The hypothesis that a causal connection exists between disturbance of energy formation in MC and the transition of shock into an irreversible phase has not yet been proved [11]. Activation of oxidative phosphorylation in MC observed in the irreversible phase of shock and maintenance of the initial rate of ATP synthesis until the animals were in an agonal state are evidence that damage to MC is not itself an essential factor causing the development of irreversibility in shock.

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PROTEOLYSIS OF NUCLEAR MATRIX PROTEINS OF THE RAT LIVER AND ZAJDELA'S ASCITES HEPATOMA

N. Vokurkova

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Much attention is currently being paid to the study of the nuclear protein matrix. In particular, comparison of the protein profile of the nuclear matrix (NM) of normal and tumor tissue is of great interest. Studies of the protein profile of NM by electrophoresis in polyacrylamide gel containing sodium laurylsulfate has shown that strong macromolecular bands are present in the 100-200 kilodaltons region of hepatoma-27 and Zajdela's ascites hepatoma, which are absent or are weak in normal liver [1, 2, 8]. Some workers consider that differences in the electrophoretic profile of NM proteins of normal liver and hepatoma are due to unequal activity of nuclear proteinases [6, 7]. These workers state that proteinase activity in liver nuclei is higher than in tumors, and that because of this, macromolecular bands are weak on electrophoresis of liver NM. The use of proteinase inhibitors, such as phenylmethylsulfonyl fluoride (PMSF) and sodium tetrathionate, during isolation of nuclei and NM, according to their data, leads to a sharp increase in the yield of NM proteins and, what is very important, to disappearance of virtually all differences between hepatoma and normal liver [7]. However, the use of sodium tetrathionate was shown to cause the formation of disulfide bonds, as a result of which some nuclear proteins may be firmly bound with skeletal structures of the nucleus [11].

Data have been published on the action of chromatin-bound proteinases [3, 5, 9, 10, 12-14]. These proteinases act mainly on histones. Most of them belong to the class of serine proteinases, and they are very active at a high ionic strength (2 M NaCl). Proteolysis of histones was not observed at acid pH values and in the presence of EDTA, but this process was observed during prolonged incubation of chromatin (about 20 h).

Laboratory of Biochemistry, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow.

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TABLE 1. Yield of NM (in Percent) from Liver and Zajdela's Ascites Hepatoma after Incubation of Nuclei for 20 h

Conditions of incubation	Liver	Zajdela's hepatoma									
		No. of experiments									
		1	2	3	4	5	6	7	8	9	10
Control	9,5±1,9 (n=4)	2,4	2,2	2,3	6,9	9,6	—	—	—	—	8,0
Buffer A:											
20 °C	8,6±1,4 (n=3)	3,9	—	7,0	—	—	—	—	—	4,6	13,4
4 °C	5,9±0,6 (n=3)	3,1	—	5,1	—	—	—	—	—	—	6,2
2 M NaCl:											
20 °C	3,0±0,3 (n=5)	—	1,1	—	5,0	5,6	5,9	—	—	—	4,9
4 °C	1,8±0,3 (n=3)	—	1,1	—	—	—	—	1,3	2,7	—	—

Legend. n) Number of experiments.

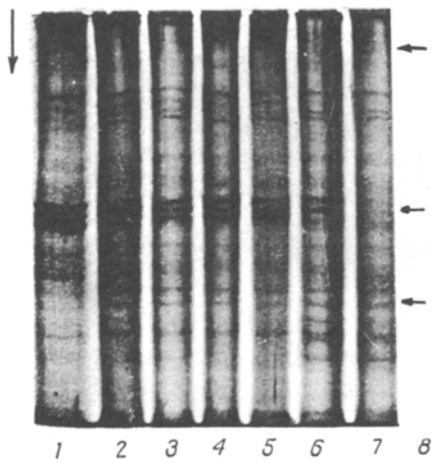

Fig. 1

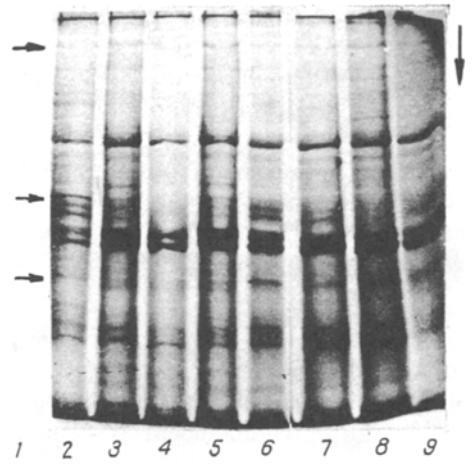

Fig. 2

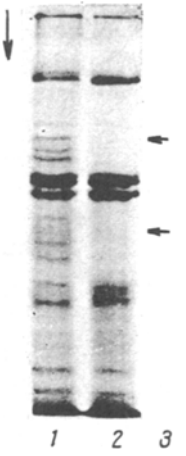

Fig. 3

Fig. 1. Electrophoresis of proteins of rat liver NM obtained after incubation of nuclei for 20 h. 1) Control; 2, 3) incubation in buffer at 20°C and 4°C respectively; 4, 5) incubation with 2 M NaCl respectively at 20 and 4°C; 6) incubation of control in buffer A: 2 h, 37°C; 7) incubation of control in buffer A: 15 h, 20°C; 8) reference substances: ferritin (220 kilodaltons, serum albumin (68 kilodaltons), egg albumin (43 kilodaltons).

Fig. 2. Electrophoresis of proteins of Zajdela's hepatoma NM obtained after incubation of nuclei for 20 h. 1) reference substances: 220, 68, and 43 kilodaltons; 2, 6) control preparations; 3, 4) incubation with 2 M NaCl respectively respectively; 5) incubation with 2 M NaCl: 20°C, 0.2 mM PMSF; 7, 8) incubation in buffer A at 4 and 20°C respectively; 9) incubation of control in buffer A: 15 h, 20°C.

Fig. 3. Electrophoresis of proteins of Zajdela's hepatoma NM. Preparation obtained after incubation of nuclei for 20 h with 2 M NaCl at 4°C; 1) in presence of 1 mM PMSF, 2) without PMSF; 3) reference substances: 68 and 43 kilodaltons.

Since a very long method of obtaining NM is most frequently used [6], and considering data on nuclear proteinases and the fact that in the writer's previous experiments involving the use of certain proteinase inhibitors, proteolysis of NM proteins was not observed, in the present investigation the aim was to study how prolonged incubation of the nuclei affects the protein profile of NM.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 150-200 g were used. Preparations of nuclei and NM were obtained from normal liver and from Zajdela's ascites hepatoma (5th day after transplantation) by methods described previously [2, 4]. NM were obtained by successive treatment of isolated nuclei, first with Triton X-100 (20 min, 4°C), then with nucleases (60 min, 20°C, 2 M NaCl (10 min, 4°C), and again with nucleases (30 min). Electrophoresis of NM proteins was carried out in 7.5% polyacrylamide gel containing sodium laurylsufate [2].

Control NM preparations were obtained by the standard method. Experimental samples were subjected to prolonged incubation (20 h) under different conditions. The isolated nuclei were treated first with 0.5% Triton X 100, made up in buffer A (0.2 M sucrose, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 20 mM Tris HCl, pH 7.4).

The nuclei were then carefully washed free from Triton X 100. The nuclei were treated with Triton X100 before incubation in order to remove any possible cytoplasmic impurities from the final preparations. This treatment removes nuclear membranes and proteins of the nuclear sap; the possibility cannot be ruled out that certain proteinases may also be washed out during this procedure. The nuclei were then incubated for 20 h in buffer A or with 2M NaCl, made up in the same buffer. Incubation was carried out at room temperature (20°C) and in the cold (4°C). In some cases PMSF, an inhibitor of serine proteinases, was added. NM from nuclei incubated in buffer A were obtained by consecutive extraction with nucleases, 2M NaCl, and nucleases once again. NM from nuclei incubated with 2M NaCl, were treated with nucleases only.

EXPERIMENTAL RESULTS

Electrophoresis of rat liver NM proteins showed (Fig. 1) that prolonged incubation of the nuclei has no appreciable effect on the electrophoretic profile of the proteins and that three bands of polypeptides in the 60-70 kilodaltons region, often called "triplet proteins" or "laminae," remained a characteristic feature of NM. Although no particular difference was demonstrated by electrophoresis, to judge from the data in Table 1, NM proteins nevertheless are exposed to the action of proteolytic enzymes, especially in solution of high ionic strength. On this basis, since the quantitative yield of NM was reduced under these circumstances, it can be postulated that all liver NM proteins are exposed more or less equally to proteolytic degradation. However, incubation of the NM preparation which was obtained under standard conditions has the result that the characteristic proteins of the triplet (lamina) become indistinct and cannot be distinguished among the remaining bands. Bands in the 20-30 kilodaltons region become more distinct. This result indicates that proteolytic enzymes are present in NM.

Somewhat different results were obtained with material from Zajdela's ascites hepatoma. Electrophoresis of proteins from Zajdela's hepatoma NM showed (Fig. 2) that during incubation of the nuclei, especially in the presence of 2M NaCl, considerable degradation of NM proteins took place. Incidentally the action of proteinases was particularly marked against laminae which disappeared virtually completely on incubation under conditions of high ionic strength. There was a sharp increase in the intensity of bands in the region of about 50 and 54 kilodaltons and a small increase in the intensity of bands in the low-molecular-weight region. Probably polypeptide bands in the 50-54 kilodaltons region are breakdown products of laminae. Possibly in the rapidly growing hepatoma specific breakdown of laminae plays some sort of regulating role. Incubation of the control NM preparations of Zajdela's hepatoma, just as in the case of liver, led to breakdown of the matrix proteins. PMSF, an inhibitor of serine proteinases, in high concentrations partially inhibits proteolytic activity (Figs. 2 and 3). It must also be noted that preparations of hepatoma NM (5th day after transplantation) obtained under standard conditions did not always give the same electrophoretic profile. They differed from one another mainly in the 50-70 kilodaltons region (Fig. 2). These differences are possibly the result of metabolism of the rapidly growing tumor cells and the precise time of transplantation and of sacrifice of the animals is relevant. Data in Table 1 show the yield of NM from Zajdela's hepatoma (because of the wide scatter, the results were not subjected to statistical analysis).

It will be clear from Table 1 that the yield of NM after incubation of the nuclei at room temperature was higher than after incubation at 4°C (this is particularly obvious in preparations from the liver). This effect is difficult to explain at present. Perhaps some form of stabilization of proteins takes place at a higher temperature. The preliminary data show that after incubation of liver nuclei for 1 h at 37°C the yield of NM rises sharply. This phenomenon is now being studied.

Data obtained by the writer in this investigation and previously are evidence that absence of high molecular weight polypeptides characteristic of tumor tissues in NM from liver is not the result of higher proteolytic activity in the liver nuclei, since the electrophoretic pattern was virtually unchanged, although after prolonged incubation of the nuclei the yield of NM is reduced. The protein profile of liver NM differs from that of Zajdela's hepatoma even after prolonged incubation. Mainly triplet proteins undergo proteolytic degradation in the rapidly growing Zajdela's tumor.

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SPECIFICITY OF LIPOSOME UPTAKE FROM LIPIDS OF TARGET CELLS

O. A. Rozenberg, V. Yu. Bekreneva,
L. V. Loshakova, S. P. Rezvaya,
E. F. Davidenkova, and K. P. Khanson

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Liposomes have been intensively studied in recent years as a new therapeutic form and a tool for biological research [11]. The most hopeful prospects are offered by the use of liposomes for directional transport of drugs [6]. The wide possibilities for choice of size, structure, and membrane composition of the vesicles provide ample scope for the search for optimal solutions to this complex problem.

One of the most important tasks in directional transport of liposomes is making their uptake by nonphagocytic cells more effective. This can be done in various ways, using affinity pairs on the contacting membranes as the model: antigen–antibody [7], hormone–receptor [4], lectin–glycolipid [13]. Manipulations of this kind are aimed primarily at making more effective contact between liposomes and cells. However, a simple increase in the binding of vesicles with the cell surface is insufficient to ensure intracellular placing of the drugs. This requires taking account of the mechanism of interaction between liposomes and the cell. Among these mechanisms, some of the most important are stable adsorption, diffusion of the internal contents of the adsorbed liposomes, endocytosis, and fusion of the contacting membranes [3].

The use of a fusion mechanism is the most promising method for cells with weak endocytosis.

One possible way of making more effective contact and subsequent fusion of liposomes with target cells may be by ensuring similarity of structural characteristics of the contacting membranes [5]. Two arguments at least can be advanced in support of this view: first, general ideas on participation of three-dimensional structures of carbohydrate components of cell membrane glycolipids in processes of intercellular recognition and adhesion; second, information showing that similarity in the phase state of the lipid bilayer of vesicles and cells facilitates fusion [10]. From our point of view, these demands are satisfied most completely by a mixture of membrane lipids of target cells.

To verify the effectiveness of this approach the writers compared uptake by target cells of liposomes made from lipids of the same cells and from lipids of other cells. Cells of ascites lymphatic leukemia NKLy/LL cells and Ehrlich's ascites carcinoma cells (EAC) were studied as models. Uptake of liposomes from homologous lipids also was compared with incorporation of vesicles from egg phosphatidylcholine (PC).

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