuged 15 min at 860 G. The sediment was washed with ice-cold saline and centrifuged 10 min at 620 G, washed again and centrifuged 7 min at 420 G, washed again and centrifuged 5 min at 220 G. The final sediment contained pure nuclei having a good affinity for vital stains.

Isolation of thyroid cell chondriome. 100 g of cleaned swine thyroids were minced, frozen, minced again and homogenized in 200 ml of ice-cold water as in the previous procedure. In the course of the homogenization in the next 10 minutes, 8.4 ml of 0.1 *M* citric acid solution and solid ice were added. The homogenizate was filtered through a cheese-cloth, stirred for 5 minutes and filtered through a flannel. The filtrate was centrifuged 20 min at 860 G, the sediment discarded and the supernatant centrifuged for 15 min at 5500 G. The sediment formed contained the fraction of "large granules", *i.e.* mitochondria and chondrioconts (chondriome I.). Now, the supernatant was centrifuged 15 min at 16,000 G; the sediment from this centrifugation contained the fraction of "small granules", *i.e.* microsomes (chondriome II.). The final supernatant served for analyses as a cytoplasmic fraction. Each of the two granular fractions was washed three times with ice-cold saline.

Analytical procedures. Thyroxine and diiodotyrosine were determined according to the method of ROCHE AND MICHEL[14]. For extraction and estimation of pentose nucleic acid (PNA), the procedure of VON EULER AND HAHN[7] has been used. Desoxypentose nucleic acid (DNA) was extracted by the same method, and estimated according to DISCHE[4]. In some analyses of nuclear fractions, the extractions were performed by SCHNEIDER's[17] trichloracetic acid method. The analytical results, obtained by VON EULER's phloroglucinol (for PNA) and DISCHE's diphenylamine (for DNA) reaction, were mostly checked by cysteine-photometrical procedure of STUMPF[18] and DISCHE[5].

RESULTS

The fractionation of thyroid cells offers greater difficulties than the same procedure carried out with liver. Thyroid gland contains relatively much connective tissue coating the follicles, and has a bigger solidity than liver. Thus, it was found to be necessary to mince the organ twice, the second procedure being made after freezing the tissue. Later, the homogenization of the minced organ required a longer period to loose the glandular epithelium and to decompose the cells which are more resistant than those of the liver.

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The process of cellular disintegration by stirring was compared in water and in 0.88 *M* sucrose. In the latter medium, a great part of the cells resisted and was not broken in spite of a prolonged stirring, so that the final yields of isolated organoids were relatively small. Because of the density of the medium, the fractional centrifugation of chondrioconts and microsomes required a very great centrifugal force which was unattainable by the apparatus employed. The obtained isolates never were quite homogenous containing admixtures, the removing of which by different speed of cen-

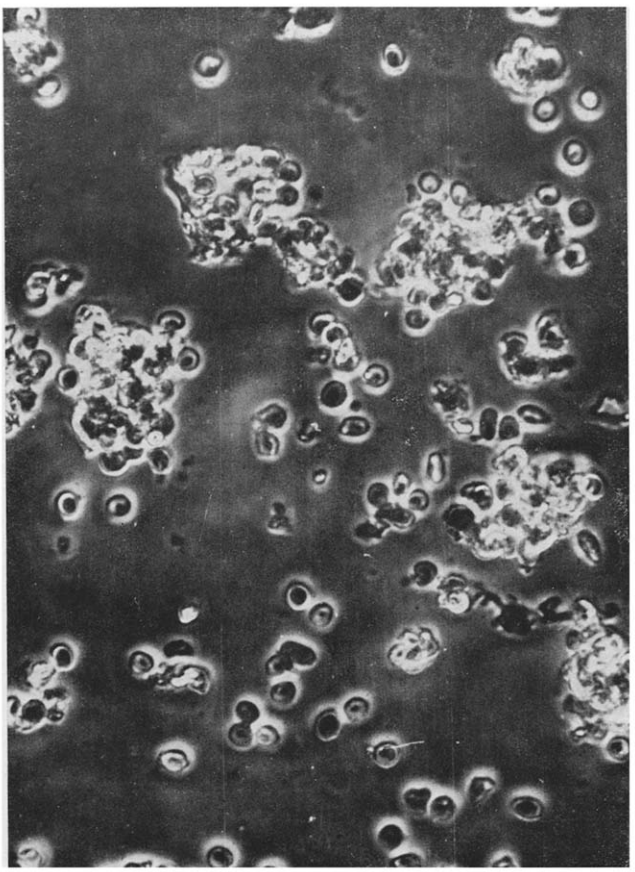

Fig. 1. Isolated thyroid cell nuclei. Dark phase-contrast. × 320

trifugation was impossible. On the contrary, the preparation in ice-cold water resulted in a more rapid disintegration of the cells, the fractions were good separable and the procedure gave greater yields. Nevertheless, for isolation of pure thyroid nuclei the molarity of citric acid must be enhanced to remove the cytoplasmic residues, as was described in the previous part. In respect of the mentioned advantages, all isolations in the present experiments were performed in water, although the possibility of a slight alteration of the isolated organoids must probably be taken into consideration.

Concerning the microscopic characteristics, the isolated nuclei (Fig. 1) were somewhat smaller than those of liver, they stained well with Janus green B and their

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caryoplasm was homogenous without any internal structure. The fraction of mitochondria and chondrioconts (chondriome I) was represented by spherical and rod-like bodies taking the characteristic greenish blue colour by the supravital stain and having a very great tendency to agglutinate. The same properties had the second fraction containing the greater and microscopic visible microsomes (chondriome II). The submicroscopic bodies could not be separated by the employed supercentrifuge, so that they remained in the supernatant fluid which contained also the follicular colloid.

The content of the individual fractions (dry weight) in thyroxine and diiodotyrosine is given in Table I. As it appears, both compounds are present partly in the bodies of the chondriome, partly in the cell nuclei, although a preparatively caused contamination was excluded by the careful final washing of each fraction. The major part of thyroxine seems to be localized outside of all the visible organoids, *i.e.*, in the supernatant liquid or in the submicroscopic bodies at most. On the other hand, the large amount of the supernatant in diiodotyrosine is probably due by the presence of the glandular colloid in this fraction.

TABLE I

THYROXINE AND DIIODOTYROSINE IN ISOLATED THYROID CELL FRACTIONS

Fraction	No. of experiments	Thyroxine mg/100 g dry w. of fraction	S*	Diiodotyrosine mg/100 g dry w. of fraction	S*
Nuclei	5	47.16	11.0	93.83	9.76
Chondriome I.	4	33.55	3.14	66.30	5.10
Chondriome II.	4	42.98	10.68		—
Supernatant	6	163.40	60.4	183.00	56.80

* $S^2 = \frac{\Sigma(X - \bar{X})^2}{n - 1}$

Concerning the nucleotides, both nucleic acids of the cell were found within all the fractions (Table II). Thyroid nuclei are relatively rich in pentose nucleic acid (PNA), besides their main content in desoxypentose nucleic acid (DNA) which reaches 20% approximately. With respect to the chondriome, the large bodies have a high content of PNA, whereas the small ones have less; both have a low but distinct content of DNA, however, which cannot be due to contamination with DNA of nuclear origin in course of the preparation. The very low concentration in PNA of the last fraction (supernatant) demonstrates that submicroscopic bodies like the hyaloplasm do not contain PNA in such quantities as the great mitochondria, and that the relative concentration in PNA decreases proportionally with the decreasing size of the cytoplasmic organoids—a fact to be assumed on the grounds of microscopic examination of the stained cells. The DNA found in the supernatant certainly does not represent its true component, and comes most probably out of the glandular colloid, as it has been previously supposed for diiodotyrosine.

In the course of the preparations of cell organoids, the amount of each fraction was carefully estimated by weighing. The results, given in Table III, give information about the quantitative distribution of organoids in the thyroid tissue. It is apparent

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TABLE II

PENTOSE NUCLEIC ACID (PNA) AND DESOXYPENTOSE NUCLEIC ACID (DNA) IN ISOLATED THYROID CELL FRACTIONS

Fraction	No. of experiments	PNA g/100 g dry w. of fraction	S*	DNA g/100 g dry w. of fraction	S*
Nuclei	5	1.56	0.22	19.6	3.66
Chondriome I.	4	4.54	0.72	1.32	0.28
Chondriome II.	5	2.68	0.84	1.83	0.84
Supernatant	4	1.69	0.68	1.81	0.79

* $S^2 = \frac{\Sigma(\bar{X} - X)^2}{n - 1}$

that both fractions of chondriome represent only a very slight share of the whole tissue weight, and that the greatest majority of the thyroid consists of hyaloplasm containing submicroscopic bodies, irrespective of the nuclear fraction amounting to more than 8%. Of course, it must be emphasized that these statements are of approximative value only, owing to two main causes: the fraction of hyaloplasm includes an unknown and unascertainable amount of the glandular colloid, and, in general, the whole process of cell fractionation is too delicate to make true quantitative conclusions possible. Nevertheless, an attempt was made to calculate the distribution of thyroxine, diiodotyrosine, PNA, and DNA among the individual fractions originating in a known amount of thyroid tissue. Table III demonstrates that only a very slight amount of these compounds occurs in both fractions of the chondriome, whereas nuclei comprise distinctly more of thyroxine and diiodotyrosine. The total amount of all cell fractions of DNA is the same as was found for the rat's thyroid formerly[3], so that the mentioned calculations seem to be correct. Therefore, the surprising fact that the sum of PNA appears to be relatively small may probably be explained by ribonuclease activity of the glandular tissue only.

TABLE III

QUANTITATIVE DISTRIBUTION OF ORGANOIDS AND OF ANALYZED COMPOUNDS IN THE THYROID TISSUE

Fraction	Weight of fraction g/100 g of gland(dry w.)	Content of the whole fraction in PNA g	DNA g	Thyroxine mg	Diiodotyrosine mg
Nuclei	8.60	0.13	1.68	4.05	8.07
Chondriome I.	1.57	0.07	0.02	0.53	1.04
Chondriome II.	1.02	0.03	0.02	0.44	—
Supernatant	88.81	1.50	1.61	145.12	162.52
Sum total	100.00	1.73	3.33	150.14	171.63

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DISCUSSION

The quantitative distribution of the analyzed compounds in the thyroid tissue can be applied to the single thyroid cell also, if the amount of every compound found in individual fractions is expressed as a percentage of the sum of the same compound being present in all cell fractions together. The percentage distribution so obtained (Fig. 2) illustrates the topography of this compound inside of the cell, demonstrating that less than 1% of the total amount of thyroxine is localized in mitochondria and chondrioconts, whereas there is considerably more of both thyroxine and diiodotyrosine in the cell nucleus. A similar picture offers the topography of pentose and desoxypentose nucleic acid.

From these facts some conclusions concerning the origin of the secretion and the rôle of the cell organoids can be drawn. The coincidence in the topography of nucleic acids and of thyroxine or diiodotyrosine suggests that there exists a close relation between both types of compounds. One manifestation of such an interrelationship may

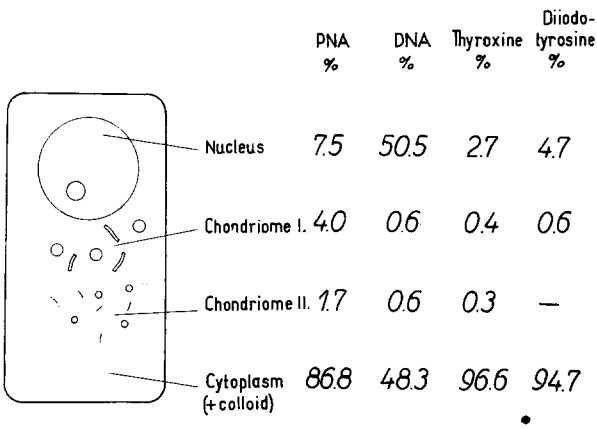

Fig. 2. Chemical topography of the thyroid cell

be seen in the elaboration of thyroglobuline which corresponds to the CASPERSSON's[1] general theory relating protein synthesis with metabolism of nucleic acids in all living cells. The fact that thyroxine seems to be present in greater quantity inside the cell nucleus than in the chondriome makes it evident that the thyroid hormone does not probably originate in cell chondriome. This is contradictory to the "chondriosome theory" of secretion according to which the mitochondria may be transformed into secretion droplets. On the other hand the distinct amount of thyroxine and diiodotyrosine present in the cell nucleus supports the idea that this organoid participates in the elaboration of secretion in the sense of the "nuclear theory". There is a good accordance of the analytical statements with older cytomorphological signs in favour of the mentioned theory too. However, this conclusion never will affirm that the nucleus is alone responsible for the secretion. The process of secretion is a matter of the cell as a whole, in which the nucleus has only a rôle of a co-operator, although its participation seems to be of leading importance.

The attempt to elucidate the intimate processes of the thyroid cell by analysis of its isolated organoids meets with some essential difficulties. The starting material for

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every fractionation represents a sum of glands from various animals having a variable degree of secretory activity. Similar secretory differences are shown in individual follicles of a single thyroid as well. The phases of secretion never are quite synchronized in all follicles and all cells together, and the cells do not expel their content simultaneously. In a single thyroid, groups of cells elaborating secretion border on those that are in a state of repose, and various phases of secretory activity can be found. Therefore the isolated cell fractions could be considered as inhomogenous, since the individual organoids originated in asynchronic functioning cells. This obstacle could not be surmounted by an artificially established functional synchronization. Nevertheless, a valuable help was found in a carefully performed selection of the employed animals. Only a perfectly healthy fattened swine was used, the thyroids of which were in a resting state, as was checked by cytomorphological examinations. Thus, the isolated organoids belong to the same phase of secretory cycle, although slight differences must be taken into account.

The mentioned difficulties are combined with those originating in the method of fractionation which has been discussed elsewhere in this paper. The presence of colloid in the cytoplasmic fraction could not be excluded and characterizes the obstacles given by experiments with thyroid in comparison with those being performed with liver or any other organ. A certain alteration of cell organoids must be considered as an unavoidable consequence of the method which most probably will be further perfected by the latest development of the technique of cell fractionation, as was given recently by WILBUR AND ANDERSON[21].

SUMMARY

1. The fractionation of thyroid cells and the analyses of cell organoids have been performed.
2. Thyroid nuclei contain more thyroxine and diiodotyrosine than the mitochondria or microsomes.
3. The concentration of PNA decreases with the diminishing size of the chondriomal bodies, the amount of DNA is altogether low.
4. The intracellular distribution is characterized by greater contents of thyroxine, diiodotyrosine, PNA, and DNA in the cell nucleus than in the chondriome.
5. The conclusions concerning the origin of secretion are briefly discussed.

RÉSUMÉ

1. Les organelles des cellules thyroïdiennes ont été fractionnées par centrifugation et le teneur des fractions en thyroxine, diiodotyrosine, l'acide ribonucléique (PNA) et thymonucléique (DNA) a été examiné.
2. Les noyaux de la thyroïde contiennent plus de thyroxine et de diiodotyrosine que de mitochondries et microsomes.
3. Le teneur en PNA s'abaisse avec les dimensions s'abaissantes des mitochondries, celui ci en DNA est en tout très bas.
4. Les conclusions lesquelles se rapportent a l'explication de la formation de l'hormone sont discutées.

ZUSAMMENFASSUNG

1. Organellen der Schilddrüsenzellen wurden mittels fraktionierter Zentrifugation isoliert und auf den Gehalt an Thyroxin, Dijodtyrosin, Ribonukleinsäure (PNA) und Desoxyribonukleinsäure (DNA) untersucht.
2. Die Kerne der Schilddrüsenzelle enthalten mehr Thyroxin und Dijodtyrosin als die Mitochondrien und die Mikrosomen.

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3. Der Gehalt an PNA sinkt mit abnehmender Grösse der Mitochondrien, die Menge an DNA weist insgesammt einen niedrigen Wert auf.

4. Betreffs der intrazellulären Verteilung ist der Zellkern an Thyroxin, Dijodtyrosin, PNA und DNA reicher, als das Chondriom.

5. Folgerungen im Hinblick auf den Mechanismus der Sekretion werden kurz besprochen.

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