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AN ENZYMIC ANALYSIS OF A NUCLEAR ENVELOPE FRACTION

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SUMMARY

A rat liver nuclear envelope fraction isolated essentially by the technique of Monneron *et al.* (J. Cell Biol. 55, 104–125 (1972)) is characterized by high levels of glucose-6-phosphatase and 5′-nucleotidase. A broadly specific nucleoside triphosphatase activity is present. Cytochromes b_5 and *P*-450 as well as NADPH- and NADH-cytochrome *c* reductase activities are present but at lower levels than found in microsomes. Cytochrome *c* oxidase activity is low. RNA polymerase activity is absent from the nuclear envelope fraction. Cytochemistry shows that glucose-6-phosphatase activity is strong and restricted to the nuclear envelope of nuclei. 5′-Nucleotidase shows weak reaction deposit in whole nuclei but in contrast gives clear reaction deposit in isolated nuclear envelopes. Cytochemical reaction deposit due to nucleoside triphosphatase activity is not restricted to the nuclear envelope but is found to a larger extent within the nucleus.

INTRODUCTION

Monneron *et al.* [1] have previously reported the isolation of a rat liver nuclear envelope fraction by a relatively simple procedure. This fraction has been characterized morphologically and chemically [1] but not enzymically. We have undertaken an enzymic analysis of this fraction as compared to microsomes and report differences in the biochemical properties of the two fractions. A preliminary report of this work has been previously presented [2].

MATERIALS AND METHODS

Animals. Subcellular fractions were prepared from livers of 120–150 g male Sprague-Dawley rats which were fasted overnight prior to sacrifice.

Preparation of subcellular fractions. Nuclei were isolated from rat liver homogenates as described by Blobel and Potter [3] (without detergent treatment) except that a Beckman SW27 rotor operating at 96 000 $\times g_{av}$ for 90 min was used.

Nuclear envelopes were prepared by the procedure described by Monneron *et al.* [1], with the following slight modification. The resuspended and vortexed

nuclei (equivalent to approx. 20 g of liver) in 5 ml of 1.8 M sucrose, 0.5 M $MgCl_2$ and 0.05 M Tris · HCl (pH 7.5) was then overlayed with a disontinuous sucrose gradient consisting of 15 ml of 1.2 M sucrose, 0.5 M $MgCl_2$, 0.05 M Tris · HCl (pH 7.5) and 16 ml of 0.4 M sucrose, 0.5 M $MgCl_2$, 0.05 M Tris · HCl (pH 7.5). The gradients were centrifuged in the Beckman SW27 rotor for 260 min at $96\,000 \times g_{av}$. The membrane pellet was washed three times *in situ* in distilled water and resuspended using a glass homogenizer.

Microsomes were prepared from rat liver homogenates (1 : 5, w/v) in 0.25 M sucrose or in 0.25 M sucrose, 0.05 M Tris · HCl (pH 7.5), 0.025 M KCl, 0.005 M $MgCl_2$) by centrifugation for 10 min in a Beckman Type 30 rotor operating at $8800 \times g_{av}$. The supernatant was decanted and centrifuged for 90 min in a type 30 rotor at $78\,000 \times g_{av}$ to obtain the microsomal pellet. The microsomal pellet was removed and resuspended in distilled water or in the buffer of the appropriate enzyme assay system.

Biochemical assays. All phosphatase activities were determined by previously published procedures with occasional minor modifications. Glucose-6-phosphatase was assayed by the method of Leskes *et al.* [4] using 200 mM Tris/maleate (pH 6.6) and 30 mM glucose 6-phosphate and 5'-nucleotidase by the method of Widnell and Unkeless [5] using 83 mM Tris · HCl (pH 8.5), 17 mM $MgCl_2$ and 17 mM AMP. Adenosine triphosphatase was measured by the method of Swanson *et al.* [6] using 80 mM Tris · HCl (pH 7.4), 3 mM $MgCl_2$ and 3 mM ATP. For the $(Na^+ + K^+ + Mg^{2+})$-activated adenosine triphosphatase, 30 mM KCl and 100 mM NaCl were added.

Uridine triphosphatase was measured in the presence of 80 mM Tris · HCl (pH 7.4), 3 mM $MgCl_2$ and 3 mM UTP. Cytidine triphosphatase, guanosine triphosphatase and inosine triphosphatase activities were measured as described for uridine triphosphatase but with 3 mM CTP, GTP, ITP as respective substrates. Inorganic phosphate (P_i) was determined in all cases by the procedure of Ames and Dubin [7].

NMN adenylyltransferase was assayed as described by Kornberg [8] and Hogeboom and Schneider [9] with a 50 mM glycylglycine buffer (pH 7.6), 150 mM $MgCl_2$, 6 mM ATP, 200 mM nicotinamide and 8 mM NMN. At the end of the reaction (30 min), NAD was measured with alcohol dehydrogenase as described by Klingenberg [10].

RNA polymerase activity in isolated nuclei and nuclear envelopes was determined essentially according to Shields and Tata [11] but with saturating substrate concentrations. The assay contained 62.5 mM Tris · HCl (pH 8.0), 25 mM KCl, 25 mM β-mercaptoethanol, 5 % glycerol and 10–800 μg of protein (nuclei or nuclear envelopes) in a final volume of 80 μl. The four nucleotides (ATP, GTP, CTP, UTP) were at a concentration of 0.4 mM and the assay included enough [^{3}H]UTP to give a final specific activity of 62 Ci/mol. For the Mg^{2+}-activated enzyme, $MgCl_2$ was present at a final concentration of 6.25 mM. The concentrations of $MnCl_2$ and $(NH_4)_2SO_4$ were 2.5 mM and 0.2 M, respectively, for the Mn^{2+}-stimulated polymerase assayed at high ionic strength.

Cytochrome *c* oxidase was assayed by the method of Smith [12]. Cytochrome *c* reductases were assayed essentially in the manner described by Dallner *et al.* [13] and rotenone (4 μM) was added where necessary. The extinction coefficient of $18.5 \cdot 10^3\ l \cdot mol^{-1} \cdot cm^{-1}$ at 550 nm for reduced minus oxidized cytochrome *c* [14] was

used for calculations. Cytochrome b_5 was determined taking a value of 163 $mM^{-1} \cdot cm^{-1}$ as the extinction coefficient between 424 and 409 nm in the difference spectrum between the dithionite-reduced and the oxidized form [15]. With dithionite-reduced biological material in both cuvettes, the cytochrome *P*-450 assay was run as a difference spectrum following the passage for 20 s of CO into the sample cuvette. The extinction coefficient of 91 $mM^{-1} \cdot cm^{-1}$ was used for cytochrome *P*-450 based upon the absorbance difference at 450 nm minus 490 nm [16]. A control for dithionite loss due to CO bubbling was carried out by bubbling N_2 an equal time in the reference cuvette. For all enzymic assays, controls were run to verify the linearity of each enzyme assay with time and amount of material. All assays using spectral analysis were carried out on a Gilford model 2000 spectrophotometer or an Aminco DW-2 UV-VIS spectrophotometer very generously made available by Dr. J. G. Joly, St. Luke's Hospital, Montreal.

Cytochemical analysis. Fixed and unfixed nuclei and nuclear envelope fractions were processed similarly, as described previously for Golgi fractions [17].

For fixed fractions, 7-ml aliquots of resuspended nuclei (equivalent to 2.5 g liver) were placed in Beckman No. 30 cellulose nitrate centrifuge tubes containing 7 ml of ice-cold 1 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). After 30 min on ice, the tubes were filled with 0.1 M cacodylate buffer (pH 7.4) containing 5 % sucrose and centrifuged at $1000 \times g_{av}$ for 10 min in an MSE Mistral 4L centrifuge. After decanting the supernatants and rinsing the pellets three times in the cacodylate buffer, 14 ml of incubation medium were added to each tube and shaken at 25 °C for 90 min. After incubation, the pellets were rinsed three times in 0.1 M acetate/veronal buffer and postfixed in 1 % OsO_4 in acetate/veronal buffer (pH 7.4) for 1 h, rinsed three times with buffer and treated with uranyl acetate block stain [17] for 60 min and dehydrated in ascending alcohols and propylene oxide. Oriented pellets [17] were embedded in Epon.

For unfixed fractions, a 7 ml suspension (as above) of nuclei or nuclear envelopes were placed in Beckman No. 30 centrifuge tubes and 14 ml of a concentrated (1.5 times) solution of incubation medium (see below) were added to each tube. The tubes were incubated for 60 min at 25 °C. The reaction was stopped by filling the tubes with cold 1 % glutaraldehyde in 0.1 M cacodylate buffer and letting them stand at 0 °C for 10 min. The tubes were either centrifuged at $78\,000 \times g_{av}$ for 40 min in the case of nuclear envelopes, or for $1000 \times g_{av}$ for 10 min in the case of nuclei (for the latter, the fixation time was extended to 20 min). The pellets were then postfixed in OsO_4 and processed as described above.

Cytochemical incubation media. For glucose-6-phosphatase cytochemistry, fractions were incubated in the modified medium of Leskes *et al.* [4] as described previously [17]. For 5′-nucleotidase, fractions were incubated in Widnell's medium [18] again as described previously [17] except that incubations were extended to 90 min. For adenosine triphosphatase, fractions were incubated in a modification of the media described by Marchesi and Palade [19] prepared with 80 mM Tris/maleate (pH 7.0), 4.5 mM $MgCl_2$, 0.6 mM ATP and 0.4 mM $Pb(NO_3)_2$. The low concentration of ATP was used to ensure efficient trapping of phosphate by the lead (see Figs. 11 and 12 of Marchesi and Palade [19]). The low concentration of $Pb(NO_3)_2$ was to ensure minimal damage to the enzyme [19] and to minimize lead-catalyzed hydrolysis of ATP (reviewed in ref. 20).

Electron microscopy. For routine assessment of morphological purity of both nuclei and nuclear envelope fractions, they were either pelleted, fixed and processed as described for the cytochemical method, or processed for random sampling by a slight modification of the procedure described by Baudhuin *et al.* [21]. The sections were stained for 1 min in ethanolic uranyl acetate [22] and then in lead citrate for 5–10 min [23]. Grids were examined in a Philips EM 300 electron microscope operated at 80 kV or a Siemens 1A Elmiskop also operated at 80 kV. For most of the cytochemical experiments, grids were examined unstained.

Freeze-fracture. The nuclear envelope fraction was identified using routine freeze-fracture procedures [24].

RESULTS

Morphology of isolated preparations. The detailed structural features and purity

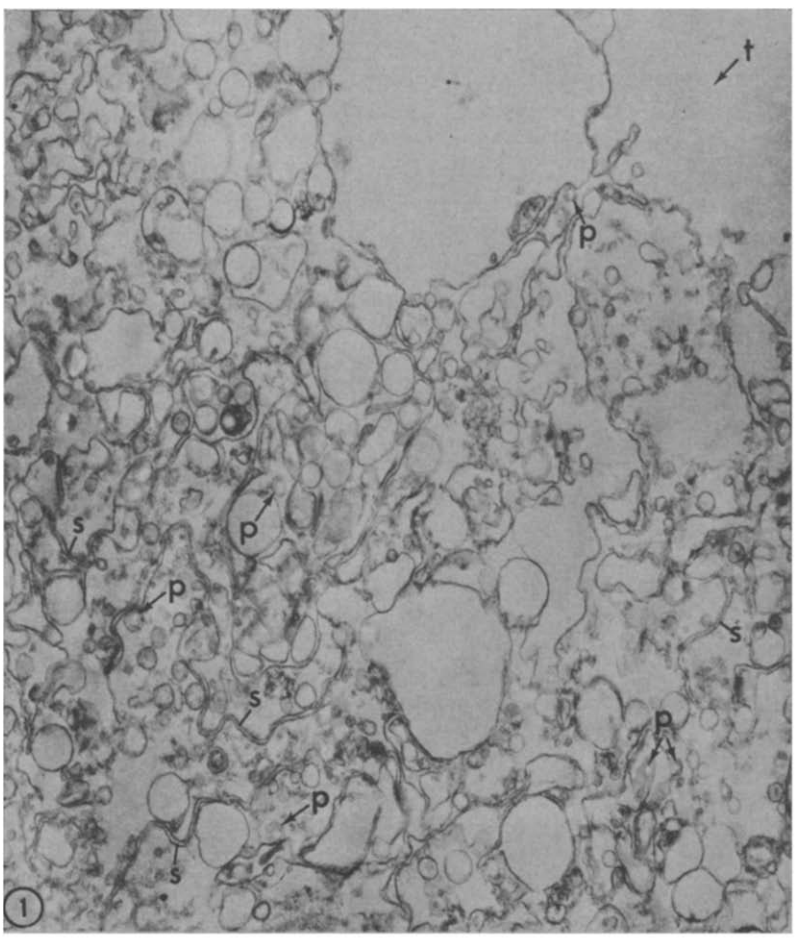

Fig. 1. Random sample of nuclear envelope fraction prepared by the filtration technique of Baudhuin et al. [21]. The fraction consists of vesicles of various sizes with some long sheets of double membranes (s). Occasional pore-like elements (p) can be seen. The top of the filter is marked t. × 24 800.

Fig. 2. Replica of freeze-fracture specimen of nuclear envelope fraction with direction of shadowing indicated by encircled arrow. The presence of nuclear pores appears as both craters and mounds. × 80 000.

of the nuclear fraction isolated by the method of Blobel and Potter [3] are substantially documented [1, 3]. The nuclear envelope fraction derived from these nuclei (Fig. 1) consists of double sheets and vesicles of various sizes free of ribosomes (which are removed by the treatment with 0.5 M $MgCl_2$ used for the preparation of nuclear envelopes [1]). Grazing sections show a variety of empty annular structures of about 50–100 nm in diameter which are considered to be remnants of nuclear pores. The freeze-fracture replica (Fig. 2) of the nuclear envelope pellet shows nuclear pores in the form of convex and concave surfaces on membranes. As already noted by Monneron *et al.* [1] nuclear pores were much more evident in the nuclear envelope fraction analyzed by freeze-fracture technique than by thin sectioning.

Recovery. On a protein basis (Table I) we isolate 2 % of the homogenate as nuclei and 0.04 % as nuclear envelope. Further, the data on the marker enzyme for nuclei, NMN adenylyltransferase [9, 25] indicate a recovery of 73 % of the total enzymic activity present in the homogenate. Using DNA as a basis for recovery of nuclei, published values vary from 70 to 90 % [1, 3].

TABLE I

PROTEIN CONTENT AND NMN ADENYLYLTRANSFERASE ACTIVITIES OF FRACTIONS

Number of experiments are in parentheses. Specific activity units: nmol NAD/20 min per mg protein. n.d., not detectable.

Fractions	Protein (6) (mg/g liver)*	NMN adenylyltransferase (3)	
		Specific activity	Recovery (%)
Homogenate	205	3.3	100
Nuclei	4.5	110	73
Nuclear envelopes	0.073	n.d.	–
Microsomes	31	–	–

* Wet weight

TABLE II

ENZYME ASSAYS OF ISOLATED FRACTIONS

Number of experiments in parentheses. Specific activity for glucose-6-phosphatase and 5′-nucleotidase, μmol P_i/20 min per mg protein; for $(Mg^{2+}+Na^{+}+K^{+})$-adenosine triphosphatase, μmol P_i/30 min per mg protein. Recoveries were calculated on the basis of total enzymic activities in each subcellular fraction.

Fractions	Glucose-6-phosphatase (5)		5′-Nucleotidase (5)		Mg^{2+}-adenosine triphosphatase (6)		$(Mg^{2+}+Na^{+}+K^{+})$-adenosine triphosphatase (6)	
	Specific activity	Recovery (%)	Specific activity	Recovery (%)	Specific activity	Recovery (%)	Specific activity	Recovery (%)
Homogenate	1.6	100	0.59	100	0.98	100	1.55	100
Nuclei	1.2	1.6	0.3	1.1	0.44	1	0.4	0.6
Nuclear envelopes	17	0.4	3.87	0.23	1.59	0.05	1.6	0.04
Microsomes	4.9	46	1.3	33	1.98	30	2.15	21.0

Phosphatases: biochemical

Glucose-6-phosphatase. Glucose-6-phosphatase is a marker enzyme of the endoplasmic reticulum of the hepatocyte [4, 26, 17]. The isolated nuclear and nuclear envelope fractions both contain glucose-6-phosphatase activity (Table II). Although the total activity recovered from the homogenate is minute (1.6 % in the case of nuclei and 0.4 % in the case of the nuclear envelope fraction), the specific activity in nuclear envelopes is exceptionally high. It is higher than values reported for nuclear envelope fractions isolated by other workers using rather different methods of isolation [27–31] and considerably higher than the specific activity in the microsomal fraction (Table II).

5'-Nucleotidase. Until recently, 5'-nucleotidase has been commonly considered a plasmalemma marker. However, Widnell's demonstration of this enzyme activity in the endoplasmic reticulum [18] and previous work on the Golgi [17, 32] prompted an analysis of 5'-nucleotidase activity. Only a small portion of the total activity is recovered in the nuclear (1.1 %) and nuclear envelope (0.23 %) fractions (Table II). The specific activity of the enzyme in nuclear envelopes is greater than that of the microsomal fraction. A number of workers in the field consider 5'-nucleotidase activity to be absent from rat liver nuclei (refs. 33 and 34 for example) but we find the activity to be readily detectable in purified preparations of nuclei and nuclear envelopes.

Adenosine triphosphatases. Adenosine triphosphatase activity in the rat hepatocyte is distributed between the plasmalemmal and microsomal fractions. The plasmalemmal activity is increased by the addition of Na^+ and K^+ [35, 36] but hardly any increment is noted in rat liver microsomes [36]. In the studies described here (Table II), however, the specific activity in the nuclear envelope fraction was very similar to that of the microsomal fraction. The addition of Na^+ and K^+ to the incubation medium had an effect on the homogenate enzyme activity but hardly any change was noted in the microsomal fraction. No activation was noted in the nuclei and nuclear envelope fractions. Further testing (Table III) of the activity showed a broad specificity of the enzyme for nucleoside triphosphatases and thus suggested a similarity to the non-specific nucleoside triphosphatase of microsomal membranes described by Ernster and Jones [37].

However, whole nuclei showed a different specificity for the substrates than the nuclear envelope fraction (Table III) indicating the possibility that different

TABLE III

NUCLEOSIDE TRIPHOSPHATASE ACTIVITY IN NUCLEI AND NUCLEAR ENVELOPES

Results are the mean of three experiments. Specific activity units: μmol P_i/30 min per mg protein.

Substrate	Specific activity	
	Nuclei	Nuclear envelope fraction
ATP	0.45	1.53
GTP	1.1	1.74
UTP	0.6	2.02
CTP	0.5	1.08
ITP	0.78	1.94

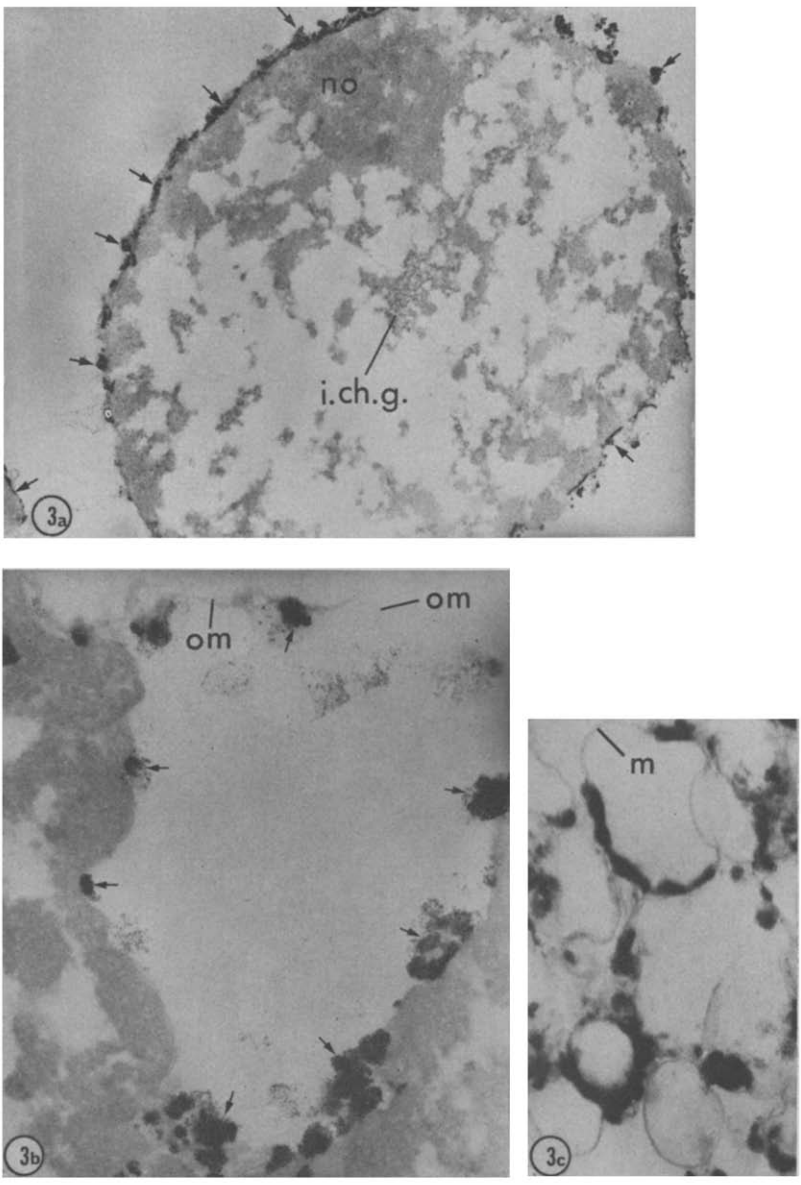

Fig. 3. (a) Glucose-6-phosphatase cytochemistry. Section through a pellet of nuclei incubated without fixation in glucose-6-phosphatase medium. A representative field showing reaction deposit along the nuclear envelope (arrows). The nucleolus (no) is devoid of deposit as are the interchromatin granules (i.ch.g.) and chromatin. × 19 200. (b) Detail of a preparation similar to (a). Where the outer membrane (om) has come away from the nucleus reaction deposit is found on the cisternal face. As well, reaction deposit is seen close to the nucleus where the inner nuclear membrane is usually found (arrows). × 52 000. (c) Nuclear envelope fraction incubated without fixation in glucose-6-phosphatase medium. The reaction deposit is closely associated with most membrane profiles (m). × 61 600.

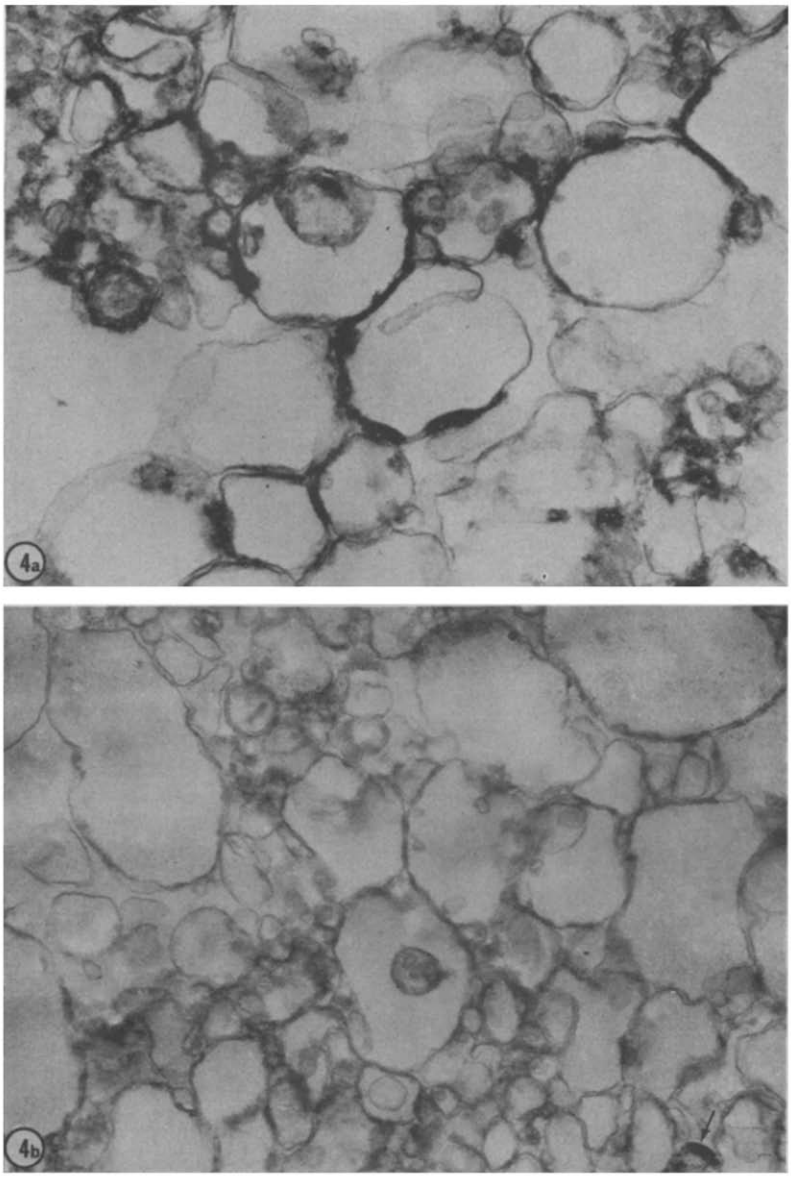

Fig. 4. (a) Nuclear envelope fraction incubated for 5′-nucleotidase without prefixation. A section through the middle of the pellet shows reaction product associated with the membrane of a number of vesicles. × 52 000. (b) Sectioned pellet of nuclear envelope fraction incubated as for Fig. 5a, except 2′-AMP (a competitive inhibitor) was substituted for 5′-AMP. Most vesicles throughout the entire pellet are negative with respect to reaction deposit. A rare vesicle showing a deposit is marked (arrow). × 40 800.

nucleoside triphosphatase activities are present within the nucleus as compared to the nuclear envelope.

Phosphatases: cytochemical

Glucose-6-phosphatase. The incubation of prefixed nuclei resulted in reaction deposit. However, the amount of deposit varied from one part of the pellet to another. In contrast, experiments with unfixed nuclear fractions when incubated in the modified cytochemical medium of Leskes *et al.* [4] (Fig. 3a) showed strong reaction deposit within the nuclear envelopes of virtually all nuclei. Although we can confirm the observation of Kartenbeck *et al.* [31] that reaction deposit can be seen associated with the cisternal face of both the inner and outer membrane of the nuclear envelope (Fig. 3b), occasionally reaction deposit was found in selective association with the cisternal face of the outer membrane (not shown).

The nuclear envelope fraction incubated for glucose-6-phosphatase showed reaction deposit associated with virtually all vesicular profiles (Fig. 3c).

5'-Nucleotidase. The cytochemical results for 5'-nucleotidase were inconclusive. When unfixed nuclei were incubated in Widnell's medium for 5'-nucleotidase (not shown), only faint and dubious reaction deposit was observed*. In contrast, no difficulty was obtained in locating reaction deposit in the nuclear envelope fraction (Fig. 4a). Parallel experiments with 2'-AMP (a specific inhibitor of 5'-nucleotidase [18]) revealed rare profiles with reaction deposit (Fig. 4b).

Nucleoside triphosphatase. When nuclei were incubated in the adenosine triphosphatase medium described in Materials and Methods, only a minor reaction deposit was seen on the nuclear envelope with a stronger deposit found associated with the nucleolus as well as deposit scattered in the nucleoplasm (Fig. 5a). The cytochemical incubation of the nuclear envelope fraction showed reaction deposits associated with approx. 50 % of the membraneous profiles (Fig. 5b).

RNA polymerase. The nuclear envelope has an intimate relationship with chromatin and could play a role in the expression of genetic information, perhaps by having an RNA polymerase. When assayed for RNA polymerase by the method of Shields and Tata [11], whole nuclei showed a specific activity of 5.4 nmol nucleotide incorporated/15 min per mg protein; but nuclear envelopes showed no activity.

Cytochrome c oxidase. The cytochrome *c* oxidase activity of nuclei (0.005 s^{-1}/mg protein) was considerably less than that found in the homogenate (Table IV). The value found for nuclear envelopes was 0.027 s^{-1}/mg protein which is of the same order as found for the homogenate but much less than the activity of 0.75 s^{-1}/mg protein reported for purified rat liver mitochondria [38]. The above activities correspond to a possible mitochondrial contamination of 0.6 and 3.6 % (on a protein basis) in the nuclear and nuclear envelope fractions, respectively. Such low values are in accord with the electron microscopic analysis of the nuclear and nuclear envelope fractions which showed no recognizable mitochondria.

Cytochromes b_5 and P-450. The value obtained for cytochrome b_5 in the nuclear envelope fraction (0.28 nmol/mg protein) is somewhat higher than values appearing in the literature [27, 39] for rat liver using different separation procedures.

* The low concentration of $Pb(NO_3)_2$ used in the incubation media (essential for retention of enzymic activity [18]) may not have been sufficient to prevent a diffusion artifact [50–52].

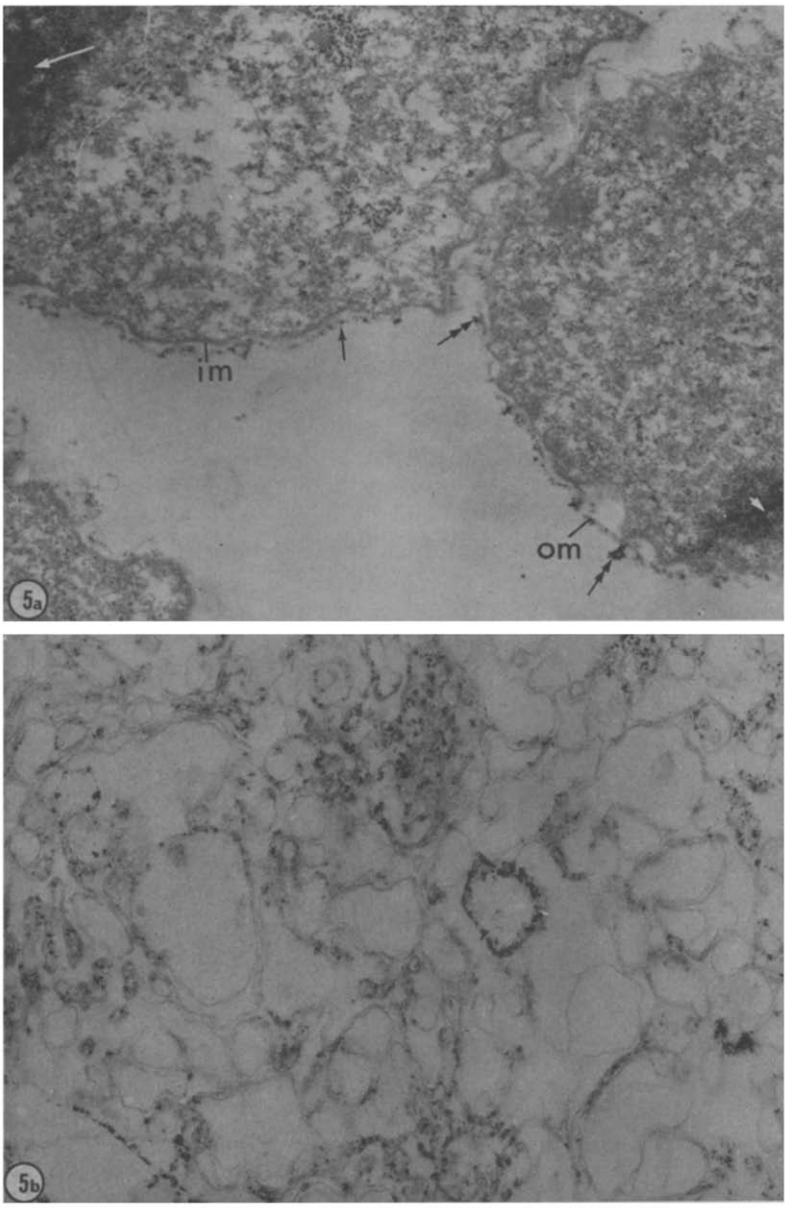

Fig. 5. (a) Nucleoside triphosphatase reaction, unfixed nuclei. Two varieties of reaction deposit can be observed on the outer membrane (om). A small generally dispersed deposit (arrows) with rarer larger deposits (double arrows) not so tightly associated with the membrane. Most of the reaction product is present in nucleoli (long and short white arrows). As well, dispersed product is present throughout the body of the nucleus. The inner membrane (im) is devoid of deposit. × 18 400. (b) Nuclear envelope fraction, unfixed, incubated in nucleoside triphosphatase medium. A section through the middle of the pellet showing reaction deposit associated with several membrane vesicles. × 38 400.

TABLE IV

CYTOCHROME AND ASSOCIATED ENZYME ASSAYS OF ISOLATED FRACTIONS

Units of specific activity for cytochrome *c* oxidase, s^{-1}/mg protein; for NADH- and NADPH-cytochrome *c* reductase, nmol cytochrome *c* reduced/min per mg protein. Units of content for cytochromes b_5 and *P*-450, nmol/mg protein. Number of experiments in parentheses. n.d., not determined.

Fractions	Specific activity			Content	
	Cytochrome *c* oxidase	NADPH-cytochrome *c* reductase	NADH-cytochrome *c* reductase	Cytochrome b_5	Cytochrome *P*-450
Homogenate	0.025 (4)	n.d.	n.d.	n.d.	n.d.
Microsomes	n.d.	104 (8)	754 (9)	0.74 (8)	0.87 (7)
Nuclei	0.005 (9)	5.0 (6)	62.7 (6)	0.031 (6)	0.033 (6)
Nuclear envelopes	0.027 (3)	46.1 (3)	488 (4)	0.28 (8)	0.099 (9)

The value, however, is considerably less than found in the microsomal fraction (Table IV). Similarly the concentration found for cytochrome *P*-450 in microsomes was much more than in nuclear envelopes, although enzyme lability probably has an influence in apparent variations of this enzyme [41–43].

NADH- and NADPH-cytochrome c reductases. The activities of the NADH- and NADPH-cytochrome *c* reductases (Table IV) in the microsomal fraction were similar to published values [13, 44]. The activities of the reductases in the nuclear envelope fraction were lower than those found in the microsomes. The assayed NADH-cytochrome *c* reductase activity for the nuclei and nuclear envelope fractions showed no inhibition from rotenone again indicating little or no contamination by mitochondrial inner membrane components.

DISCUSSION

The available literature is characterized by a variation of the concentration of many enzyme activities in the nuclear envelope fraction (see recent review ref. 39). Hopefully this represents an evolution in the assay and separation methods and improvement in the correlation between a given cell structure and its isolated fraction. Glucose-6-phosphatase in nuclear membranes has been reported at varying concentrations from zero [27, 33, 45] to values half that reported for microsomes [28], to values equal to that of microsomes [31], and finally to values greater than were found in microsomes [46]. Our data indicate that the nuclear envelope fraction is highly enriched in glucose-6-phosphatase (values even higher than found in ref. 46) and the cytochemical results show that this activity is restricted to the nuclear envelope of isolated nuclei. It is thus possible to quantitate the amount of nuclear envelope present in the hepatocyte from the study of glucose-6-phosphatase. There is a 14-fold increase in specific activity of the enzyme in the nuclear envelope fraction relative to nuclei. This indicates that the nuclear envelope fraction comprises approx. 7 % of the total nuclear protein. The data for NMN adenylyltransferase (Table I) allow a conversion of 100 % recovery of nuclei to 6.1 mg protein/g wet weight of liver (or 3 % of the homogenate protein). Accordingly, the nuclear envelope would comprise approx. 1/14th of this total value, more precisely, 0.43 mg/g liver (or 0.2 % of the homogenate protein). The lack of correspondence in the cytochemistry of 5′-nucleotidase in the nuclear and nuclear envelope fractions does not enable the use of the 5′-nucleotidase data in such a calculation.

The cytochemistry of nucleoside triphosphatase activity showed, as expected from the biochemical data, the presence of a major nucleoside triphosphate-hydrolyzing activity within the nucleus with only a minor reaction present on the nuclear envelope. Such an intranuclear activity has been reported previously both biochemically [47] as well as cytochemically [48]. However, the significance of all lead salt adenosine triphosphatase cytochemistry is controversial* and although the cyto-

* The cytochemical nucleoside triphosphatase has been analyzed in greater depth. The enzymic activity during the conditions of the cytochemical incubation has been determined and sufficient (> 50 %) enzyme activity remains to enable cytochemical detection. GTP, CTP, or UTP either singly or together with ATP result in a similar cytochemical result as is shown with ATP. The cytochemical result is unaffected by inhibitors of RNA synthesis (actinomycin D and α-amanitin) or inhibitors of poly(ADP-ribose) synthesis (thymidine). The reaction deposit has been isolated and examined chemically and consists of lead phosphate but no pyrophosphate or nucleotide is found in the precipitate.

chemistry of glucose-6-phosphatase and 5′-nucleotidase has been validated by others [4, 17, 18, 49], the possibility of diffusion artifacts in these and the nucleoside triphosphate cytochemical enzyme assays cannot be excluded [50–52].

Taken together, our results show that the nuclear envelope fraction contains a higher glucose-6-phosphatase activity than microsomes, a higher 5′-nucleotidase activity, similar nucleoside triphosphatase activities, a considerably lower content of cytochromes b_5 and *P*-450 and lower NADPH- and NADH-cytochrome *c* reductase activities than in the microsomal fraction. Different concentrations for a given enzyme in the two subcellular fractions suggest that the enzymes are not free to equilibrate between the two membrane systems, the nuclear envelope and the endoplasmic reticulum, in the cell.

Additional evidence for this conclusion is shown by the results of Monneron *et al.* (see Fig. 16 of ref. 1) who demonstrated different gel electrophoretic patterns for the polypeptides of rough microsomes as compared to the nuclear envelope fraction. All of these differences imply that some mechanism of discrimination prevents the mixing of the protein constituents of the endoplasmic reticulum and the nuclear envelope. This can only occur if the two membrane systems are not in permanent direct continuity or if these membranes are less fluid than is at present accepted [53, 54].

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