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Cytophotometric Determination of Nuclear Size and DNA Distribution in Different Hyperfunctioning Thyroid Lesions

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Summary. Nuclear enlargement in hyperfunctioning thyroid lesions which has been found in earlier cytophotometric studies is also one of the criteria in the subjective histological estimation of thyroid function. Histopathological assessment is, however, often unreliable. In the present study stage scanning cytophotometric measurements in Feulgen-stained tissue sections were used to determine the nuclear changes encountered in non-toxic and toxic nodular goitre, and in toxic diffuse goitre. To ensure optimal selection of specimens for measurements autoradiography was used. Specimens of toxic diffuse goitre invariably had enlarged nuclei, but no difference was found between nodules in non-toxic and toxic nodular goitre. In fact, the same nuclear area was found in hot nodules, warm nodules and perinodular tissue in non-toxic nodular goitre, and in hot nodules in toxic nodular goitre.

Thus there are lesions with clear-cut clinical, biochemical, and autoradiographic hyperfunction that do not have enlarged nuclei. Against this background it is possible that the nuclear enlargement present in toxic diffuse goitre reflects the disorder in itself and not the hyperfunctioning state.

Hyperdiploid cell nuclei were found in all cases of toxic diffuse goitre and in a higher precentage than in the other lesions. It was not possible to distinguish nontoxic and toxic nodular goitre on this basis.

Key words: Thyroid disease – Feulgen-DNA – Scanning microdensitometry – Autoradiography

Introduction

Conventional histological criteria of increased functional activity of the thyroid gland include nuclear enlargement, small follicles with scanty colloid, and high follicular cells. The occasional prescence of extremely large follicular cell nuclei has been noted in Graves' disease, thyroiditis, and adenomatous goitre (Meissner and Warren 1969). The subjective histological estimation of thyroid functional activity is, however, unreliable as was shown in a study on autoradio-

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graphically confirmed lesions (Risberg et al. 1981). Nuclear enlargement in hyperfunctioning thyroid lesions has been noted in objective cytophotometric studies. Nilsson (1972) who studied fine-needle biopsies from toxic and non-toxic goitre found a slightly larger mean nuclear diameter in toxic goitre. He also found an increased incidence of hyperdiploid nuclei in toxic goitre. In a cytophotometric study, Tasca and Stefaneanu (1977) found that the mean DNA content was higher in toxic diffuse goitre than in euthyroid goitre (3.5 C:2C). Calculated nuclear volumes were almost twice as large in TDG than in euthyroid goitre. In a morphometric study, Fontolliet-Girardier (1972) suggested a linear relation between nuclear size and functional activity. Thus there seems to be little doubt concerning the nuclear enlargement in toxic goitre, although the explanation for this is unclear. The question whether it is the underlying disease or the hyperfunctioning state in itself that causes the nuclear enlargement has not been touched upon so far.

Principally there are 3 types of hyperfunctioning thyroid lesions, (i) toxic diffuse goitre, (ii) hot nodules in non-toxic nodular goitre, and (iii) toxic nodular goitre. Toxic adenomas are most probably not true neoplasms, but variants of toxic nodular goitre (Meissner 1978).

Previous cytophotometric studies concern only toxic diffuse goitre or unspecified toxic goitre, and the conclusions reached are thus not valid for the entire spectrum of hyperfunctioning thyroid lesions. Thus, the reasons for the nuclear changes encountered in hyperfunctioning thyroid lesions remain obscure.

The aim of this investigation was to determine the nuclear area and DNA distribution in morphologically and functionally well defined thyroid lesions, with special reference to hyperfunctioning lesions. Measurements were done by stage scanning cytophotometry in tissue sections to enable the selection of functionally defined areas.

Material and Methods

The material for the study was obtained from surgically removed specimens from 12 patients. Basic clinical data for the patients are summarized in Table 1. All patients with nodular lesions were scintigraphed and had all received ^{131}I 48 h before operation. This fact enabled us to use autoradiography for selecting functionally different lesions within each specimen. The resected thyroid lobes from these patients (cases 1–5 in Table 1) were perfusion-fixed with neutral buffered formaldehyde, followed by overnight immersion in the same fixative. The lobes were subsequently prepared for whole-organ-sectioning as described by Bergman et al. (1980).

Autoradiography was performed directly on the whole-organ-sections, using LKB ^{3}H film. The lesions were classified as follows: hot – blackening stronger than surrounding normal thyroid tissue; warm – blackening equal to normal thyroid tissue (Fig. 1). From each lobe specimens for cytophotometry were taken from several nodules and perinodular tissues.

Specimens of toxic difffuse goitre were obtained from patients 6–12. Due to the diffuse nature of this lesion normal reference tissue is unlikely to be found, and therefore neither whole-organ-sectioning nor autoradiography was performed.

On histological examination 3 of the 7 cases of toxic diffuse goitre had prominent lymphoid infiltrates with germinal follicles (Table 1).

As thyrostatics and iodine treatment have been shown to induce nuclear changes (Eggen and Seljelid 1972), only specimens from patients receiving adrenorecptor blocking drugs alone were included.

Cytophotometry. For the cytophotometric investigation the areas selected by autoradiography were cut out from the whole-organ paraffin blocks and re-embedded in ordinary sized blocks.

Table 1. Clinicopathological a data for the 12 cases studied

Case	Age/Sex	Thyroid function	Morphology	Autoradiography
1	61/F	Euthyroid	Nodular goitre	hot nodules, 8 and 10 mm warm nodules, 6 and 9 mm
2	39/F			hot nodule 15 mm warm nodule 6 mm
3	68/M			hot nodules, 6 and 20 mm
4	59/F			hot nodules, 8, 9 and 27 mm
5	34/F			hot nodules, 8 and 25 mm
6	50/F	Hyperthyroid	Diffuse hyperplasia with lymphoid stroma	
7	43/F			
8	46/F			
9	53/F		Diffuse hyperplasia without lymphoid stroma	
10	56/M			
11	29/F			
12	43/F			

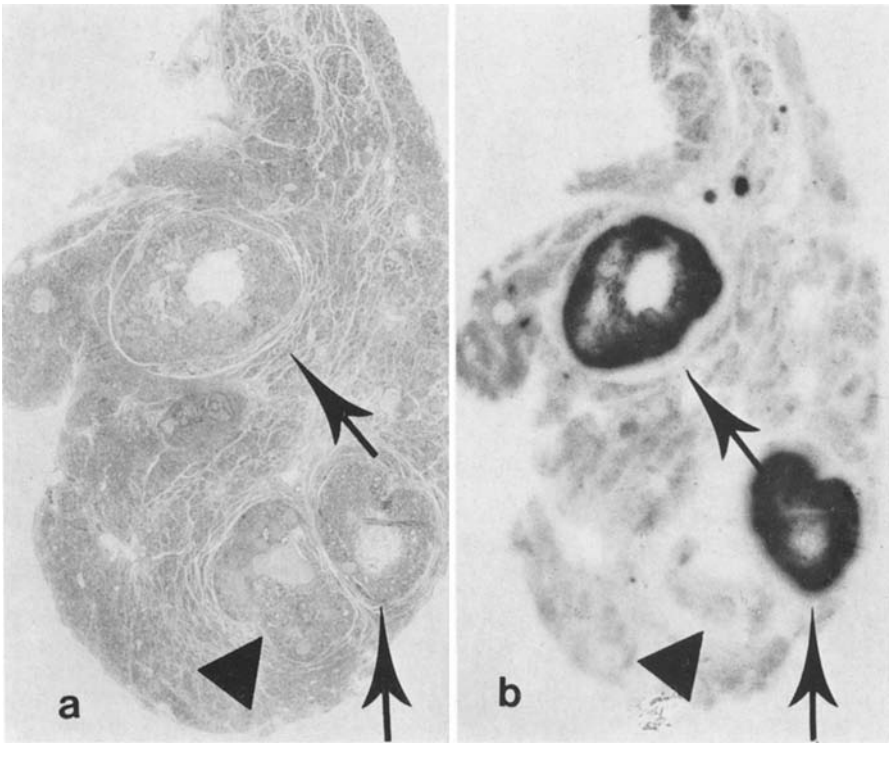


Fig. 1a, b. Whole-organ-section of a non-toxic nodular goitre (case 1) (**a**). The corresponding autoradiograph with arrows indicating hot nodules and an arrowhead indicating a warm nodule (**b**). The nodules were cut out from the whole-organ paraffin block for the cytophotometric measurements

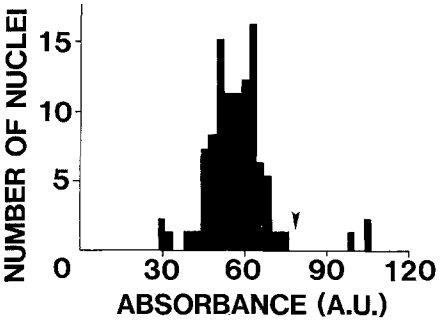


Fig. 2. Absorbance histogram from one of the specimens measured. The arrow indicates the estimated upper range of diploidy. All nuclei with higher DNA content are classified as hyperdiploid, in this instance 3 nuclei, i.e. 3%

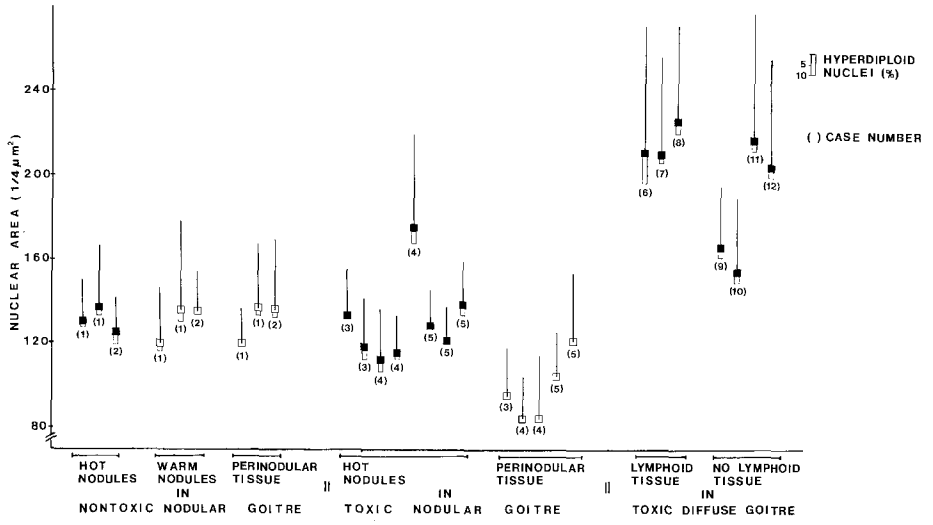


Fig. 3. Results of cytophotometric measurements for all specimens. Hot lesions are indicated by filled squares. Standard deviation for the area value is layed out upwards, and the percentage of hyperdiploid nuclei downwards

Care was taken to orientate the specimen so as to correspond to the original section. From each block 2 consecutive sections were cut using a Leitz 1212 microtome, constantly set at 5 μm section thickness. One section was stained with Hematoxylin and Eosin (H&E), and was compared with the original whole-organ-section to ensure precise orientation. The H&E sections was also used to improve localization in the Feulgen-stained section if necessary. Feulgen-staining was done as described by Duijndam and Van Duijn (1973 and 1975) after acid hydrolysis in 5 N HCl for 1 h. Quantitation of nuclear area and DNA content was performed by computerized stage scanning absorbance cytophotometry, using a Leitz MPV 2 cytophotometer and the HISTOSCAN program (Bjelkenkrantz et al. 1981). Hundred follicular cell nuclei were measured in each lesion. Owing to nuclear cutting it is not possible to determine the absolute DNA content in individual nuclei, but using the method described by Kreicbergs and Zetterberg (1980) a coarse measure of the number of hyperdiploid nuclei can be obtained, because nuclei of homogeneous size (as in each specimen in this study) form one single peak in the absorbance histogram (Fig. 2). Nuclei with clearly higher DNA content can thus safely be regarded as hyperdiploid. The percentage of these hyperdiploid nuclei in each lesion was recordered.

Nuclear area is the number of measuring points (pixels = picture elements) within the nucleus. As the scanning is done with 0.5 μm resolution the area values are therefore most conveniently presented as the number of pixels, each of which represents 1/4 μm^2.

Results

The results of the cytophotometric measurements are summarized in Fig. 3.

Non-Toxic Nodular Goitre (Cases 1–2)

In this group 3 specimens each from the hot nodules, warm nodules, and perinodular tissue have been studied. Nuclear area does not differ between the 3 types of specimens, the mean area being 130 pixels in all instances.

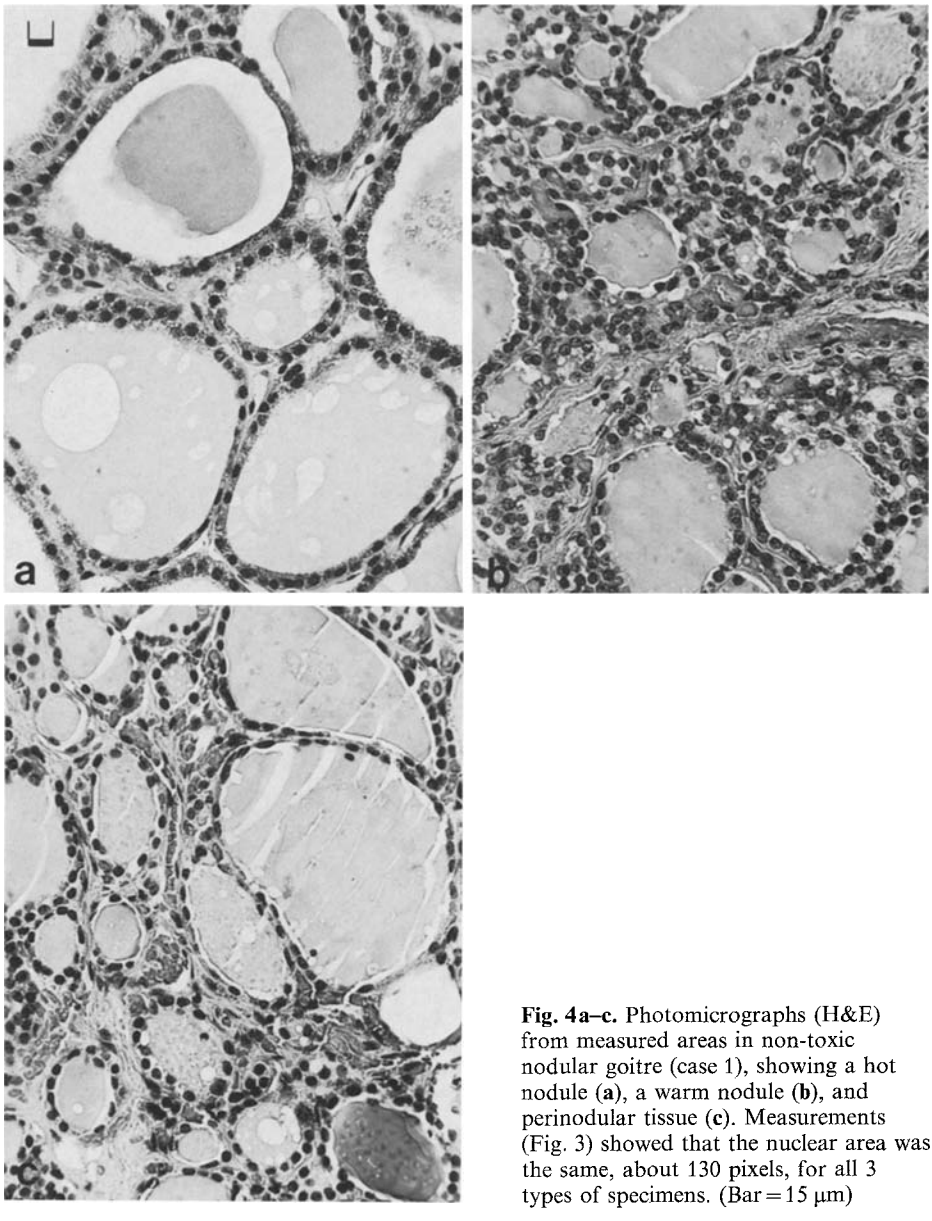
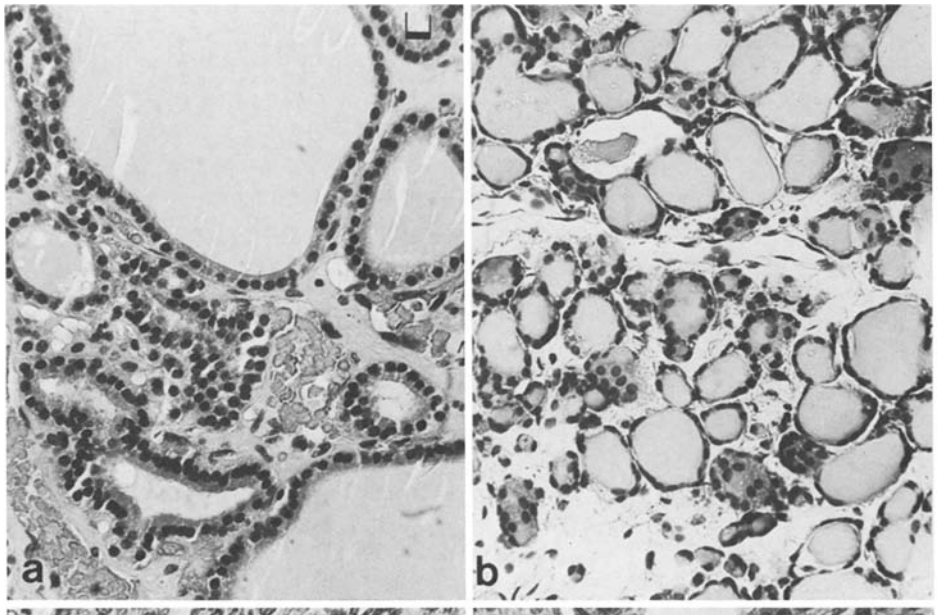


Fig. 4a–c. Photomicrographs (H&E) from measured areas in non-toxic nodular goitre (case 1), showing a hot nodule (**a**), a warm nodule (**b**), and perinodular tissue (**c**). Measurements (Fig. 3) showed that the nuclear area was the same, about 130 pixels, for all 3 types of specimens. (Bar = 15 μm)



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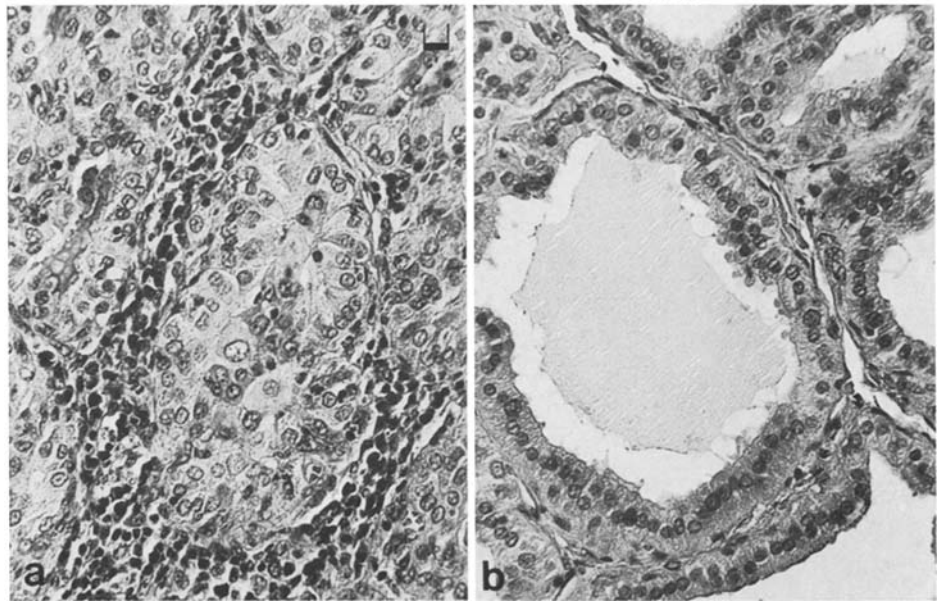

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Fig. 5a, b. Photomicrographs (H&E) of measured areas in toxic nodular goitre, showing a hot nodule (**a**), and perinodular tissue (**b**). Measured nuclear area was 138 pixels for the hot nodule and 84 pixels for the perinodular tissue. (Bar = 15 μm)

Fig. 6a, b. Photomicrographs (H&E) of measured areas in toxic diffuse goitre showing a specimen with lymphoid stroma (case 8) (**a**), and a specimen without lymphoid stroma (case 11) (**b**). The measured areas were 226 and 217 pixels respectively. (Bar = 15 μm)

The variability between specimens is small with all 9 specimens within the range of 119–137 pixels. The standard deviation (S.D.) for the area value is within 17–29 for the hot nodules, 19–42 for the warm nodules, and 17–32 for the perinodular tissue. In 7 of the 9 specimens, including all hot nodules there are a small number of hyperdiploid cell nuclei, ranging from 1–4%. Relevant histopathological photographs from the measured areas in the H&E section of case 1 are shown in Fig. 4a–c.

Toxic Nodular Goitre (Cases 3–5)

8 specimens of hot nodules, and 5 specimens of perinodular tissue have been studied. The hot nodules have a mean area of 130 pixels. Seven of the 8 specimens show grouping with means for individual specimens within 112–138, while one nodule has a much higher value, 175 pixels. The S.D. values for the 7 grouped nodules are between 16–24, and for the nodule with the larger nuclei 44. In 5 of the 8 specimens hyperdiploid nuclei have been detected in up to 4% in the aligned group and in 6% in the nodule with larger nuclei. The area values for the perinodular tissue are lower ranging from 84–121 pixels, mean value 98 pixels. S.D. within 19–33. No hyperdiploid nuclei were detected. Microphotographs from measured areas in the H&E section clearly demonstrates the difference in nuclear size between hot nodules and perinodular tissue in toxic nodular goitre (Fig. 5a, b).

Toxic Diffuse Goitre (Cases 6–12)

The area values for the 7 cases in this group are considerably higher with a mean value of 198 pixels. The 3 specimens with lymphoid stroma forms a closely aligned group with mean area value 216 pixels, while the 4 specimens without lymphoid stroma have area values ranging from 154–217 pixels. S.D. values for the whole group are variable, within 28–60. Hyperdiploid nuclei are present in all lesions, ranging from 2–13%. Photomicrographs of measured areas in H&E sections from case 8 with lymphoid stroma, and from case 11 without lymphoid stroma, are shown in Fig. 6a, b.

Discussion

Nuclear enlargement in toxic goitre is a widely accepted phenomenon, and constitutes one of the histological criteria of thyroid hyperfunctioning. Objective cytophotometric studies have confirmed this enlargement. As the basis of the enlargment is not clear and the histological criteria of hyperfunction in general are unreliable (Risberg et al. 1981), a study comprising different types of hyperfunctioning lesions was undertaken, the aim being to establish whether the nuclear enlargement is associated with hyperfunction per se or whether it is related to the type of thyroid lesion. The findings in this study lends no support to the notion that nuclear size reflects the functional state of the lesion. The same nuclear area, 130 pixels, was re-

corded in autoradiographically hot nodules in cases of biochemically active, toxic goitre, as in not, and warm, nodules in non-toxic goitre.

From the small number of cases studied we can of course not disprove the hypothesis that nuclear size correlates with functional activity, but the results show that such a correlation is not invariably present.

In this context it is interesting to note that the perinodular tissue in toxic nodular goitre have clearly smaller nuclei – 98 pixels – than recorded in the hot nodules – 130 pixels. This difference between nodules and perinodular tissues was not present in non-toxic goitre. This difference may therefore provide an indirect clue in histopathological work. In the individual case however this nuclear sizediscrepancy may very well be inconspicuous, as is evident from Fig. 3.

The perinodular tissue in toxic nodular goitre is TSH depleted, a change which in experimental work in rats has been shown to diminish nuclear size (Alfert et al. 1955; Stötzer 1976).

The nuclei in toxic diffuse goitre are enlarged with a mean area value of 198 pixels. Since nuclear enlargement is not present in toxic nodular goitre this enlargement most probably reflects the lesion in itself and not the hyperfunctioning. The finding of large nuclei in toxic diffuse goitre is consistent with the results of Tasca and Stefaneanu (1977) and Nilsson (1972).

We were not able to correlate the coarse measure of the frequency of hyperdiploid nuclei with the functional activity, but we belive that this aspect deserves closer investigation using techniques enabling precise ploidy determination which is not possible in tissue sections. In conclusion, the results obtained suggests that hyperfunction is not necessarily followed by nuclear enlargement but that increase in nuclear size reflects the primary disorder causing the hyperfunction. Hyperdiploid cell nuclei were present in all cases of toxic diffuse goitre but were otherwise an inconsistent finding, without obvious correlation to the functional state.

References

Alfert M, Bern HA, Kahn RH (1955) Hormonal influence on nuclear synthesis. IV. Karyometric and microspectrophotometric studies of rat thyroid nuclei in different functional states. Acta Anat 23:185–205

Bergman C, Gröntoft O, Olofsson J, Risberg B (1980) A technique for whole-organ sectioning and its applications. Sience Tools, the LKB Journal 27:46–50

Bjelkenkrantz K, Stål O, Gröntoft O, Olofsson J, Herder A, Hellquist H (1981) HISTOSCAN: Computer program for cytophotometry in tissue sections and its application in the evaluation of nuclear atypia. Histochemistry 73:353–362

Duijndam WAL, Van Duijn P (1973) The dependance of the absorbance of the final chromophore formed in the Feulgen-Schiff reaction on the pH of the medium. Histochemistry 33:373–375

Duijndam WAL, Van Duijn P (1975) The interaction of apurinic acid aldehyde groups with paraosaniline in the Feulgen-Schiff and related staining procedures. Histochemistry 44:67–85

Eggen PC, Seljelid R (1973) The histological appearance of hyperfunctioning thyroid following various pre-operative treatments. Acta Pathol Microbiol Scand (A) 81:16–20

Fontolliet-Girardier C (1972) Morphologie et morphométrie de l'adénome toxique de la thyroide. Virchows Arch [Pathol Anat] 353:253–263
Kreicbergs A, Zetterberg A (1980) Cytophotometric DNA measurements of chondrosarcoma. Methodologic aspects of measurements in tissue sections from old paraffin embedded specimens. Anal Quant Cytol 2:84–92
Meissner A, Warren S (1969) Tumors of the thyroid gland. Atlas of tumor pathology Second series Fasc. 4. Armed Forces Institute of Pathology, Washington DC., p 91
Meissner WA (1978) Pathology. In: Werner SC, Ingbar SH (eds) The thyroid. Harper and Row Publ. Inc. New York-San Fransisco-London, pp 445–452
Nilsson G (1972) Nuclear size classes in fine needle aspirates from toxic goitres. Acta Endocrinol (Copenh) 70:273–288
Risberg B, Eneström S, Lennquist S, Bergman C (1982) Evaluation of thyroid scintigraphic lesions by whole-organ sections and autoradiographs. Acta Pathol Microbiol Scand (A) 90:19–26
Stötzer H (1976) Age dependent morphokinetics of the thyroid gland. Gustav Fischer Verlag. Stuttgart-New York, pp 42–46
Tasca C, Stefaneanu L (1977) Cytology and cytophotometry in the thyroid diseases. Acta Histochem (Jena) 60:261–272

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