

# BIOCHEMISTRY AND BIOPHYSICS

## DISTRIBUTION OF NUCLEIC ACIDS IN THE CELL NUCLEI OF NORMAL LIVER AND TRANSPLANTED MOUSE HEPATOMA

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I. B. Zbarskii and S. S. Debov [1-3] have shown that three protein fractions can be obtained from the cell nuclei isolated from normal tissues and malignant tumors in man; these authors called the three fractions "nucleo-protein," "acid protein" and "residual protein."

The ratio of these fractions in the cell nuclei of malignant tumors differs from the normal in the low content of nucleoprotein and acid protein and a sharp increase (5-10-fold) in residual protein content [1, 2].

These protein fractions from cell nuclei vary in their amino acid composition [3]. With respect to their content of nucleic acids, the authors only give data concerning the phosphorus content and indicate qualitative detection of desoxyribonucleic acid (DNA) in the nucleoprotein fraction only [1, 3].

The present work is concerned with investigation of the DNA and ribonucleic acid (RNA) content of the cell nuclei and nuclear protein fractions in normal liver and transplanted mouse hepatoma.

### EXPERIMENTAL METHODS

Mice of the strain "S<sub>3</sub>NA" were inoculated subcutaneously with hepatoma. They were sacrificed on the 18th-20th day after the inoculation and the tumor was removed for examination.

Cell nuclei were isolated from 8-10 g liver or tumor tissue obtained from several animals.

The cell nuclei were isolated in a neutral medium by the method of A. Dounce [7] or by the Zbarskii and Perevoshchikova modification of this method [4] and then fractionated by the procedure described earlier [1].

The content of the nuclear fractions was expressed in percentage nitrogen of the given fraction with respect to the total nuclear nitrogen.

The isolation and separation of nucleic acids was carried out according to G. Schmidt and S. I. Thannhauser [12] in the H. W. Kosterlitz [10] modification. The amount of DNA was determined in terms of desoxypentose using the colorimetric method of I. M. Webb and H. B. Levy [13] and parallelly in terms of phosphorus; RNA was determined by way of phosphorus. Nitrogen estimations on individual samples were performed by the Kjeldahl micromethod; phosphorus was estimated by the C. Fiske and G. Subbarow [9] method.

Since it is extremely difficult to determine the dry weight and to express results as percentages of dry weight when cell nuclei and nuclear fractions are isolated from small amounts of material under investigation, the calculations of nucleic acid content of such material were made in percentages of the total nitrogen content multiplied by 6.25 ("total protein").

The results obtained (see Table) indicate that the DNA content of cell nuclei in normal mouse liver is on the average  $12.3 \pm 0.7\%$  of their total protein; this corresponds to data in the literature [5, 6, 8].

In the case of the cell nuclei isolated from mouse hepatoma the DNA content is equal to  $3.8 \pm 0.2\%$  of the total protein, i.e., it is one-third of that in the nuclei of normal liver; this can evidently be explained by the raised content of the residual protein fraction in the nuclei of the tumor cells.

**DNA and RNA Content of Cell Nuclei and Nuclear Fractions in Normal Mouse Liver and Transplanted Mouse Hepatoma**

No. of expt.	Nuclei			Nucleoprotein		Acid protein	
	In % of $N_{x6.25}$		RNA DNA	in % of total pro- tein in the nuclei		in % of total pro- tein in the nuclei	
	DNA	RNA		DNA	RNA	DNA	RNA
Mouse liver							
1	10.4	1.1	0.10	98.0	61.1	1.6	37.0
2	14.7	1.0	0.06	98.2	67.0	1.0	31.7
3	10.7	0.7	0.06	98.0	61.3	1.5	38.1
4	14.0	1.2	0.08	97.4	70.1	1.2	28.8
5	12.1	1.3	0.10	95.0	55.0	3.0	45.0
6	12.0	1.4	0.11	96.0	58.0	2.8	40.3
	Average 12.3±0.7	1.1±0.1		97.1±0.5	62.0±0.3	1.8±0.3	36.8±0.8
Mouse hepatoma							
7	4.1	0.6	0.14	95.0	51.3	2.5	46.1
8	3.2	0.5	0.15	95.7	53.0	4.3	47.0
9	4.0	0.9	0.2	91.2	52.3	4.1	45.3
10	4.6	0.8	0.17	90.0	58.0	4.4	42.0
11	3.6	0.6	0.17	94.3	55.0	4.0	43.3
12	3.8	0.4	0.10	98.2	54.3	1.7	45.7
	Average 3.8±0.2	0.6±0.06		93.0±1.3	53.9±0.9	3.5±0.4	44.9±0.8

The RNA content of cell nuclei in the liver approaches the literature data [6, 8] and is about 1% of their total protein. The amount of RNA in the cell nuclei of hepatoma is nearly half that of normal liver nuclei and is, on the average, 0.6% of their total protein. However, the ratio of RNA and DNA in hepatoma is nearly double that of normal liver nuclei.

The nucleoprotein fraction extracted by 1 M NaCl solution contains almost all (90-98.2%) the cell nuclei DNA, whether the nuclei are isolated from normal liver or transplanted mouse hepatoma. These results confirm the data reported by Mirsky and collaborators [11] who stated that almost all the DNA could be extracted from nuclei by means of 1 M NaCl solution.

Nuclear RNA is found in the nucleoprotein fraction and in acid protein, its amount in the nucleoprotein fraction apparently depending to a considerable extent on admixture of cytoplasm; thus, the RNA content in the nucleoprotein fraction of less thoroughly purified nuclei with early agglutination is considerably raised.

No nucleic acids are found in the residual protein.

The results obtained thus make it possible to characterize more precisely the nuclear fractions of normal liver and transplanted hepatoma from the point of view of their nucleic acid content. It is probable that the acid protein fraction can in some measure be regarded as a ribonucleoprotein fraction, while residual protein evidently does not form complexes with nucleic acids.

No significant differences are found in the distribution of nucleic acids in the nuclear fractions of normal and tumor nuclei; the RNA-DNA ratio, however, is higher in tumor nuclei than in the normal.

#### SUMMARY

It was shown that desoxyribonucleic acid (DNA) constituted 10-14% of dry substance of cellular nuclei in normal liver and about 3-4% of the nuclei of mice hepatoma. Practically all DNA (90-98%) is found in nucleo-

protein fraction in both cases. Ribonucleic acid (RNA) constitutes 0.4-1.4% of dry substance of the cellular nuclei in normal liver as well as in transplanted hepatoma in mice. However, the ratio of RNA to DNA in the nuclei of hepatoma is greater than normal. Nuclear RNA is found both in the nucleoprotein fraction and in acid protein. Its quantity evidently depends to a great extent on the admixture of cytoplasm. No nucleic acids are found in the residual protein.

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\* Russian translation.