LITERATURE CITED

1. G. V. Elyakova, Dokl. Akad. Nauk SSSR, 202, No. 5, 1196 (1973).
2. G. V. Elyakova, Dokl. Akad. Nauk SSSR, 215, No. 3, 709 (1974).
3. K. S. Mitin, S. M. Sekamova, and N. A. Sokolina, Arkh. Anat., No. 2, 13 (1973).
4. D. B. Allbrook, J. Anat. (London), 96, 137 (1962).
5. A. Hess and S. Rosner, Am. J. Anat., 129, 21 (1970).
6. A. Mauro, J. Biophys. Biochem. Cytol., 9, 493 (1961).
7. A. R. Muir, A. N. Kanji, and D. B. Allbrook, J. Anat. (London), 99, 435 (1965).
8. M. Reznik, J. Cell. Biol., 39, 110 (1968).
9. M. Reznik, Lab. Invest., 20, 353 (1969).
10. S. M. Shafig and M. Gorycki, J. Path. Bact., 90, 123 (1965).
11. H. Teräväinen, Z. Zellforsch., 103, 320 (1970).

RNA SYNTHESIS IN RING-LIKE NUCLEOLI OF HEPATOCYTES

A. A. Pal'tsyn, A. K. Badikova, and K. V. Botsmanov

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Analysis of serial sections showed that the so-called ring-like nucleoli of hepatocytes consist of cavities with amorphous contents surrounded by fibrillary and granular material. These nucleoli are found only occasionally in normal animals; the number of ring-like nucleoli rises considerably in the chronic pathological process caused by repeated injections of CCl_4. Electron-microscopic autoradiography showed that the ring-like nucleoli can synthesize RNA.

KEY WORDS: *Nucleolus; RNA synthesis; CCl_4; electron-microscopic autoradiography.*

Ring-like nucleoli are constantly found in some normal cells: smooth-muscle and endothelial cells, cells of the sebaceous glands, oocytes, lymphocytes, and plasma cells. They are also found in the cells of some tumors (Ehrlich's ascites carcinoma cells and leukemic lymphoblasts) and in hepatocytes in cases of hepatitis or after administration of inhibitors of RNA synthesis [3, 5, 12]. Many workers consider that cells in which ring-like nucleoli are found are in the final stages of differentiation or in a state of degeneration and that they are characterized by a decrease in RNA synthesis. On this basis they regard a ring-like structure as the morphological expression of low functional activity of the nucleolus and they class such nucleoli among structures virtually not synthesizing RNA [3, 5, 7-10, 12, 12]. However, it must be remembered that an increase in the number of ring-like nucleoli during the action of inhibitors or cell differentiation can be only an indirect indication of probable inhibition of RNA synthesis in just those and not all nucleoli. The direct study of this problem has been undertaken only by light-microscopic autoradiography [10, 13], the resolving power of which is too low, and also with the use of lymphocytes in which the overwhelming majority of nucleoli, irrespective of their structure, are not labeled, as the test object.

In this investigation, in order to resolve the problem of RNA synthesis in ring-like nucleoli, the more accurate method of electron-autoradiography was used to investigate RNA synthesis in hepatocyte nucleoli in the course of a chronic pathological process.

Department of Pathological Anatomy, 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 A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 11, pp. 1388-1390, November, 1976. Original article submitted May 3, 1976.

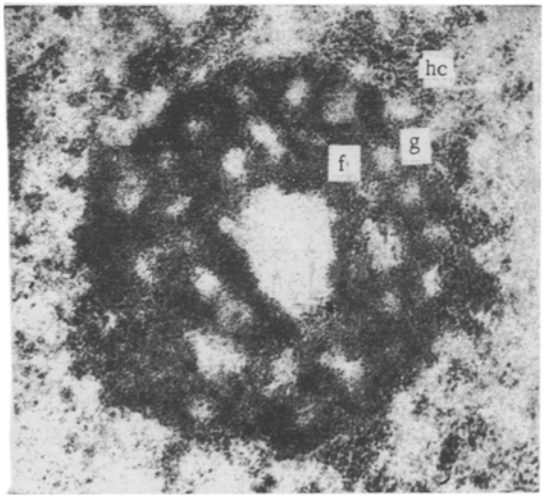

Fig. 1

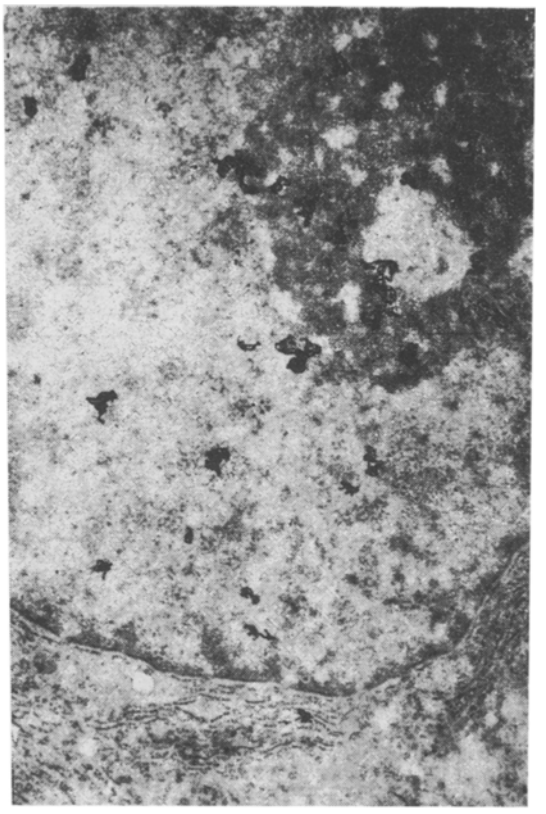

Fig. 2

Fig. 1. Ring-like nucleolus of hepatocyte of mouse receiving weekly injections of CCl_4 for 3.5 months. Central cavity and spaces in reticulum of nucleolonema contain material of low electron density. Ring-shaped body of nucleolus surrounding central cavity consists of numerous alternating areas of granular (g) and fibrillary (f) structure. Externally, ring is in contact with zone of heterochromatin (hc); 24,000×.

Fig. 2. Electron-microscopic autoradiograph of ring-like nucleolus in hepatocyte of mouse receiving weekly injections of CCl_4 for 6.5 months. Many silver grains above body of nucleolus, indicating intensive RNA synthesis in it; 20,000×.

EXPERIMENTAL METHOD

Once a week 0.2 ml of a 40% solution of CCl_4 in peach oil was injected subcutaneously into noninbred mice. On the second day after each injection the liver of two experimental animals was fixed in neutral formalin and Carnoy's mixture. Paraffin sections were stained with hematoxylin–eosin and methyl green–pyronine. In 100 randomly chosen hepatocytes the number of ring-like nucleoli was counted. These determinations were made on the animals of the experimental group in the course of 1 year and on 16 control mice aged 1-7 months. RNA synthesis in the hepatocytes was studied by electron-microscopic autoradiography 1.5, 3.5, 5.5, 6.5, 8, 10, 12.5, and 14 months after the first injection of CCl_4. For this purpose, uridine-5-^{3}H in a dose of 100 μCi/g was injected into three or four animals at each time. The liver tissue was fixed 4 h after injection of uridine with 2.5% glutaraldehyde and 1% OsO_4 and embedded in Epon. A monolayer of M emulsion was applied to the sections. The preparations were developed (D-19 developer) after exposure for 30-120 days and examined in the IEM-100B microscope.

EXPERIMENTAL RESULTS

Inspection of paraffin sections showed that ring-like nucleoli are also present in normal hepatocytes. However, their number under normal conditions is small: No such nuclei were found in half of the control animals, and only 14 ring-like nucleoli were found in 16 mice. The number of ring-like nucleoli in the experimental animals was appreciably greater than in the controls: six nucleoli per 100 hepatocytes on average.

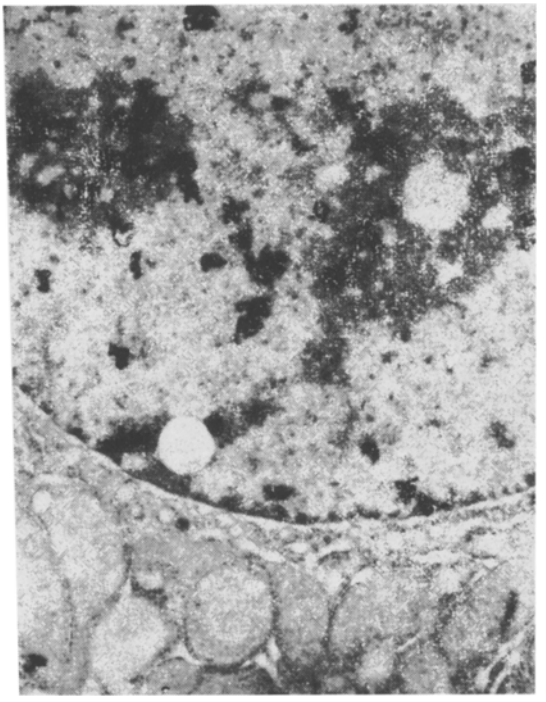

Fig. 3. Electron-microscopic autoradiograph of hepatocyte of mouse receiving weekly injections of CCl_4 for 8 months. Grains of silver located above ring-like as well as normal nucleolus. Difference between them as regards number of grains not significant; 17,000×.

The solid configuration of the ring-like nucleoli was determined in serial sections. All nucleoli studied had a cavity in their central part, and the reason for their ring-like appearance in electron-microscopic and histological sections was due to the fact that the section passed through the central part of a sphere, cutting off the upper and lower "domes." The contents of the central cavity were amorphous and were indistinguishable from the contents of the spaces within the reticulum of the nucleolonema (Fig. 1). Most probably the cavity is formed by fusion of these spaces. Administration of some antimetabolites is known to cause the characteristic change in the structure of the nucleolus known as segregation [11]. In the normal nucleolus its components — an amorphous substance, RNP granules, and RNP fibrils — interpenetrate and become as it were intermingled. During segregation they separate and the nucleolus becomes clearly divided into fibrillary, granular, and amorphous zones [1, 2]. Inhibitors of RNA synthesis have a particularly strong segregating action, and segregation can accordingly be regarded as a morphological feature of the inhibition of RNA synthesis in the nucleolus [6]. Accordingly a ring-like structure is regarded as a special type of segregation which affects mainly the amorphous material of the nucleolus and proceeds by the unification of many amorphous areas into a single wide amorphous zone. No appreciable separation of the fibrillary and granular components of the ring-like nucleolus could be observed (Fig. 1).

Analysis of the electron-microscopic autoradiographs showed that the opinion of Smetana et al. [3, 4, 7-10, 12, 13] on the blocking of RNA synthesis in ring-like nucleoli does not correspond to the true state of affairs as regards hepatocytes. RNA synthesis in the ring-like nucleolus can take place very intensively (Fig. 2) and is not significantly different from synthesis in the normal nucleolus (Fig. 3). Electron-autoradiographic investigations showed that RNA is synthesized in the fibrillary component of the nucleolus and is then transported into the granular component [4]. The normal structure of these components and the high level of RNA synthesis in ring-like nucleoli observed in the present experiments are consistent with the view that a ring-like structure is a special type of segregation of the amorphous component, without any appreciable inhibition of nucleolar function.

In some cells, such as lymphocytes, cavity formation in the nucleoli in fact reflects the final phase of differentiation, with repression of most or even all of the genes and with the cessation of RNA synthesis in the nucleolus. In hepatocytes, on the other hand, as the results now described show, the functional significance of the ring-like structure of the nucleoli is still unexplained. Very probably cavity formation in the nucleoli takes place gradually, and the preservation of a high level of RNA synthesis in ring-like nucleoli which the present writers observed applies only to the early phase of the process. If future investigations show that at some later phase the ring-like nucleolus becomes functionally imperfect, whereas the function of the remaining nucleoli of the cell is preserved, this will mean that the life span of the nucleolus may be shorter than that of the cell. Such observations would be of great importance in the study of cellular adaptation to the action of the environment.

LITERATURE CITED

1. Yu. S. Chentsov and V. Yu. Polyakov, Ultrastructure of the Cell Nucleus [in Russian], Moscow (1974).
2. W. Bernhard, Nat. Cancer Inst. Monogr., 23, 13 (1966).
3. H. Busch and K. Smetana, The Nucleolus, Academic Press, Washington, D. C. (1970).
4. N. Granboulan and P. Granboulan, Exp. Cell Res., 38, 604 (1965).
5. J. Karasek, E. Hrdlicka, and K. Smetana, J. Ultrastruct. Res., 42, 234 (1973).
6. R. Simard, Int. Rev. Cytol., 28, 169 (1970).
7. K. Smetana, E. Freireich, and H. Busch, Exp. Cell Res., 52, 112 (1968).
8. K. Smetana, F. Gyorkey, P. Gyorkey, et al., Exp. Cell Res., 60, 175 (1970).
9. K. Smetana and M. Potmesil, Z. Zellforsch., 92, 62 (1968).
10. K. Smetana and M. Potmesil, Folia Haemat. (Leipzig), 93, 16 (1970).
11. D. Svoboda and J. Higginson, Cancer Res., 28, 1703 (1968).
12. A. Rivenzon and L. Nebel, Experientia (Basel), 28, 1472 (1972).
13. K. Terao, Y. Sakakibara, and M. Yamazaki, Exp. Cell Res., 66, 81 (1971).