ATPase AND 5'-NUCLEOTIDASE ACTIVITY OF RAT LIVER NUCLEAR MATRIX

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Successive extraction of isolated cell nuclei with 0.14 MNaCl solution, 1-2 MNaCl, and dilute alkali (0.01-0.5 MNaOH) has been used to isolate skeletal structures of the cell nucleus, including nucleolar material, the intranuclear fibrillary network, and nuclear membrane, described as the "acid protein" and "residual protein" of the cell nucleus [2, 3, 13]. Similar preparations were later described in various laboratories under different names, and by the use of nonpolar detergents and nucleases the protein skeleton, usually described as the nuclear matrix, could be purified [4]. Besides its supporting or skeletal function, the nuclear matrix (NM) can play an important role in basic metabolic processes taking place in the nucleus. This is shown, in particular, by the discovery of newly replicated DNA bound with NM [5]. Activity of certain phosphohydrolases was discovered previously [9] in the

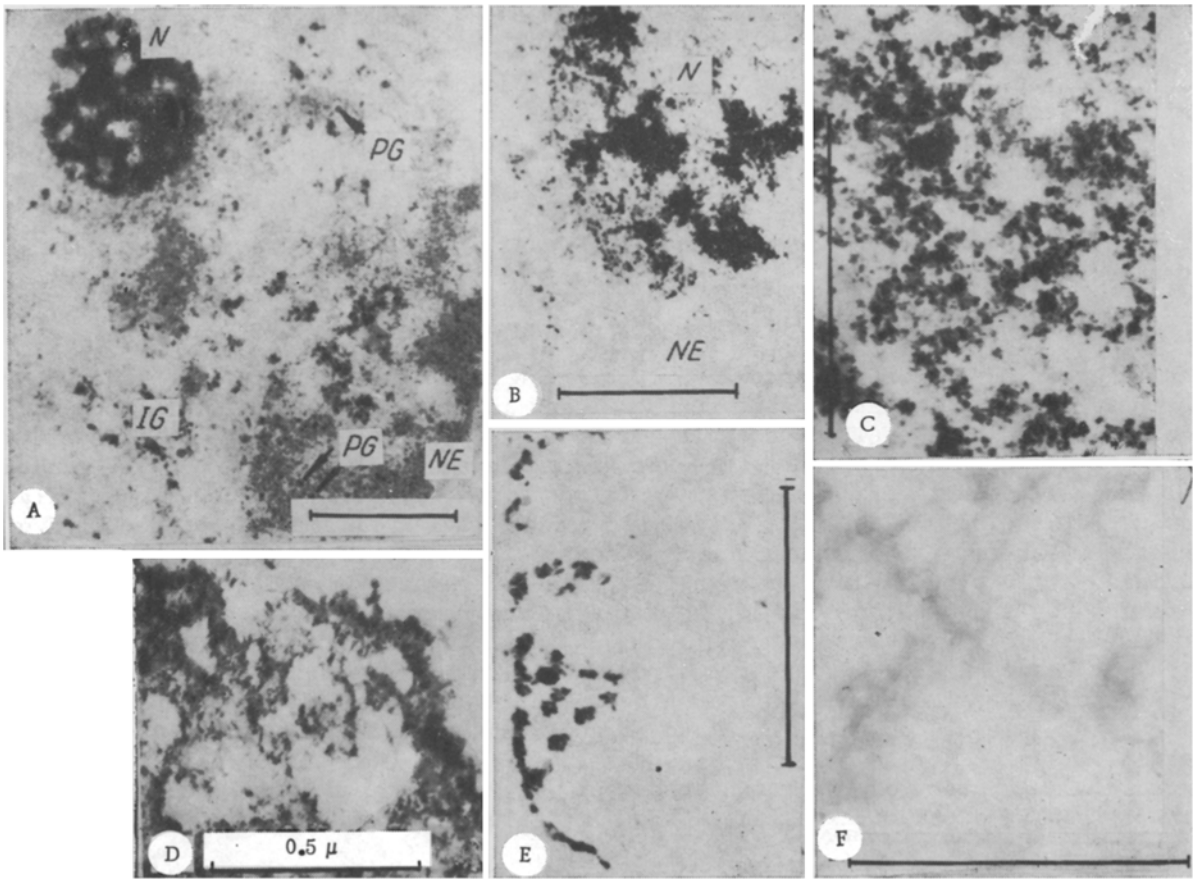

Fig. 1. ATPase and 5'-nucleotidase activity in whole nuclei and isolated NM. Explanation in text. N) Nucleolus; NE) nuclear envelope; IG) interchromatin granules; PG) perichromatin granules.

KEY WORDS: ATPase, 5'-nucleotidase; nuclear matrix; electron histochemistry.

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TABLE 1. ATPase and 5'-Nucleotidase Activity in Isolated Nuclei and NM of Rat Liver Cells (M ± m)

Enzyme	Nuclei	NM
ATPase	42,9±10,0	11,2±0,66
5'-Nucleotidase	13,2±3,3	33,3±6,6

Legend. Incubation for 30 min at 37°C; activity expressed in nanomoles inorganic phosphorus/min/mg protein.

TABLE 2. Action of Inhibitors on 5'-Nucleotidase Activity in Rat Liver NM

Experimental conditions	Nuclei	NM
Control	13,2	33,3
$ZnSO_4$	0 (100)	0 (100)
Uridine	9,1 (31)	26,2 (21,4)

Legend. Percentage inhibition of enzyme activity shown in parentheses; $ZnSO_4 \cdot 7H_2O$ used in final concentration of 100 mM, uridine in final concentration of 50 mM.

protein components of the DNP- and RNP-structures of the nucleus.

This paper describes the detection of ATPase and 5'-nucleotidase activity in isolated NM of rat liver; the specific 5'-nucleotidase activity was three times higher than in isolated nuclei, but ATPase activity only one-third as high.

EXPERIMENTAL METHOD

Isolation of nuclei from rat liver in concentrated sucrose, the conduct of the electron-histochemical reaction for ATPase and 5'-nucleotidase activity by a modified Gomori's lead method, and biochemical estimation of activity of these enzymes were described previously [9]. NM was isolated by Wunderlich's method, but the concentration of Triton X-100 was increased to 0.5% [1, 10].

Ultrathin sections were cut on the LKB III (Sweden) Ultratome and examined in the IEM-100C (Japan) electron microscope.

EXPERIMENTAL RESULTS

It can be concluded from a comparison of the results of electron-histochemical study of 5'-nucleotidase activity in rat hepatocyte nuclei and in isolated nuclei (Fig. 1A, B), with previous observations on the ATPase activity of hepatocyte nuclei [9] that the 5'-nucleotidase activity in all RNP- and DNP-structures of the nucleus and also in the nuclear membrane was weaker than ATPase activity. This was confirmed by the results of biochemical analysis, which showed that the 5'-nucleotidase activity of whole nuclei was less than one-third of the ATPase activity (Table 1). So far as the isolated NM is concerned, the ratio was different: 5'-Nucleotidase activity was three times higher than ATPase activity (Fig. 1C). Comparison of these data shows that most of the ATPase activity was removed from the nuclei during isolation of NM (Fig. 1D), whereas the 5'-nucleotidase activity was evidently bound much more firmly with NM. Hence it follows that the traditional idea of 5'-nucleotidase as an enzyme characteristic of plasma membranes only must be rejected, more especially because sufficient histochemical evidence has now been obtained to show that 5'-nucleotidase activity is present in the nuclei of various cells — lymphocytes and thymocytes [9, 11], smooth-muscle cells of the rat aorta [12], neurons of the rat hippocampus [7], and human hepatocellular carcinoma cells. There is also biochemical evidence that 5'-nucleotidase activity is present in the cell nucleus [14, 15].

The specificity of the methods of biochemical and histochemical determination of 5'-nucleotidase activity in the isolated nuclei and NM used in the present experiments was demonstrated both by the sensitivity of enzyme activity to specific inhibitors of 5'-nucleotidase (Table 2) and also by the different distribution of the product of the histochemical reaction with paranitrophenyl phosphate as substrate instead of AMP (Fig. 1E), and also by absence of reaction with sodium β-glycerophosphate as substrate (Fig. 1F). These histochemical data are in agreement with those of Borgers and Thone [8], who found a high intensity of enzymic hydrolysis of phenyl phosphate in the perinuclear space of liver cells at pH 7.0.

Morphological analysis showed that the NM preparations contained fibrillary and granular structures and also remnants of the nuclear membrane and nucleoli, and they thus corresponded to the preparations described previously [1, 4, 5, 10].

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EFFECT OF X RAYS ON DNA CONTENT AND SIZE OF CELL NUCLEI IN REGENERATING RAT LIVER

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Besides other changes, ionizing radiation also causes inhibition of DNA synthesis and mitosis in the liver of intact animals, to a degree which depends on the dose and the age of the animals [12, 13]. These changes are expressed more clearly in the regenerating liver [6, 8, 10]. Data of most investigations are concerned mainly with changes found in irradiated animals during 2 or 3 days or regeneration.

This paper describes the results of a study of the DNA content in the nuclei and measurement of the size of the nuclei in regenerating liver cells of rats irradiated for a period of 21 days after partial hepatectomy (PH), i.e., throughout the period of regeneration.

EXPERIMENTAL METHOD

Two series of experiments were carried out on 130 adult male Wistar albino rats weighing 200-220 g. In series I the rats were irradiated in a dose of 154.8 mCi/kg body weight, i.e., 600 R (on the TUP-T-250 x-ray therapy apparatus, 200 kV, 16 mA, dose rate 10.32 mCi/kg body weight/min, i.e., 45 R/min) and PH was performed on them 10-30 min later by the usual method [7]. In series II the same determinations were made on unirradiated animals subjected to PH (control).

The experimental and control rats were killed between 18 h and 21 days after the operation, always in the morning (between 6:30 and 7:30 a.m.). Films were made from liver cell suspensions [3] and stained under standard conditions by Feulgen's method. The DNA content in the nuclei was determined cytophotometrically (on the Chirana 11 cytophotometer), by a two-wave method and calculated in relative units (rel. u.). In each group, consisting of five animals, 250 measurements were made of extinctions and of the dimensions of the same nuclei. The nuclei were measured in two mutually perpendicular directions by means of an

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