Ploidy of Micronucleated Thyrocytes Induced by Methylnitrosourea Injection

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Ploidy of thyroid parenchyma was studied in adult rats in the control and on day 9 after hemithyroidectomy; operated animals received 3 injections of N-nitroso-N-methylurea. The population of follicular thyrocytes is mainly diploid; total count of polyploid cells increased from 4.4% in the control to 8.5% in experimental rats. All thyrocytes containing micronuclei were tetraploid. No diploid micronucleated elements were detected. This suggests that genetically damaged thyrocytes divide by the mechanism of acytokinetic (polyploidizing) mitosis.

Key Words: *thyroid; thyrocytes; methylnitrosourea; micronuclei; polyploidy*

Exposure of dividing cells to genotoxic factors leads among other things to the appearance of additional nuclear structures (micronuclei) in these cells after mitosis [4,9]. Chromosome aberrations and/or damage to the mitotic spindle, associated with it, inevitably lead to disorders in the distribution of genetic material and normal course of mitosis, and it is therefore important to know the extent of changes in cell ploidy, an important integral characteristic of the cell genome [8]. This is true primarily for highly specialized populations with low rejuvenation level, capable of retaining aberrant cells for a long time. For the population of follicular thyrocytes studies as a prospective test system for bioindication of genotoxic injuries to the thyroid gland (TG) [5,10] the question about ploidy of damaged cells remains not studied.

We carried out a cytophotometric study of DNA content in thyrocytes after exposure of TG to a chemical mutagen (N-nitroso-N-methylurea).

MATERIALS AND METHODS

Experiments were carried out on 10 adult male Wistar rats (200-250 g). Left-side hemithyroidectomy was used as a standard model for inducing thyrocyte proliferation [2]. The operation was carried out under hexenal narcosis. Two, four, and six days after surgery the animals were intraperitoneally injected with N-nitroso-N-methylurea (Sigma) in a dose of 6.4 mg/kg [5]. The animals were sacrificed by ether inhalation 9 days after the start of the experiment. Fragment of TG obtained during surgery served as the basal control. Thyrocytes were isolated by dissociation of TG fragments in 0.25% collagenase solution for pancreatic islets (Sigma) at 37°C for 2-2.5 h. Smears of thyroid cells were fixed in ethanol, stained after Feulgen (hydrolysis in 5 n HCl for 12 min at 37°C, staining by Schiff reagent during 1 h) with poststaining of the cytoplasm with light green.

The incidence of mononuclear, binucleated, and micronucleated thyrocytes was evaluated (1000 cells per animal were examined). Ploidy of the population in control and experimental samples was studied at λ=580 nm using an MIF-K cytophotometer (Moscow State University).

RESULTS

The population of follicular thyrocytes included mononuclear, binucleated, and micronucleated cells (Fig. 1). Quantitative analysis of these types by

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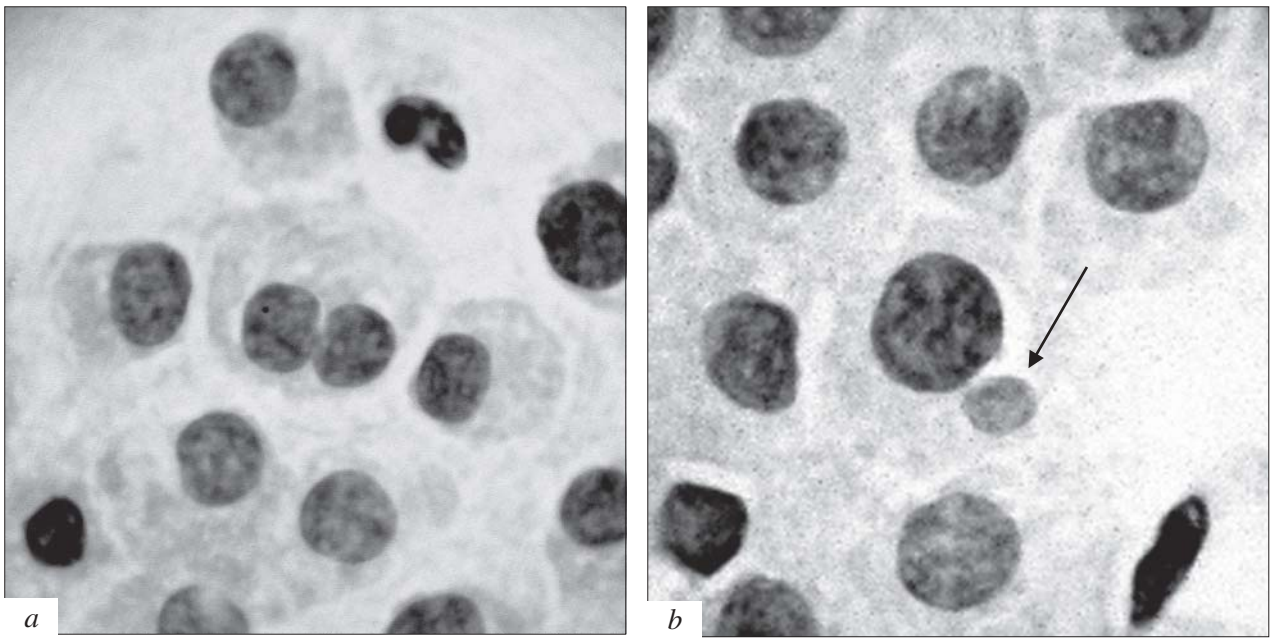

Fig. 1. Isolated binucleated and mononuclear thyrocytes (*a*), thyrocyte with micronucleus (arrow; *b*). Feulgen and light green staining, ×630.

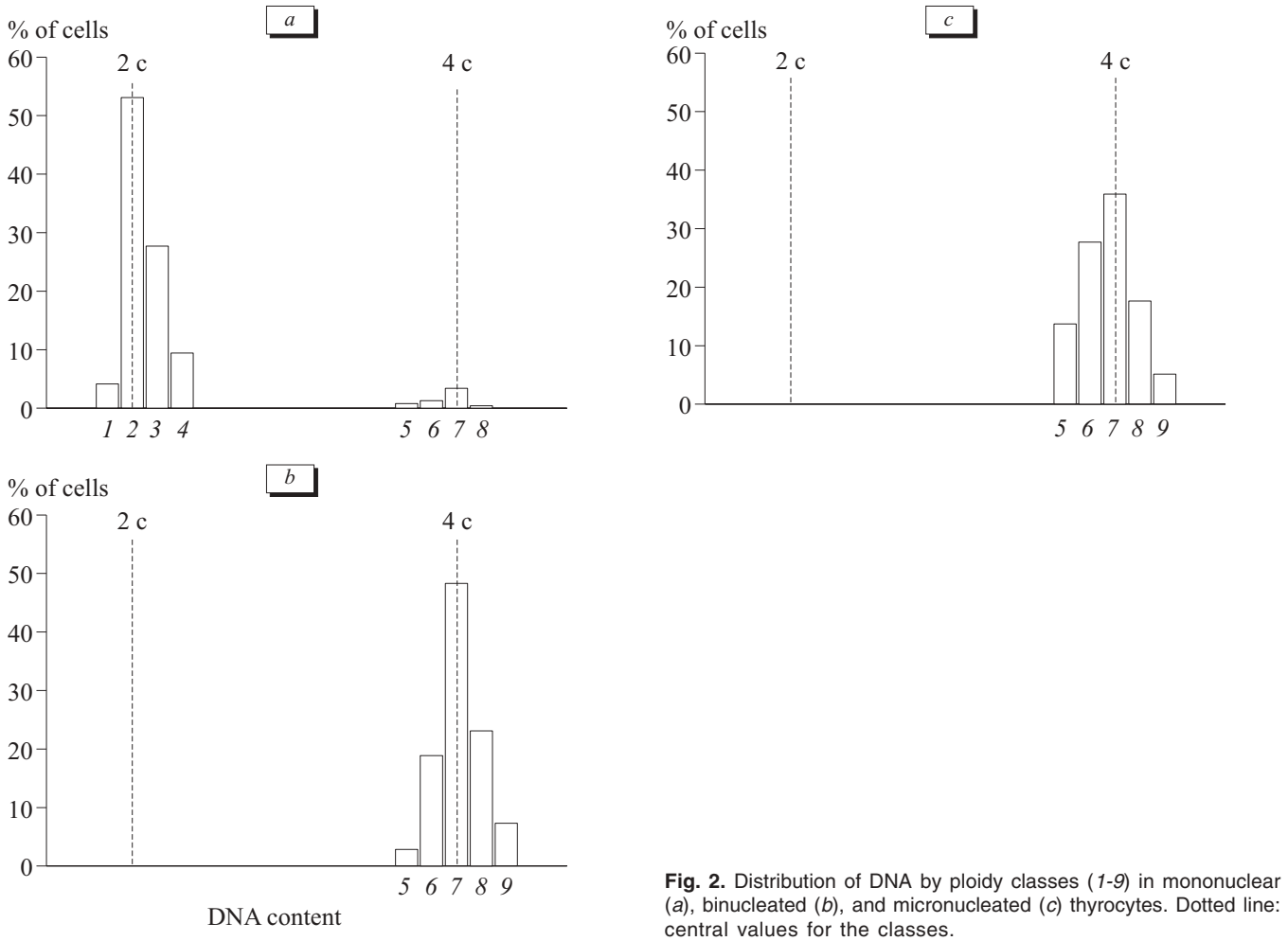

Fig. 2. Distribution of DNA by ploidy classes (*1-9*) in mononuclear (*a*), binucleated (*b*), and micronucleated (*c*) thyrocytes. Dotted line: central values for the classes.

TABLE 1. Content of Thyrocytes with Different DNA Content (per Cell)

Group	Mononuclear		Binucleated		Micronucleated	
	2c	4c	2c×2	4c×2	2c	4c
Control	95.6	4.0	0.2	0	0	0.2
Experiment	91.5	5.5	1.4	0	0	1.6

ploidy classes was carried out (Table 1; Fig. 2). Thyroid epithelium of intact animals was mainly diploid, total content of tetraploid cells is 4.4% (mononuclear thyrocytes predominated). The total content of polyploid elements in hemithyroidectomied animals treated with the mutagen reached 8.5%, the content of binucleated (2c×2) and micronucleated cells increased 7- and 8-fold, respectively. All thyrocytes containing micronuclei were tetraploid; no diploid micronucleated elements were detected.

The data of DNA cytophotometry suggest that the course of mitosis of thyrocytes exposed to the mutagen was disturbed at the stage of metaphase (last point of genetic control of the mitosis course); as a result, the cell cycle than proceeds by the acytokinetic mechanism [7,11]. Hence, micronucleated thyrocytes are formed by the mitotic mechanism underlying the formation of binucleated cell in other highly specialized cell lines: cardiomyocytes, hepatocytes, endotheliocytes [1,3,7]. All postmitotic micronucleated thyroid cells become polyploid. Presumably, this phenomenon is a specific feature of the studied cells: in the hepatocyte population micronuclei are observed mainly in the tetra- and octoploid cells, though can be sometimes seen in diploid elements [12]. In diploid lymphocyte cultures prestimulated to division, mitosis in aberrant cells is completed, and hence, in order to label the pool of proliferating lymphocytes, they are artificially transformed into polyploid ones by means of cytochalasin B (cytotomy blocker) [9].

In general, retention of an extra chromosome set in genetically damaged thyrocytes can improve their viability and provide compensation for the negative consequences of the developed genome defects [7,8,13].

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