A morphometric study of nuclei, nucleoli and nuclear bodies in goitres and papillary thyroid carcinomas[1]

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Summary. Significant positive correlations were found between the nuclear surface-to-volume ratio and the volumetric density of nucleoli, as well as between the volumetric density of nucleoli and the volumetric and numerical densities of complex nuclear bodies in goitres and papillary thyroid carcinomas.

The ground-glass appearance of the nuclei of papillary thyroid carcinomas has been related to a particular physical state of the chromatin during the interphase[3], a hypodiploidia of the nuclei[4], a high frequency of cytoplasmic evaginations into the nuclei[5], or, finally, a despiralization of chromatin, a process which is generally connected with an increase of the nuclear activity[6]. On the other hand, the finding in these carcinomas of several alterations concerning the fine structure of nuclei, nucleoli and nuclear bodies[7] has raised the possibility of the interference of a disturbed RNA metabolism in their pathogenesis.

The purpose of this study was to determine, using morphometric methods, whether any correlation could be established between the characteristics of nuclei of thyroid lesions and those of the nucleoli and of the nuclear bodies.

Material and methods. Surgical specimens were obtained from 4 patients with diffuse hyperplastic goitres (group I), from 3 patients with papillary carcinomas of the thyroid (group II) and from 6 patients with occult sclerosing carcinomas of the same gland (group III). In 1 patient of group II it was possible to obtain surgical specimens from 2 independent tumors of both lobes of the gland (cases 6A and 6B), and in 1 patient of group III it was possible to obtain tissue fragments from a lymph node metastasis (case 11M). For light microscopy, tissue fragments were routinely processed. For electron microscopy, tissue fragments were fixed in 2% phosphate buffered osmium tetroxide for 2 h, dehydrated in graded ethanols and embedded in epoxy-resin.

From each case, 6 tissue blocks were selected at random. From each block, a section of approximately 1 μm was stained with toluidine blue and used to determine the mean diameter of the nuclei of the cells (D). D was calculated measuring 200 nuclear profiles from each case with an eyepiece with a micrometer incorporated at a magnification of approximately 1250, and the value thus

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Summary of morphometric results

Group	Case No.	Sex age	Nuclei D (μm)	Nuclei S/V (μm^{-1})	Nucleoli V_v (%)	Nucleoli Na (No./100 μm^2)	Nuclear bodies V_v (%)	Nuclear bodies Na (No./100 μm^2)	Nuclear bodies N'a* (No./100 μm^2)
I	1	♀, 54	9.0	0.91	0.96	1.69	0.19	1.09	0.0
	2	♀, 17	8.6	0.88	1.05	1.92	0.15	0.80	0.06
	3	♀, 40	7.8	0.88	0.62	1.28	0.10	0.57	0.0
	4	♀, 43	7.5	1.06	0.94	0.88	0.11	0.47	0.07
	Mean ± SE		8.2 ± 0.3	0.93 ± 0.04	0.89 ± 0.09	1.44 ± 0.23	0.14 ± 0.02	0.73 ± 0.14	0.03 ± 0.02
II	5	♀, 51	8.4	0.92	0.85	0.71	0.18	1.42	0.09
	6A	♀, 42	8.2	0.95	1.65	1.01	0.55	1.97	0.59
	6B	♀, 42	8.0	0.74	1.74	1.42	0.27	1.56	0.26
	7	♀, 14	8.4	1.00	1.51	1.37	0.54	1.60	0.23
	Mean ± SE		8.3 ± 0.1	0.90 ± 0.06	1.44 ± 0.20	1.13 ± 0.17	0.39 ± 0.09	1.64 ± 0.12	0.29 ± 0.11
III	8	♂, 50	8.4	1.05	1.47	1.26	0.29	0.85	0.46
	9	♀, 65	8.2	1.07	1.72	1.51	0.55	1.91	0 46
	10	♀, 16	7.8	1.69	1.92	1.84	0.37	1.82	0.42
	11	♀, 30	8.1	1.49	2.29	1.79	0.35	1.79	0.30
	11M	♀, 30	8.3	1.32	1.73	1.37	0.65	1.40	0.45
	12	♂, 59	9.5	0.94	1.95	1.27	0.53	1.48	0.40
	13	♀, 40	9.3	1.04	1.25	0.83	0.11	0.34	0.17
	Mean ± SE		8.5 ± 0.2	1.23 ± 0.11	1.76 ± 0.17	1.41 ± 0.13	0.41 ± 0.07	1.37 ± 0.22	0.38 ± 0.11
p-value (I vs II)			n.s.	n.s.	$p < 0.05$	n.s.	$p < 0.05$	$p < 0.005$	$p < 0.05$
p-value (I vs III)			n.s.	n.s.**	$p < 0.005$	n.s.	$p < 0.02$	n.s.**	$p < 0.001$
p-value (II vs III)			n.s.	$p < 0.05$	n.s.	n.s.	n.s.	n.s.	n.s.

* Number of complex nuclear bodies (types III and IV of Bouteille et al.[10]) per unit area of the nuclear profile. ** $p < 0.1$.

obtained was corrected according to Bach[8]. From each block, a silver ultrathin section was double stained with uranyl acetate and lead citrate and microphotographed at a primary magnification of 4000. The exact final magnification for each set of electron micrographs was calibrated by means of a carbon grating replica; as an extreme variation of less than 5% of the mean was found, all the calculations were made using only the mean value (×12,000). From each ultrathin section, 5 electron micrographs were recorded; fields were chosen at random and those not containing nuclear profiles of thyroid cells were discarded. A double quadratic lattice test system of 400 points and 225 cm^2 was used to calculate the fractions of the nuclear volume occupied by nucleoli and by nuclear bodies (V_v), the surface-to-volume ratio of nuclei (S/V) and the number of nucleolar and nuclear bodies profiles per unit area of nuclear profiles (Na). In order to determine all these parameters, the techniques described by Weibel and Bolender[9] were used; no corrections were made concerning Holmes effect. The classification of the nuclear bodies was made according to Bouteille et al.[10]. Individual morphometric data were averaged and SD and SE were calculated. In order to compare the results, Student's 2-sided test was used; 2 means were considered significantly different if the probability of error (p) was smaller than 0.05.

Results and discussion. At the optic level, most of the nuclei of the carcinomas displayed a ground-glass appearance and contained prominent nucleoli, while those of goitres had coarse chromatin and rarely contained prominent nucleoli.

The statistical analysis of the results obtained in the morphometric study is summarized in the table and shows several significant differences between the nuclei of the goitre group and those of both types of thyroid carcinomas, as well as a significantly higher surface-to-volume ratio of the sclerosing carcinoma nuclei in comparison with those of common papillary carcinomas. Furthermore, it shows that the increased volumetric density of nucleoli in carcinomas, when compared with goitres, does not depend on an increased number of nucleoli, but on an increase of their individual volumes, which is in keeping with their prominence in light microscopy.

The significant positive correlation ($r = 0.555$; $p < 0.05$) that was found between the surface-to-volume ratio of the nuclei and the volumetric density of their nucleoli, matches Burns et al.[11] statement about the close relationship between the size of the nucleo-cytoplasmic contact and the degree of nucleolo-cytoplasmic interaction, and points to increased nuclear and nucleolar activities in papillary thyroid carcinomas.

Significant positive correlations ($r = 0.701$; $p < 0.005$ and $r = 0.770$; $p < 0.001$) were also found between the volumetric density of nucleoli and the volumetric density of nuclear bodies, as well as between the volumetric density of nucleoli and the number of complex nuclear bodies per unit area of nuclear profiles. Since it is known that the central core of complex nuclear bodies is made of ribonucleoproteins[12], these findings also point to an increased nucleolar activity in papillary thyroid carcinomas, although they do not rule out the hypothetical involvement of a disturbed RNA metabolism in their pathogenesis[7].

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Invasive properties of the ovarian cortex in birds

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Summary. In quail/chicken ovarian cortex associations , grown on chicken chorioallantoic membrane (CAM), epithelial and/or germ cells from the quail ovary may invade the cortical rim of the chicken ovary. In certain experimental conditions, ovarian cortical cells may leave a transplanted ovary and grow over or invade the mesenchyme of the surrounding CAM. So a germinal epithelium completely free from other ovarian cell groups can be obtained.

In a recent study[2] we have found experimental evidence that, in the embryonic Japanese quail ovary (transplanted on CAM), surface epithelial cells penetrate into the ovigerous cords and finally give rise to the development of follicle cells. The present investigation demonstrates that the penetration potentialities of this surface epithelium and/or its derivatives are not necessarily limited to its own cortex.

Material and methods. A. Quail-chicken ovarian cortex associations: left ovaries of 9- to 11-day-old chicken or Japanese quail embryos were used for this study. First the quail ovary, with its cortical side directed upwards, is transplanted on the CAM of an 8- to 9-day-old chicken embryo, according to the technique of Harris[3]. 1 day later, a chicken ovary of the same age is placed crosswise over the quail ovary, taking care that part of the ovarian cortex of both ovaries remains in close contact.

B. Quail ovarian cortex in contact with the chorionic epithelium of chicken CAM: ovaries (with their cortical side downwards) from 16-day-old quail embryos or parts from 9-day-old quail ovaries, already grown for more than 1 week on CAM, are grafted on the CAM of 8-day-old chicken embryos. 7–10 days later, grafts from both experimental group A and B were excised and fixed in acetic-alcohol (1:3) for 1 h. After embedding in paraffin, the transplants were sectioned at 7 μm thickness. After deparaffination, the sections were stained with the PAS

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