IODIDE-PEROXIDASE ACTIVITY IN HUMAN THYROID.

I. STUDIES ON NON-TOXIC NODULAR GOITRE

By

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ABSTRACT

Eighteen nodules from thirteen patients with non-toxic nodular goitre were studied. The nodules were classified, according to the $^{131}$I scintiscan, into »cold« and »warm« types. The histological diagnosis of all the glands was multinodular colloid goitre.

An enzymatic system with iodide-peroxidase activity was prepared from nodular tissue obtained by surgical thyroidectomy. The enzymatic activity was determined by spectrophotometry at 287.5 nm by measuring the formation of triiodide ion.

The group of »cold« nodules showed an average of 333 units of enzymatic activity and the »warm« nodules 940 units.

The significance of the correlation between the capacity for iodide-uptake, the iodide-peroxidase activity and the involution of the metabolic steps in the nodular goitre is discussed.

Since intrathyroidal oxidation of iodide is one of the first steps in the biosynthesis of thyroxine and triiodothyronine, and since thyroidal iodide-peroxidase plays an important role in such process, a correlation between this enzymatic activity and the different pathological conditions of the thyroid gland has been studied. Previous data were presented in a communication (Niepomniszcze et al. 1968) showing a correlation between thyroidal scinscanning and iodideperoxidase activity in patients with non-toxic nodular goitre. Our findings and the report of Alexander et al. (1968), on peroxidase activities of

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mitochondria from goitrous cretins, present further information on the physiopathology of the thyroid gland.

**MATERIALS AND METHODS**

Eighteen nodules from 13 patients with non-toxic nodular goitre were studied. Of these patients, eleven were females and two males between 21 and 63 years of age. Of the eleven females, eight patients presented uninodular goitre (5 »warm« and 3 »cold« nodules); the three patients with multinodular goitre were distributed as follows: two patients each with 2 »warm« nodules, and one patient with 2 »cold« and 1 »warm« nodules. Of two male patients: one had a »cold« uninodular goitre while the other patient with a multinodular goitre presented 2 »cold« nodules.

The patients were euthyroid according to clinical and laboratory examinations and the histological diagnosis of the glands was multinodular colloid goitre. The clinical data of the patients allowed of their classification into uninodular or multinodular goitres. By means of 131I scintiscanning the nodules were classified into »cold« and »warm« types, and this diagnosis was confirmed by triiodothyronine-inhibition and TSH-stimulation tests. Thyroidectomy was performed at least two months after the last test and none of the patients received any treatment. After operation, the nodule that was palpable in the clinical examination, was divided into two sections, one for histology and the other for enzymatic studies.

**Enzyme preparation**

Nodular tissue, obtained by surgical thyroidectomy, was frozen immediately after operation and stored at -15°C until it was homogenized with 0.15 M KCl (1:4 p/v) in a teflon Potter-Elvehjem homogenizer.

The supernatant obtained after centrifugation at 600 g for 10 min, was then ultracentrifuged at 15 000 g for 30 min when a mitochondrial precipitate was obtained. This was rinsed thrice with the KCl solution and homogenized with 1% digitonine solution in 0.05 M sodium phosphate buffer, pH 7 and kept at 4°C for 30 min with continuous shaking.

The digitonine-treated suspension was centrifuged at 40 000 g for 20 min. The enzymatic activity was found to be present in the supernatant.

**Protein and nucleic acid determination**

Protein was determined by the method of Warburg and Christian and an estimate of the nucleic acid content was obtained from the ratio of the optical densities at 280 and 260 nm (Layne 1957).

**Assay of peroxidase**

Iodide peroxidase activity was determined by an assay which depends on the oxidation of I- to I3- by H2O2, in the presence of excess I-. The change in the concentration of I3- was followed by means of spectrophotometry at 287.5 nm according to Alexander (1962). A stock solution containing 2 ml of 0.05 M phosphate buffer (pH 7) and 0.5 ml of 0.08 M KI was prepared. The stock solution (2.5 ml) and the enzyme (0.5 ml) were mixed in a cuvette with a 1 cm light path and a temperature of 23°C, and the absorbance was set at zero. The reaction was started with the addition of 20 µl of a
solution of 0.08 M H₂O₂ and spectrophotometric readings were taken every 15 seconds. A control run, without peroxidase, was prepared in which the enzymatic solution was replaced by buffer, in order to obtain the spontaneous oxidation value.

Plotting the optical density against time (Fig. 1) and applying the following formula: \( V_{15} = 3y_1 + \frac{y_3}{3} - 1.5y_2 \), where \( V_{15} \) represents the initial rate of the reaction extrapolated at 15 s; \( y_1 \), \( y_2 \) and \( y_3 \) are the optical density values at 15, 30 and 45 s respectively, according to a modification of Algranati's method (Algranati 1963). Three plots were obtained: a) the curve of enzymatic activity, b) the tangent to the initial rate and c) the spontaneous oxidation curve of iodide in the absence of enzyme.

The units of iodide-peroxidase activity were defined in the following way: the 15 s

![Fig. 1](image)

**Spectrophotometric assay of iodide-peroxidase activity.**
- - - - - activity of the complete enzymatic system.
- - - - - activity in the absence of the enzymatic system (spontaneous).
- - - - - tangent to the initial rate.

The double vertical lines indicate the value of the difference between \( V_{15} \) and spontaneous O.D. at 15 s (see »Materials and Methods«).
value of optical density of the spontaneous oxidation run was substracted from $V_{12}$, and this result related to 1 mg of protein content for the enzymatic solution and multiplied by a factor of 1000.

**RESULTS**

As can be seen in Fig. 2 the values for peroxidase activity of the »cold« nodules group ranged from 0 to 946 units with an average value of 333 and the range for »warm« nodules was from 125 to 1384 units with an average of 940 units. Six of the eight cold nodules (75 %) presented values between 0 and 433 units and seven of the ten »warm« nodules (70 %) had values between 1006 and 1384 units.

The spectrophotometric data showed that the protein concentration of the

![Fig. 2.](image-url)

Iodide-peroxidase activity (units) for the »cold« and »warm« nodules.
enzymatic preparation ranged between 0.36 and 0.71 mg/ml for the »cold« nodules and 0.63 and 1.70 mg/ml for the »warm« nodules. The nucleic acid content varied from 2.8 to 3.8 % in 83 % of the total number of cases, none of them being more than 10 %.

DISCUSSION

As stated by Frantz (1962) hyperplasia, hypertrophy and involution of the thyroid are part of a cyclic pattern, representing alternate phases of activity and inactivity, illustrated by Marine’s classical diagram. When this cyclic process continues, a nodular goitre results. The last steps of these changes have a common pathology as manifested by a colloidal goitre image.

The modern technique of scintiscaner with radioisotopes introduces a functional differentiation in the nodular tissue even though the histological picture is of the same pattern. By this technique only variations in $^{131}$I uptake by the tissue are shown. The so-called »cold« nodule loses its capacity for iodide uptake and nothing is known about other parameters which are related to hormonal biosynthesis. In the same way, the »warm« nodule retains the capacity of iodide uptake but the scintiscaner does not give any evidence which might exclude the possibility of other metabolic steps being affected. The measure of the iodide-peroxidase activity could be a parameter that might help the understanding of this pathology.

The data presented in this paper show, in general, that a loss in enzymatic activity corresponds to a loss in capacity to take up iodide, and that 30 % of the »warm« nodules lose iodide-peroxidase activity. Due to this fact, a nodule with a radioiodide uptake of the same proportion as the surrounding tissue and which behaves as »warm« in the scanner, has the possibility of being altered as determined by the enzymatic activity. These facts support the clinical view that »warm« nodules should be treated in a similar way as the »cold« nodules.

Trunnell & Wade (1955) and Pitt-Rivers et al. (1957) reported that nodular goitre contain less thyroxine in relation to iodotyrosine and less diiodotyrosine in relation to monoiiodotyrosine and they suggested that these findings indicate a reversion towards a more primitive type of biosynthesis. If this hypothesis is correct and bearing in mind that in the hormonal biosynthetic sequence, the process of iodide uptake is followed by the peroxidation of this ion, it is possible to consider theoretically, the following alternatives: There are nodules with both activities unaltered, or with the second step, i.e. the enzymatic activity diminished or there are nodules with both processes altered. Our findings show that there are nodules having the three functional possibilities cited above. Among the eighteen nodules studied, seven correspond to the first possibility, three to the second one, and seven to the third. The remaining
nodule was "cold" as judged by scanning, i.e. with a decreased uptake and with peroxidase activity close to the modal frequency of the "warm" nodules.

All these data suggest the existence of an involution line of the biochemical steps related to the metabolism of the nodular tissue, which should be considered as the reverse image of the ontogenetical process of the thyroid gland.

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REFERENCES


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