PATTERN OF RNA PRODUCTION IN THE BODY OF THE NUCLEOLUS STUDIED BY ANALYSIS OF SERIAL ELECTRON-MICROSCOPIC AUTORADIOGRAPHS

E. Ya. Sanovich, A. A. Pal'tsyn,
V. P. Tumanov, and V. A. Zelenskii

UDC 612.822.1.015.36:547.963.32]-086.3

KEY WORDS: nucleolus; RNA synthesis; electron-microscopic autoradiography.

In the modern view the main function of the nucleolus is to form ribosomal RNA (rRNA) [5, 6, 8-10]. This product is formed by transcription of the corresponding genes [11] located in the body of the nucleolus [4, 7], and represented by a large number of copies. A problem of fundamental importance in cell biology is the organization of the work (transcription) of these genes in time, i.e., whether they all function simultaneously or in a certain order. The solution to this problem is important not only in connection with the character of function of the nucleolus, but also because it would yield valuable information on the work of genome in general, and also of the various organoids in the cell as a whole. The first experiments on serial electron-microscopic autoradiography, reflecting incorporation of the RNA precursor 5-^{3}H-uridine [1, 3], showed that the labeling density of two consecutive sections may differ substantially. This difference may reflect a real difference in the content of newly synthesized RNA in neighboring sections, but it may also be the result of errors of the method. The sources and limits of possible errors have been discussed in detail in the literature [2, 3]. In particular, one cause of the mistaken detection of a difference in labeling density may be unequal thickness of the sections or of the layer of emulsion above them; it has also been emphasized that if the difference in density of the grains of silver above the nucleolus were due to these causes, corresponding differences ought to be found in the density of the grains above the nucleoplasm of the sections. When serial autoradiographs are examined, usually no clear correlation is found between the densities of the grains of silver above the nucleolus and nucleoplasm.

This paper gives the results of quantitative analysis of this correlation.

EXPERIMENTAL METHOD

Serial sections of neurons in layer V of the cerebral cortex of albino rats weighing 180 g were studied. Under ether anesthesia the RNA precursor 5-^{3}H-uridine (specific activity 26 Ci/mmole) was injected into the animals with a special needle into the region of the sensorimotor cortex in area PA^m. The labeled uridine (50 μCi) was dissolved in 0.5 ml of Ringer's solution. Pieces of tissue 3 h after injection of 5-^{3}H-uridine were fixed in 2.5% glutaraldehyde solution made up in phosphate buffer, pH 7.4. In the course of the next 24 h the pieces of tissue were washed with buffer with frequent changes of solution and then postfixed in 1% OsO_4 solution. After dehydration in alcohols the pieces were embedded in Epon, light-microscopic autoradiographs were prepared from semithin sections, and on the basis of the results of their analysis the region for thin section cutting was chosen. Serial electron-micrographs were prepared with type M emulsion [2, 3]. After exposure for one month the preparations were developed and examined in the IEM-100B microscope.

It was not an aim of the investigation to cut all nucleoli of the neuron into serial sections. Only those sections which included the body of the nucleolus [the nucleolonema (in subsequent description called "the nucleolus")] and which had no significant technical defects were chosen for statistical analysis. The area of cross section (the product of the maximal and minimal diameters) of the nucleolus and of the extranucleolar zone of the nucleus (subsequently called the "nucleus") was measured on negatives. The labeling density in the nu-

Department of Pathological Anatomy and Laboratory of Cybernetics, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR M. I. Kuzin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 7, pp. 102-105, July, 1982. Original article submitted March 25, 1982.

 0007-4888/82/9407-0982$07.50

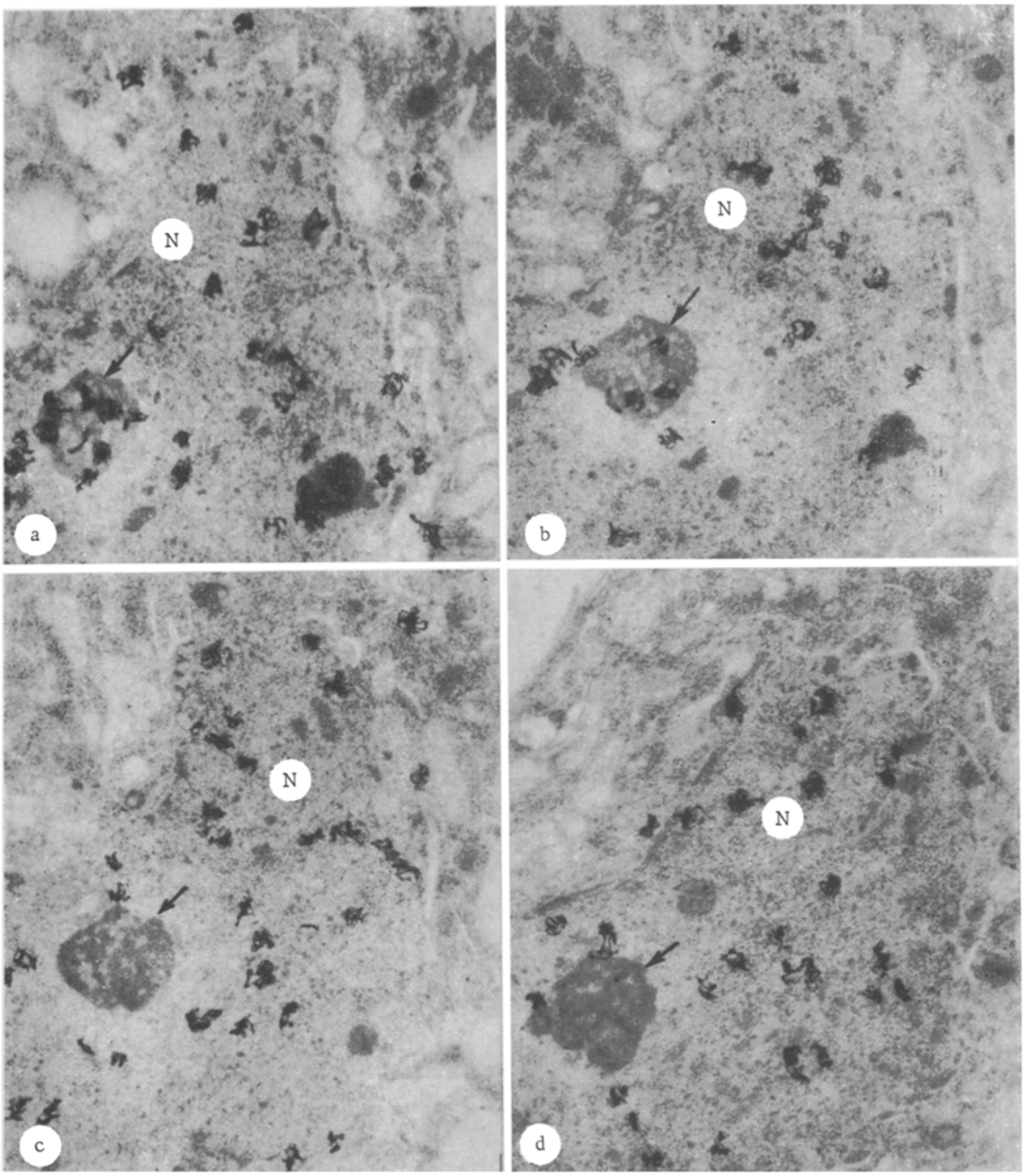

Fig. 1. Serial sections through rat cerebral cortical neuron. Distribution of grains of silver reveals small differences in concentration of labeled RNA in sections of nucleus (N) and a significant difference in content of labeled RNA in sections of the nucleolus (arrow). Magnification 22,000. a) 8 grains of silver above nucleolus; b) 5 grains of silver above nucleolus; c) no grains of silver above nucleolus; 2) 2 grains of silver above nucleolus.

cleolus and nucleus was determined in conventional units (the ratio between the number of grains of silver found above these zones and their area of cross sections). Altogether 374 serial sections were investigated in 50 neurons. The quantitative results of the experiments were processed by M-220 computer.

EXPERIMENTAL RESULTS

Analysis of serial sections through one neuron showed differences in the concentration of grains of silver between neighboring sections. Above the nucleus this difference was usually very small (Fig. la-d), and only rarely did neighboring sections differ sharply. The change in labeling density in the nucleolus was of a different character. In this case the difference between neighboring sections was very sharp, and often a dense accumulation of grains could be seen in some sections (Fig. la, b), whereas in sections next to them there

TABLE 1. Mean Density of Labeling in Nucleolus and Extranucleolar Zone of Nucleus in Serial Sections through Neurons in Layer V of Rat Cerebral Cortex (M ± m)

Serial	Volume of sample (number of sections studied in each neuron)	Mean density of labeling in extranucleolar zone of nucleus	Mean labeling density in nucleolus	Coefficient of correlation (r)	Significance of coefficient of correlation, %
1	7	250,350±28,651	1579,366±361,161	0,85	1
2	7	217,146±20,346	620,956±190,786	—0,22	35
3	8	39,948±10,609	76,191±25,705	0,16	30
4	10	52,554±0,431	138,596±43,619	0,51	7
5	11	62,335±13,753	192,785±39,831	0,77	0,5
6	9	88,319±7,125	294,409±50,728	0,27	20
7	8	75,980±20,571	325,423±74,466	0,60	6
8	11	77,395±17,181	414,234±99,897	0,53	5
9	6	30,395±3,757	156,085±82,902	—0,4	20
10	10	98,445±21,722	311,756±71,034	0,75	1
11	7	65,299±3,697	428,573±118,223	0,53	10
12	10	72,849±9,056	168,216±48,368	0,48	7
13	8	139,966±22,547	400,370±92,138	0,66	4
14	5	34,824±10,232	450,000±238,939	0,27	30
15	7	261,677±20,302	586,107±314,484	0,42	17
16	12	119,914±19,449	375,810±137,014	0,5	5
17	6	120,305±27,092	659,717±411,202	—0,11	50
18	9	89,598±7,106	512,071±140,616	0,52	9
19	10	34,314±7,220	374,060±92,801	0,90	0,05
20	12	32,247±7,603	272,845±46,334	0,28	30
21	8	129,081±71,788	223,425±43,129	0,41	15
22	6	68,807±13,406	297,620±143,353	0,52	15
23	9	32,667±4,578	130,363±37,498	0,35	20
24	11	93,675±7,458	291,559±74,741	0,06	50
25	5	47,488±5,178	225,696±69,878	—0,13	45
26	11	75,744±10,835	464,655±102,011	0,41	10
27	7	24,631±15,673	254,291±102,246	0,08	50
28	11	102,375±7,309	547,137±63,973	0,04	50
29	10	42,748±8,617	115,837±33,148	—0,01	50
30	5	99,368±10,838	405,556±141,531	—0,50	15
31	6	18,968±4,589	136,365±45,455	—0,27	30
32	6	100,198±12,018	311,197±51,077	0,07	50
33	6	96,912±5,972	263,760±40,231	0,70	6
34	8	163,115±12,031	970,180±140,671	0,67	3
35	5	149,732±34,325	587,810±222,408	0,01	50
36	7	120,650±13,750	658,594±69,666	0,09	40
37	8	176,913±18,597	1455,263±254,006	0,57	8
38	6	74,265±6,916	885,417±143,584	0,97	0,1
39	5	96,378±4,378	1030,000±128,062	0,37	25
40	5	127,460±19,927	1489,650±146,805	—0,27	35
41	5	83,334±11,651	1071,814±82,470	0,50	15
42	9	119,274±9,666	952,827±72,429	0,33	20
43	5	77,596±5,226	553,870±127,018	—0,05	40
44	6	108,142±27,206	956,445±116,637	—0,57	12
45	5	221,754±22,133	1382,654±218,317	0,34	25
46	6	442,182±42,348	1378,597±247,769	0,48	20
47	5	239,552±22,839	948,148±206,612	0,17	30
48	6	110,405±14,680	950,948±158,193	0,63	8
49	9	34,217±4,316	189,830±42,294	0,26	25
50	6	106,402±28,699	777,777±220,362	0,91	0,01

were only one or two grains or none whatever (Fig. 1c, d). Preliminary analysis of the preparations thus suggests that it is unlikely that the difference in the content of label in neighboring sections of the same nucleolus is due to an error of the autoradiographic technique, namely that the labeling density depends on the thickness of the section and of the layer of the emulsion above it. If this were so, there ought to have been a corresponding difference in the concentration of grains of silver above the nucleus also, for the thickness of the layer of the nucleolus and nucleus in the same section is identical. A difference in the thickness of the layer of emulsion above the nucleolus and nucleus of the same section is also extremely unlikely. For verification a quantitative correlation analysis was made of the labeling density in the nucleolus and nucleus, with calculation of the coefficient of correlation for each separate neuron. The following assumption was made: If variations of labeling density in sections through the same nucleolus are due to an error of the method, i.e., to a difference in the thickness of the sections of layer of emulsion, correlation should be observed between changes in labeling density in the nucleolus and those in the nucleus of the same cell.

The results of analysis are given in Table 1. Of 50 neurons tested, changes in labeling density in the nucleolus and nucleus correlated in only 11 cells (22%), and in the remaining

39 (78%) there was no correlation between these values. Statistical analysis thus showed that the content of newly synthesized RNA in the nucleolus is not uniform. This nonuniformity is most probably not attributable to an error of the method but must exist objectively. Differences in the content of newly synthesized RNA in different zones of the nucleolus can be due to the following causes: 1) a nonhomogeneous distribution of rRNA genes in the mass of the nucleolus; 2) the existence of definite sites in the nucleolus for accumulation of newly synthesized RNA; 3) an alternating pattern of work of the rRNA genes.

Further investigations are necessary to determine the real worth of these hypotheses.

LITERATURE CITED

1. A. A. Pal'tsyn, E. Ya. Sanovich, and V. P. Tumanov, Byull. Eksp. Biol. Med., No. 3, 364 (1977).
2. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, Adaptive Reorganization of Biorhythms [in Russian], Moscow (1975).
3. D. S. Sarkisov, A. A.Pal'tsyn, and B. V. Vtyurin, Electron-Microscopic Autoradiography of the Cell [in Russian], Moscow (1980).
4. W. Bernhard and N. Granboulan, in: Ultrastructure in Biological Systems, Vol. 3, New York (1968), pp. 81-149.
5. D. Brown and J. Gurdon, Proc. Natl. Acad. Sci. USA, 51, 139 (1964).
6. J. E. Edstrom, J. Biophys. Biochem. Cytol., 8, 47 (1960).
7. N. Granboulan and P. Granboulan, Exp. Cell Res., 34, 71 (1964).
8. R. P. Perry, Exp. Cell Res., 29, 400 (1963).
9. R. P. Perry, Natl. Cancer Inst. Monogr., 14, 73 (1964).
10. R. P. Perry, Natl. Cancer Inst. Monogr., 23, 527 (1966).
11. V. M. Ritossa and S. Spiegelman, Proc. Natl. Acad. Sci. USA, 53, 737 (1965).

DETACHMENT OF THE RETINA IN EXPERIMENTAL HEMOPHTHALMIA

E. G. Rapis

UDC 617.747-003.215-06:617.735-002.281

KEY WORDS: intravitreal hematoma; collapse of the vitreous body; early detachment of the retina.

Irreversibility of pathological changes in the retina in hemophthalmia is often attributable to its total detachment [4, 9, 12]. It has recently been shown by ultrasonic B scanning that clinically observed detachment of the retina may show particular structural features incompatible with the recovery of visual functions [9]. However, the causes of these detachments have not been sufficiently studied. Information in the literature do not indicate conclusively whether it is blood or the trauma accompanying hemophthalmia that leads to the development of detachment of the retina [2, 8, 11]. Until recently, when the causes of detachment of the retina in hemophthalmia were analyzed, the role of intravitreal hematoma was ignored, although my own previous investigations showed that it is invariably present should hemorrhage into the vitreous body (VB) arise because of rupture of a blood vessel [5].

It was accordingly decided to make a special study of the state of the retina in experimental intravitreal hematoma.

EXPERIMENTAL METHOD

Experiments were carried out on 52 albino and chinchilla rabbits of both sexes weighing 1.5-2.5 kg (97 eyes). Under local anesthesia (1% procaine solution, subconjunctival or retrobulbar injection) a model of experimental hemophthalmia was produced by injecting different quantities of autologous blood (from 0.1 to 1.2 ml) taken from an auricular vein, into VB by means of a syringe and needle. The sclera was punctured in the flat part of the cil-

Turkmenian Research Institute of Eye Diseases, Ashkhabad.

(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 7, pp. 105-109, July, 1982. Original article submitted March 22, 1982.

0007-4888/82/9407-0985$07.50