Polypeptides of the Nuclear Envelope†

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ABSTRACT: A simple procedure for the purification of nuclear membrane from chicken erythrocytes is described. Purified erythrocyte nuclei are briefly digested with pancreatic DNase I and extracted with 2 M NaCl. Protease inhibitors are included throughout the purification procedure to prevent proteolysis. Nuclear membrane is obtained from purified nuclei in less than 2 h in a yield of 0.55 mg/ml of packed erythrocytes. Contamination of the nuclear membrane fraction by chromatin and plasma membrane is minimal, as assessed by DNA assay and (Na^+,K^+)ATPase activity, respectively. The buoyant density of the nuclear membrane fraction is 1.21 ± 0.01 g/cm^3. The dodecyl sulfate polyacrylamide gel electrophoresis pattern of purified erythrocyte nuclear membrane is dominated by four major bands with apparent polypeptide molecular weights of 80 000, 74 000, 62 000, and 50 000. Bands with apparent molecular weights of 29 000, 14 000, and 10 000 are ascribed to histone contamination of the nuclear membrane fraction. These polypeptides comigrate with purified erythrocyte histones both in the low pH urea gel system of Paynim and Chalkley (Paynim, S., and Chalkley, R. (1969), *Biochemistry 8*, 3972–3979) and in a high-resolution dodecyl sulfate slab-gel system. The dodecyl sulfate gel pattern of the nuclear membrane fraction differs radically from that of a sample of purified erythrocyte plasma membrane. Furthermore, the erythrocyte nuclear membrane contains no detectable glycopeptides, as determined by periodate–Schiff staining, whereas a number of the plasma membrane glycopeptides are stained, albeit weakly, with this reagent. Extraction of the erythrocyte nuclear membrane fraction with 0.1 M NaOH removes 43% of the membrane protein but less than 5% of the membrane phospholipid. The 80 000- and 74 000-dalton polypeptides are extracted by this procedure, while the 62 000- and 50 000-dalton polypeptides remain membrane bound. These observations (minimal extraction of phospholipid and differential extraction of polypeptides) are consistent with the proposal of Steck and Yu (Steck, T. L., and Yu, J. (1973), *J. Supramol. Struct. 1*, 220–232) that NaOH extractions of membranes solubilizes peripheral membrane proteins. It is concluded that the 80 000- and 74 000-dalton polypeptides are peripheral, whereas the 62 000- and 50 000-dalton polypeptides are integral erythrocyte nuclear membrane polypeptides. The polypeptide composition of a sample of chicken liver nuclear membrane, prepared by a procedure analogous to that used to prepare the erythrocyte nuclear membrane, is compared to that of the erythrocyte nuclear membrane. The dodecyl sulfate gel pattern of liver nuclear membrane contains seven major polypeptide bands. Three of these bands (80 000, 74 000, and 62 000 daltons) comigrate with erythrocyte nuclear membrane polypeptides.

The plasma membranes of several eukaryotic cells, notably of the human red blood cell, have been characterized with regard to chemical composition and polypeptide chain distribution. The results show that approximately half of the mass of the membrane is protein and that several (8–20) polypeptide chains of different molecular weights account for at least 80% of the protein. In the case of the red blood cell, functions have been assigned to five of the polypeptide chains of the plasma membrane. On the other hand, there is much less information on the other membranes present in eukaryotic cells. The membranes of mitochondria have been analyzed to some extent, those of secretory vesicles have also been studied, and attempts have been made to characterize the membranes of the nuclear envelope. The nuclear envelope differs from other membranous structures of the cell in two principal ways. One is that it is permeable to macromolecules of molecular weight up to approximately 40 000 (Bonner, 1975a,b; Paine et al., 1975), which, apparently, can pass freely through the pore complexes that connect the inner and outer membranes of the envelope. The other is that the inner membrane of the nuclear envelope apparently is associated with chromatin. One might envisage that these drastic functional differences between the nuclear envelope and other cellular membranes should be reflected in a considerable difference in the polypeptide chain compositions of these membranes. Inversely, the functional and morphological similarity of the nuclear envelopes of all eukaryotic cells suggests that the polypeptide chain composition of the nuclear envelope should be relatively invariant.

This paper deals with nuclear envelope derived from two sources: chicken erythrocytes and chicken liver. Zentgraf et al. (1971) were the first investigators to utilize the chicken erythrocyte as a source of nuclear membrane; however, their purification procedure was lengthy and the problem of proteolysis during the prolonged preparation was not addressed. Zentgraf et al. did not examine the polypeptide chain composition of their nuclear membrane sample. Blanchet's (Blanchet, 1974) preparative procedure was more simple than that of Zentgraf et al. (1971) but he also did not consider the problem of proteolysis. However, Blanchet did examine the polypeptide chain composition of his fractions via gel electrophoresis in dodecyl sulfate. Surprisingly, he found that, despite their functional dissimiliarity, the polypeptide chain compositions of the erythrocyte plasma and nuclear membrane fractions were very similar. A number of investigators have prepared nuclear membrane from liver and other fully functional tissues (Kay et al., 1972; Monneron et al., 1972; Zbarsky

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et al., 1969; Kashnig and Kasper, 1969; Bornens, 1973; Franke et al., 1970; Berezney et al., 1972; Price et al., 1972).

This paper presents a simplified method for the preparation of bulk quantities of chicken erythrocyte nuclear membrane, and demonstrates, in contrast to the finding of Blanchet (1974), that the polypeptide composition of the nuclear membrane differs radically from that of the plasma membrane, thus supporting the proposition that the functional difference between the two membranes is reflected in their polypeptide chain compositions. In addition, it is demonstrated that nuclear membrane fractions derived from two different tissues (chicken erythrocyte and chicken liver) have similar polypeptide chain compositions.

Materials and Methods

Preparation of Erythrocyte Nuclear Membrane. A mature rooster (Spafas Farms, Norwich, Conn.), which had been fasted 24 h, was given an intravenous injection containing 1000 USP units of heparin and 15 mg of pentobarbital each per kg of body weight in Tris[1]-buffered saline (150 mM NaCl, 10 mM Tris-HCl, pH 7.5). The anesthetized bird was exsanguinated from the jugular vein. Blood was collected into a beaker containing 2000 USP units of heparin and chilled in an ice bath. Seventy to one hundred milliliters of blood was obtained per rooster. The blood was filtered through two layers of cheesecloth to remove any debris and washed by three cycles of centrifugation (3000*g* for 10 min in a Sorvall RC-2B) and resuspension. The buffy coat was removed by aspiration after each centrifugation. The red cell pellet from the third wash was resuspended in 0.1 M sucrose–TKM buffer[1] and washed once more. A 5–10% cellular suspension was prepared by resuspending the red cell pellet in approximately 9 volumes of 0.1 sucrose–TKM, containing 1 mg/ml of sodium tetrathionate (K & K Laboratories) and 0.1 mM PMSF. Aliquots of the suspension were homogenized in a single-speed Waring blender equipped with a semimicro stainless steel jar. To prevent foaming, air space above the homogenate was eliminated by floating a polyethylene bottle, with a diameter slightly less than that of the stainless steel jar, on the surface of the homogenate. If not prevented, foaming greatly reduced nuclear yields. Each minute of blending was followed by 3 min of cooling in an ice bath. After 2 min of blending time, the homogenate was centrifuged at 300*g* for 10 min. Plasma membrane is isolated from the supernatant as described below. The pellets consisting of intact cells and nuclei were resuspended in 0.1 M sucrose–TKM buffer, containing PMSF and sodium tetrathionate, and the sample was rehomogenized. The homogenate was centrifuged once more at 300*g* for 10 min and the nuclear pellets were resuspended in 150 ml of 67% sucrose prepared in 50 mM Tris-HCl, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$, 0.1 mM PMSF with 1 mg/ml of sodium tetrathionate. Any nuclear aggregates were dispersed by homogenizing the viscous suspension for 30 s in the blender. This suspension was layered over 10 ml of the 67% buffered sucrose solution and the interface between the suspension and the 67% buffered sucrose solution was disturbed by stirring with a glass rod, to prevent an accumulation of nuclei at the interface. The suspension was centrifuged at 23 000 rpm in the SW25.2 rotor for 4–6 h. The purified nuclear pellets were resuspended in 0.1 M sucrose–TKM containing PMSF and sodium tetrathionate with the aid of a Dounce homogenizer fitted with a loose pestle. The yield of purified nuclei, based upon recovery of DNA, was 72.3 ± 1.4%. The resuspended nuclei were divided into six portions, centrifuged at 700*g* for 10 min, and digested with bovine pancreatic DNase I as follows. Each nuclear pellet was resuspended in 5 ml of 0.1 mM $MgCl_2$ containing 0.1 mM PMSF and 1 mg/ml of sodium tetrathionate by vigorous vortexing. Twenty milliliters of 10 mM Tris-HCl, pH 7.5, 1 mg/ml of sodium tetrathionate, 0.1 mM PMSF, and 200 μl of a 1 mg/ml solution of DNase I (Worthington, grade DPFF, pretreated with 1 mM PMSF prior to use) in TKM were added to each tube. The suspension was homogenized with a Dounce homogenizer (three to four strokes, tight pestle) to ensure nuclear lysis and incubated at room temperature for 15 min. The digestion was stopped by the addition of one-half volume of ice-cold 0.1 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.5, and the suspension was centrifuged at 27 000*g* for 20 min.

The gelatinous pellets were resuspended in 100 ml of 2 M NaCl with three to four strokes of the tight-fitting Dounce pestle and recentrifuged (27 000*g* for 15 min). Pellets were resuspended in 10 mM Tris-HCl pH 7.5, 0.1 mM $MgCl_2$, and centrifuged once more as above. The final pellet was resuspended in 2–4 ml of 10 mM Tris-HCl, pH 7.5, 0.1 mM $MgCl_2$, frozen in a dry ice–acetone bath, and stored at approximately −70 °C.

Preparation of Erythrocyte Plasma Membrane. The supernatant from the first homogenization step (see above) was centrifuged at 300*g* for 10 min to remove residual nuclear fragments and then at 12 000*g* for 30 min. This produced a two-layered pellet (Oye and Sutherland, 1966, Bilezikian and Aurbach, 1973). The light-brown bottom layer was tightly packed and rich in DNA, while the milky-white upper layer was loosely packed. After removal of the supernatant, the loosely packed layer containing plasma membrane was separated from the well-packed bottom layer by gently swirling the tube with a small amount of 0.1 M sucrose–TKM buffer, containing 1 mg/ml of sodium tetrathionate and 0.1 mM PMSF. After dilution of the resuspended plasma membrane fraction with 100 ml of buffer, the centrifugation and resuspension steps were repeated twice more. The final membrane pellet was resuspended in 10–15 ml of 10 mM Tris-HCl, pH 7.5, 0.1 mM $MgCl_2$, frozen in a dry ice–acetone bath, and stored at approximately −70 °C.

Preparation of Chicken Liver Fractions. A rooster was sacrificed as described above. The liver was perfused with approximately 500 ml of ice-cold 10 mM Tris-HCl, pH 7.5, 150 mM NaCl. Nuclei were prepared by a modification of the procedure of Blobel and Potter (1966). Briefly, the minced liver was suspended in 2 volumes of 0.25 M sucrose–TKM buffer, containing 1 mg/ml of sodium tetrathionate and 0.1 mM PMSF, and homogenized with a motor-driven Teflon pestle homogenizer. The homogenate was diluted with another 3 volumes of buffer and centrifuged (700*g* for 10 min). Mitochondria and microsomes were prepared from the postnuclear supernatant as described below. The nuclear pellet was resuspended in 60% sucrose–TKM, containing tetrathionate and PMSF, and dispersed with the Teflon-pestle homogenizer. The suspension was layered over 10 ml of 60% sucrose–TKM, containing sodium tetrathionate and PMSF, and centrifuged at 23 000 rpm in a Beckman SW 25.2 rotor for 4–5 h. The liver nuclei were washed, digested with DNase I, extracted with 2 M NaCl, 10 mM Tris-HCl, pH 7.5, as described above in the erythrocyte nuclear membrane preparation procedure, washed with 10 mM Tris-HCl, pH 7.5, 0.1 mM $MgCl_2$, resuspended

[1] Abbreviations used are: TKM, 50 mM Tris-HCl, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$; PMSF, phenylmethanesulfonyl fluoride; ATPase, adenosine triphosphatase; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; EDTA, (ethylenedinitrilo)tetraacetic acid.

in 2–4 ml of this buffer, and stored at approximately −70 °C.

The postnuclear supernatant was centrifuged at 10 000*g* for 10 min. The mitochondrial pellet was washed once with 0.25 M sucrose–TKM (10 000*g* for 10 min) and resuspended in the wash buffer. The postmitochondrial supernatant was centrifuged at 100 000*g* for 2 h and the microsomal pellets were resuspended in 0.25 M sucrose–TKM buffer.

Enzyme Assays. ATPase activity was assayed at 37 °C for 15 min. Samples (250 μl) were buffered with 40 mM Tris-HCl, pH 7.5, and contained 80 mM NaCl, 16 mM KCl, 4 mM $MgCl_2$, 2 mM [γ-^{32}P]ATP (approximately 10^6 cpm). [γ-^{32}P]ATP, prepared according to the method of Glynn and Chapel (1964), was a gift of Dr. L. Waxman, Harvard University.

The reaction was quenched with 1 volume of ice-cold 10% trichloracetic acid. The precipitated protein was pelleted by centrifugation (6000*g* for 5 min) and aliquots of the supernatant were assayed for inorganic ^{32}P, as described by Pollard and Korn (1973). (Na^+,K^+)ATPase activity was determined from the difference between samples incubated in the absence and presence of 2×10^{-4} M strophanthidin. Heat-inactivated samples (100 °C × 3 min) were included, in order to control for nonenzymatic ATP hydrolysis. All samples were prereduced with 25 mM β-mercaptoethanol at 37 °C for 15 min prior to assay.

5′-Nucleotidease was determined as described by Hinton et al. (1970). Succinate dehydrogenase was assayed according to the procedure of Pennington (1961), as modified by Porteus and Clark (1965). Acid phosphatase was measured by the *p*-nitrophenyl phosphatase assay of Mitchell et al. (1970).

Chemical Determinations. Protein was determined by the method of Lowry et al. (1951), as described by Bailey (1967). Bovine serum albumin ($E_{279nm}^{1\%}$ = 6.7; Foster and Yang, 1954) was used to standardize the assay. Since sodium tetrathionate interferes with this assay, samples containing tetrathionate were precipitated with trichloroacetic acid and resuspended in a tetrathionate-free buffer prior to assay. Lipids were extracted with $CHCl_3$–methanol, as described by Hajra et al. (1968), except that sulfuric acid was substituted for phosphoric acid. Lipid phosphate was determined by the method of Ames (1966). Phospholipid was calculated by assuming that 1.0 μg of lipid phosphate corresponds to 25 μg of phospholipid (Kopaczyk et al., 1966). DNA and RNA were determined according to the procedure suggested by Munroe and Fleck (1966). RNA was extracted into 1 M NaOH by incubation at 37 °C for 30 min and quantitated colorimetrically using the orcinol reaction (Munroe and Fleck, 1966). Chicken embryo RNA (gift of Dr. P. Williamson, Harvard University) was used to standardize the assay. DNA was extracted into 0.5 M $HClO_4$ during three successive 15-min incubations at 90 °C. Extracted DNA was quantitated by the diphenylamine method (Burton, 1956). Salmon sperm DNA (Sigma) was used to standardize the assay.

Subfractionation of Membrane Protein with NaOH. The procedure for NaOH extraction of erythrocyte nuclear membrane was similar to that of Steck and Yu (1973). The membrane sample (1–2 mg of protein) was brought to a volume of 0.9 ml with ice-cold distilled water and 100 μl of 1 M NaOH was added. The suspension was vortexed and incubated in an ice bath for 20–40 min. Finally, the membranes were pelleted at 27 000*g* for 30 min. The supernatant was removed and the membranous pellet was resuspended in 50 mM Tris-HCl, pH 7.5. The supernatant and pellet fractions were assayed for protein and phospholipid and their polypeptide composition was analyzed by gel electrophoresis in dodecyl sulfate.

Electron Microscopy. Nuclear pellets (3000*g* for 5 min) were prepared for microscopy as described by Olins and Olins (1972). Nuclear membrane samples were fixed with glutaraldehyde (2%) and OsO_4 (1%) in Caufield's Veronal acetate buffer (Caufield, 1957), as described by Monneron et al. (1972). The fixed pellets were dehydrated in acetone and embedded in Spurr's medium (Spurr, 1969). Thin sections were cut on a Reichert OM-U2 ultramicrotome and stained either with uranyl acetate (Stempak and Ward, 1964) and lead citrate (Reynolds, 1963), or according to the method of Monneron and Bernhard (1969). Microscopy was done with a Phillips EM-300 electron microscope.

Other Methods. Chicken erythrocyte histones were prepared from isolated nuclei as described by Ruiz-Carrillo et al. (1974). The low pH urea gel system used to analyze histones was that of Paynim and Chalkley (1969). The dodecyl sulfate disc gel electrophoresis system used was previously described (Jackson, 1975). Gels were 7.5% acrylamide, 0.1% methylenebisacrylamine. All samples were reduced in 20 mM β-mercaptoethanol prior to electrophoresis. The dodecyl sulfate slab gel electrophoresis system used has been described by Studier (1973). Apparent molecular weights were determined as described by Weber et al. (1972). Molecular weight standards used in assigning subunit molecular weights were myosin (220 000), *Escherichia coli* β-galactosidase (130 000), transferrin (80 000), bovine serum albumin (68 000), ovalbumin (43 000), DNase I (31 000), and cytochrome *c* (11 700). The periodate–Schiff staining method used was that of Glossmann and Neville (1971).

Results

Purification of the Erythrocyte Nuclear Membrane. Although a variety of enzymes are reportedly located in the membranes of the nuclear envelope (for reviews see Kasper, 1974; Kay and Johnston, 1973; Berezney, 1974), including glucose-6-phosphatase, rotenone-insensitive NADH cytochrome *c* reductase and cytochrome *c* oxidase, the localization of these activities in the nuclear envelope relies principally on cell fractionation and may, in fact, be due to cross-contamination with mitochondria and endoplasmic reticulum. This is particularly true for cytochrome *c* oxidase (see Jarasch and Franke, 1974). Furthermore, all the enzymatic activities ascribed to the nuclear membrane are found in even higher specific activity in other cellular fractions.

Since chicken erythrocytes contain little, if any, endoplasmic reticulum and mitochondria, one might argue that any of these activities should serve as a suitable nuclear membrane marker; however, Zentgraf et al. (1971) could detect no glucose-6-phosphatase nor cytochrome *c* oxidase in any of their chicken erythrocyte fractions. They did detect NADH cytochrome *c* reductase, but its specific activity was so low that one must question whether it was the result of an endogenous nuclear membrane enzyme or of a small amount of endoplasmic reticulum derived either from leucocytes or immature erythrocytes. In view of these difficulties, no marker enzyme for nuclear membrane was used in the nuclear membrane purification; instead, nuclear membrane was characterized as the membranous fraction (by the criteria of electron microscopic appearance, high phospholipid content, low buoyant density, and low DNA content) derived from plasma membrane free nuclei.

The contamination of erythrocyte nuclear membrane preparations by plasma membrane was ascertained by assaying

TABLE I: ATPase Activity of Chicken Erythrocyte Fractions.[a]

	Fraction	(Na^+,K^+)ATPase[b]	% Plasma Membrane
1	Homogenate	9.7 ± 3.5 (4)	1.0
2	Plasma membrane	923 ± 133 (4)	100
3	Plasma membrane (No PMSF or $Na_2S_2O_6$)	879 ± 16 (1)	
4	Nuclear membrane	33 ± 9.5 (4)	3.6
5	Plasma membrane	1180 ± 145	
6	Plasma membrane (DNase/NaCl)[c]	1102 ± 112	

[a] ATPase activity was determined as described under Methods. Unless otherwise indicated, all samples were prepared in the presence of protease inhibitors (1 mg/ml of $Na_2S_2O_6$ (sodium tetrathionate) and 0.1 mM PMSF) and prereduced with 25 mM β-mercaptoethanol at 37 °C for 15 min prior to assay. [b] Activities are given in nmol mg of protein^{-1} h^{-1} ± average deviation of mean (number of independent preparations assayed). Each individual preparation was assayed at least in duplicate. [c] Sample was treated with DNase and extracted with 2 M NaCl (see text).

TABLE II: Chemical Composition of Chicken Erythrocyte and Liver Fractions.

Fraction	Component Assayed	Relative Proportions[a]	pg/Cell[b]
Erythrocytes	Protein	1.00 ± 0.04 (6)	45 ± 3 (6)
	Phospholipid	0.012 ± 0.001 (3)	0.53 ± 0.03 (3)
	DNA	0.042 ± 0.005 (6)	1.8 ± 0.2 (6)
	RNA	0.0008 ± 0.0002 (3)	0.03 ± 0.01 (3)
Erythrocyte nuclei	Protein	1.00 ± 0.05 (3)	
	Phospholipid	0.034 ± 0.002 (2)	
	DNA	0.86 ± 0.07 (3)	
	RNA	Nd[c]	
Erythrocyte plasma membrane	Protein	1.00 ± 0.05 (6)	
	Phospholipid	0.36 ± 0.06 (6)	
	DNA	0.002 ± 0.001 (3)	
	RNA	0.003 ± 0.001 (2)	
Erythrocyte nuclear membrane	Protein	1.00 ± 0.04 (5)	
	Phospholipid	0.284 ± 0.002 (5)	
	DNA	0.063 ± 0.002 (5)	
	RNA	0.015 ± 0.001 (2)	
Chicken liver nuclear membrane	Protein	1.00 ± 0.05 (2)	
	Phospholipid	0.26 ± 0.04 (2)	
	DNA	0.031 ± 0.005 (2)	
	RNA	0.042 (1)	

[a] Mass ratio of component assayed to protein ± average deviation of mean (no. of determinations). [b] Picograms/cell ± average deviation of mean (no. of determinations). [c] Nd signifies that the amount of this component was not determined.

(Na^+,K^+)ATPase activity. In order to do this accurately, it was necessary to determine the (Na^+,K^+)ATPase activity of a purified sample of erythrocyte plasma membrane. Although the sodium tetrathionate, routinely used to inhibit proteolysis during the preparation of plasma and nuclear membrane, also inhibits (Na^+,K^+)ATPase (Tobin and Akera, 1975), the *S*-sulfenylsulfonate derivative formed by the reaction of tetrathionate with protein sulfhydryls is readily reversed by mercaptans (Liu, 1967). Control experiments showed that prereduction of samples with 25 mM β-mercaptoethanol at 37 °C for 15 min produced maximal stimulation of (Na^+,K^+)ATPase activity; consequently, all samples were prereduced prior to assay of (Na^+,K^+)ATPase. Although the absolute (Na^+,K^+)ATPase activity varied somewhat from one preparation to the next, the average (Na^+,K^+)ATPase activity corresponded closely to that of a preparation of plasma membrane purified in the absence of protease inhibitors (Table I, lines 2 and 3). Measurement of the (Na^+,K^+)ATPase activity of a purified sample of plasma membrane allows a determination of the percentage of plasma membrane protein in the homogenate and nuclear membrane fractions. As reported in Table I, plasma membrane comprises approximately 1% of the homogenate protein and 3.6% of the protein of the erythrocyte nuclear membrane fraction. To show that the differential treatment of the plasma and nuclear membrane fractions did not affect these results, a sample of plasma membrane prepared in the presence of protease inhibitors was divided into two aliquots. One aliquot was treated with DNase I and extracted with 2 M NaCl as if it were nuclear membrane, the other sample was not treated. Both samples were assayed for (Na^+,K^+)ATPase activity. As shown in Table I (lines 5 and 6), the decrease in the specific activity of the DNAse-NaCl treated sample was minimal.

Chromatin contamination of the erythrocyte nuclear membrane preparation was monitored by assaying for DNA. The average mass ratio of DNA to protein in the purified nuclear membrane preparation was 0.063 compared to a ratio of 0.86 in purified nuclei (Table II) The DNA to protein ratio of the nuclear membrane fraction is comparable to that observed by others (Zentgraf et al., 1971; Kasper, 1974) and indicates a substantial purification of this membrane. The buoyant density (1.21 ± 0.01), phospholipid to protein ratio (Table II), and electron microscopic appearance (see below) of the nuclear membrane fraction corroborate its membranous nature. A yield of 0.55 ± 0.15 mg of nuclear membrane protein per ml of packed erythrocytes was obtained.

Purification of the Nuclear Membrane of Liver Cells. Liver nuclear membrane was prepared by a procedure analogous to that used to prepare erythrocyte nuclear membrane. The enzymatic data presented in Table III demonstrate that mitochondrial (succinate dehydrogenase), plasma membrane (5′-nucleotidase), and lysosomal (acid phosphatase) contamination of the nuclear membrane fraction was minimal. Although there is no satisfactory marker enzyme capable of distinguishing endoplasmic reticulum and nuclear membrane, very little endoplasmic reticulum was observed in electron micrographs of the isolated liver nuclei (see below), indicating that contamination from this source was also minimal. Electron microscopy was also used to determine the extent to which erythrocyte nuclei and ghosts contaminated the liver nuclei. Any nucleus which exhibited erythroid characteristics (oblong shape and heterochromatic appearance) was scored as erythroid in origin. By this criterion, less than 4% of the nuclei were derived from erythrocytes.

The mass ratio of DNA to protein in the purified liver nuclear membrane fraction was 0.031 (Table II). This value is approximately one-half that of the erythrocyte nuclear membrane fraction, and it is comparable to DNA to protein ratios reported by others for liver nuclear membrane (Kasper, 1974). As with the erythrocyte nuclear membrane fraction, the buoyant density (1.21 ± 0.02), phospholipid to protein ratio (Table II), and electron microscopic appearance (see below) of the liver nuclear membrane fraction corroborate its membranous nature.

TABLE III: Enzyme Activities of Chicken Liver Fractions.

Cellular Fraction	5′ Nucleotidase		Succinate Dehydrogenase		Acid Phosphatase	
	Sp Act.[a]	Relative Act.	Sp Act.[a]	Relative Act.	Sp Act.[a]	Relative Act.
Homogenate	1.07 ± 0.03(3)	1.00	1.65 ± 0.08(3)	1.00	4.47 ± 0.05(3)	1.00
Crude nuclei	0.72 ± 0.02(2)	0.67	1.58 ± 0.01(3)	0.96	3.91 ± 0.09(2)	0.87
Mitochondria	1.9 ± 0.2 (2)	1.78	2.43 ± 0.04(3)	1.47	10.6 ± 0.2 (3)	2.37
Microsomes	2.8 ± 0.3 (2)	2.62	0.09 ± 0.02(2)	0.55	8.6 ± 0.2 (3)	1.92
Nuclear membrane	0.16 ± 0.05(2)	0.15	<0.04(2)	<0.22	0.79 ± 0.08(2)	0.18
% recovery	94		75		106	

[a] Activities are given in μmol mg of protein^{-1} h^{-1} ± average deviation of mean (no. of determinations).

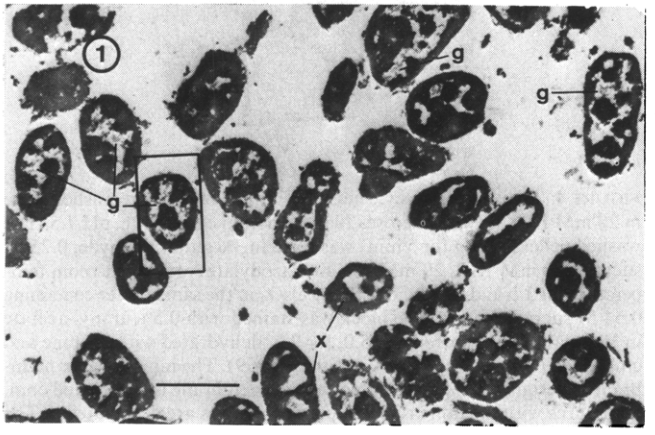

FIGURE 1: Purified erythrocyte nuclei. A nuclear pellet (3000*g* for 5 min) was fixed for 1 h at room temperature in 3% glutaraldehyde, 0.25 M sucrose, 20 KCl, 5 mM $MgCl_2$, 20 mM sodium cacodylate, pH 7.5, and postfixed with 1% OsO_4 in the same buffer for 1 h also at room temperature. After dehydration with ethanol and propylene oxide, the sample was embedded in araldite. Sections were stained with uranyl acetate and lead citrate. Intranuclear inclusions are designated X. Granular material is denoted g. The bar corresponds to 2 μm.

FIGURE 2: Erythrocyte nucleus. Portion of Figure 1 under higher magnification. Note that the granular material (g) occupying the interchromatin spaces extends to the periphery of the nucleus. The nuclear membrane is obscured by densely stained chromatin. The bar corresponds to 0.5 μm.

Chemical Composition of the Fractions. Table II presents the complete chemical composition of the erythrocyte and liver fractions. As expected, the chemical compositions of the erythrocyte and liver nuclear membrane fractions are very similar. The erythrocyte plasma membrane fraction has a DNA to protein ratio of 0.002. The hard-packed lower layer of the plasma membrane pellet, which was discarded during the plasma membrane purification (see Methods), had a DNA to protein ratio of 0.27. This indicates that the removal of the top layer of the plasma membrane pellet (see Methods) achieves a clean separation.

The number of picograms of protein, lipid, DNA, and RNA per erythrocyte are also tabulated in Table II. These values are in good agreement with those of other workers (Zentgraf et al., 1971). The small amount of RNA per cell is indicative of the extreme nuclear inactivity of the mature erythrocyte.

Ultrastructure of the Fractions. Electron micrographs (Figure 1) of the purified erythrocyte nuclei demonstrate the homogeneity of this fraction. No plasma membrane contamination is observed. Intranuclear inclusions approximately 0.15 μm in diameter (designated "x" in Figures 1 and 2) are visible within the nuclei. The function of these structures, which have been referred to as filled cavities by Walmsley and Davies (1975), is not known. Granular structures (designated "g" in Figures 1 and 2) of lower staining intensity than the heterochromatin are visible in the interchromatic spaces. As seen in Figure 2, these granular structures often extend to the periphery of the nucleus. The location of the granular structures adjacent to densely stained heterochromatin might suggest that they simply represent a more dispersed form of chromatin, i.e., euchromatin. However, this notion is dispelled when the regressive-staining technique of Bernhard (Monnerson and Bernhard, 1969) is applied (Figure 3). This staining procedure was designed to emphasize the ribonucleoprotein containing structures of the nucleus by preferentially bleaching DNA-containing structures; however, as noted by Monneron and Bernhard (1969), it is not clear whether the procedure can distinguish between a structure which contains both protein and RNA and one which contains only protein. Nevertheless, as shown in Figure 3, granular structures, with the same dimensions and distribution as those in Figure 2, are prominent in thin sections stained by the Bernhard procedure. This suggests that the granular structures extending to the nuclear periphery in Figures 2 and 3 are identical and are not simply regions of dispersed chromatin. Although the nature of the granular structures remains unknown, they bear some re-

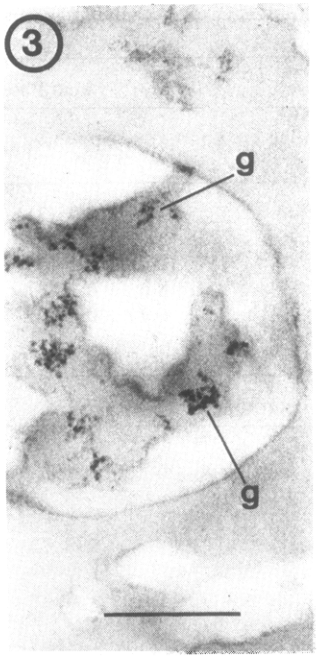

FIGURE 3: Erythrocyte nucleus stained for ribonucleoprotein. Purified nuclei were fixed with glutaraldehyde, as described in the legend to Figure 1, dehydrated with acetone, and embedded in Spurr's epoxy resin (Spurr, 1969). Sections were stained with 5% uranyl acetate, bleached with 0.2 M EDTA, and overstained with lead citrate, as described by Monneron and Bernhard (1969). Granular material (g) is prominent between the bleached masses of heterochromatin. The bar corresponds to 0.5 μm.

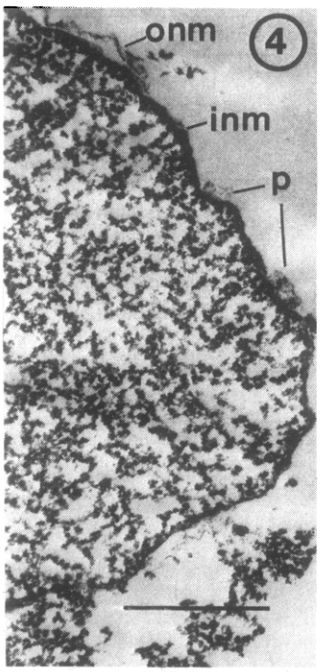

FIGURE 4: Swollen erythrocyte nucleus. Purified nuclei were washed twice in 20 mM KCl, 0.54 M sucrose, 20 mM sodium cacodylate, pH 7.5. The washed pellet (3000g for 5 min) was fixed in 3% glutaraldehyde, 0.25 M sucrose, 20 mM KCl, 20 mM sodium cacodylate, pH 7.5, at room temperature for 1 h and postfixed with 1% OsO_4 in the same buffer containing 0.54 M sucrose for 1 h. The block was stained with 0.5% uranyl acetate in Veronal acetate buffer, pH 5.0, for 2 h, dehydrated with acetone and embedded in Spurr's epoxy resin (Spurr, 1969). The inner nuclear membrane is designated inm, the outer nuclear membrane is designated onm. Structures with the dimensions of pore complexes are designated p. The bar corresponds to 0.5 μm.

semblance to the ribonucleoprotein matrix observed in rat liver nuclei (Berezney and Coffey, 1974; Zbarsky et al., 1962; Narayan et al., 1967); alternatively, they may represent remnants of the erythrocyte nucleolus. (Walmsley and Davies, 1975).

When erythrocyte nuclei are fixed in a buffer containing 5 mM $MgCl_2$, as in Figures 1, 2, and 3, densely staining heterochromatin obscures the nuclear membranes; however, when the nuclei are swollen in a buffer containing no $MgCl_2$ prior to fixation, as in Figure 4, the chromatin disperses and the membranes are visible. In accordance with the observations of Zentgraf et al. (1969) and Walmsley and Davies (1975), the Waring blender homogenization procedure removes most of the outer nuclear membrane; consequently, the erythrocyte nuclear membrane fraction prepared from such nuclei is enriched for inner nuclear membrane. However, no attempt was made to establish the exact proportion of the two membranes in the nuclear preparation, since this would require extensive electron microscopy and would not necessarily establish the proportion of the two membranes in the final nuclear membrane preparation. Structures which have the dimensions of pore complexes can be seen in cross section adhering to the inner nuclear membrane in Figure 4. Neither granular structures nor "filled cavities" comparable to those of Figures 1–3 are observed in Figure 4.

Figure 5 demonstrates the membranous nature of the erythrocyte nuclear membrane fraction. Characteristic envelope structures, comprised of inner and outer nuclear membranes, are not seen, presumably because most of the outer nuclear membrane has been removed during the nuclear preparation. Amorphous material (Figure 5 arrows), which may derive from either chromatin, the granular structures referred to above, or both, is closely associated with many of the membranes. The lack of characteristic envelope morphology and the presence of the amorphous material prevent

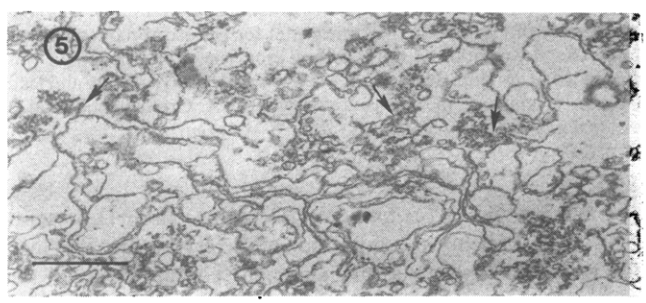

FIGURE 5: Erythrocyte nuclear membrane. An erythrocyte nuclear membrane pellet (12 000g for 30 min) was fixed with glutaraldehyde (2%) in Veronal acetate buffer at 4 °C for 1 h and postfixed with 1% OsO_4 also in Veronal acetate buffer for 1 h at room temperature. The sample was dehydrated and embedded as described in the legend to Figure 3. Sections were stained with uranyl acetate and lead citrate. Arrows denote membrane-associated amorphous materials. The bar corresponds to 0.5 μm.

the unambiguous identification of pore complexes in the nuclear membrane fraction.

As mentioned above, the liver nuclear fraction (Figure 6) appears to be free from contamination with endoplasmic reticulum and erythroid nuclei. In contrast to the erythrocyte, the liver nuclei retain most of their outer nuclear membrane; consequently, the liver nuclear membrane preparation should not be biased toward inner nuclear membrane, as is the erythrocyte nuclear membrane fraction. Nuclear pore complexes (Figure 6, inset) and granular structures resembling those observed in erythrocyte nuclei (Figures 2 and 3) are observed without the aid of the special staining techniques and hypotonic swelling used to detect these structures in erythrocyte nuclei.

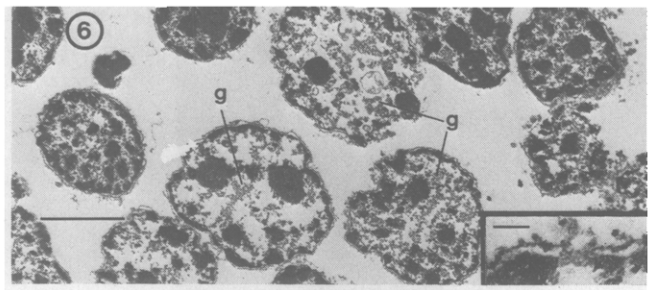

FIGURE 6: Purified liver nuclei. A pellet of liver nuclei (3000*g* for 5 min) was fixed with glutaraldehyde (3%) and postfixed with OsO_4 (1%) as described in the legend to Figure 1. Samples were embedded in Spurr's epoxy resin (Spurr, 1969) and thin sections were stained with uranyl acetate and lead citrate. Granular material is designated g. The bar corresponds to 2 μm. Inset: nuclear pore complex. The bar corresponds to 0.1 μm.

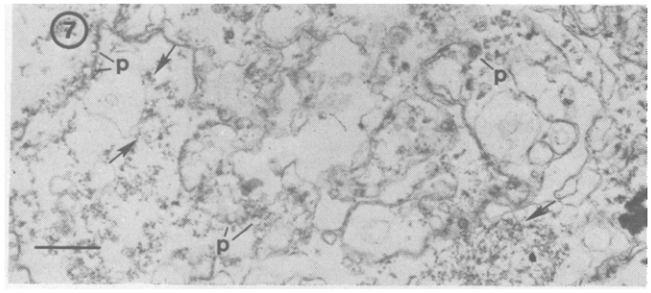

FIGURE 7: Liver nuclear membrane fraction. A pellet of liver nuclear membrane (12 000*g* for 30 min) was prepared for microscopy, as described in the legend to Figure 5. Arrows denote membrane-associated amorphous material. Structures with the dimensions of pore complexes are designated p. The bar corresponds to 0.5 μm.

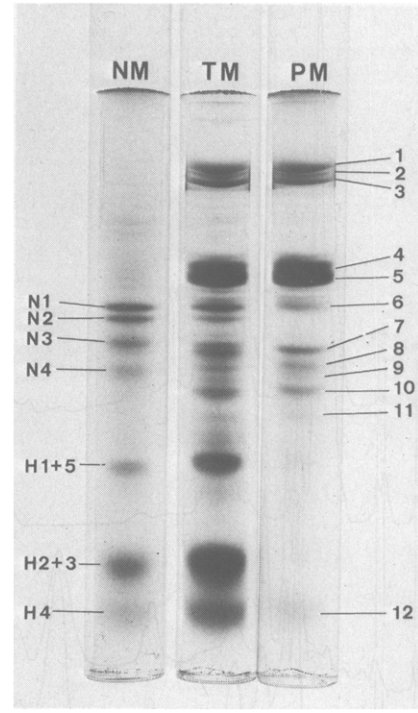

FIGURE 8: Dodecyl sulfate gel electrophoresis of chicken erythrocyte membrane fractions. Membrane fractions were prepared in the presence of protease inhibitors as described under Methods. The total membrane fraction consists of hypotonically lysed erythrocytes which have been briefly digested with DNase I (Jackson, 1975). It contains both plasma and nuclear membrane polypeptides. PM, plasma membrane fraction; TM, total membrane fraction; NM, nuclear membrane fraction. Each gel contains approximately 20 μg of protein.

The liver nuclear membrane fraction (Figure 7), like its erythrocyte counterpart (Figure 5), is composed of membranes with adherent material of amorphous appearance. The occasional observation of a nucleolar fragment in the liver membrane fraction indicates that the fraction is contaminated to a small extent with nucleoli. Typical nuclear pore complex structures are often observed in the nuclear membrane fraction (Figure 7).

Polypeptide Composition of Erythrocyte Nuclear Membrane. Figure 8 compares Coomassie blue stained gels of the erythrocyte plasma membrane (PM), total membrane (TM), and nuclear membrane (NM) fractions. The erythrocyte nuclear membrane polypeptides designated N1, N2, N3, and N4 in Figure 8 have apparent molecular weights of 80 000, 74 000, 62 000, and 50 000, respectively. Bands H1–H5 are composed primarily of histones H1–H5, respectively; however, H_2SO_4 extraction data (see below) show that these bands also contain small amounts of other polypeptides. The total membrane fraction (prepared as previously described (Jackson, 1975)) consists of DNase I digested erythrocyte ghosts. It is clear that the polypeptides of the total erythrocyte membrane are a composite of the plasma and nuclear membrane polypeptides. When protease inhibitors are omitted from the plasma membrane preparation, proteolysis produces polypeptide fragments P1, P2, and P3 (Figure 9 traces C and D). These three bands serve as sensitive indicators of the degree of degradation in plasma membrane preparations. In the lower molecular weight region of the gel, band 7 is almost completely degraded. In contrast to the plasma membrane, none of the major polypeptide bands are significantly altered in dodecyl sulfate gels of nuclear membrane prepared in the absence of protease inhibitors (Figure 9); however, there is some degradation of the faint high-molecular-weight bands (see discussion below).

When dodecyl sulfate gels of purified erythrocyte plasma membrane are stained for glycopeptides with periodate–Schiff reagent, a pattern identical to that of total erythrocyte membrane (Jackson, 1975) is obtained. On the other hand, gels of nuclear membrane do not stain with periodate–Schiff reagent, indicating that the nuclear membrane may contain no glycoprotein components. However, polypeptides with substantial amounts of covalently bound carbohydrate often stain poorly with periodate–Schiff reagent (e.g., band III of human red blood cell membranes (Ho and Guidotti, 1975)).

The origin of the faint high-molecular-weight bands (>80 000 daltons) in the erythrocyte nuclear membrane gel (Figure 9, trace A) cannot be absolutely established; however, three lines of evidence indicate that they are plasma membrane in origin. First, the pattern of the high-molecular-weight region is similar to that of the corresponding region of a plasma membrane gel (Figure 9, compare traces A and D). Each of the faint high-molecular-weight nuclear membrane bands corresponds to a recognizable plasma membrane band. Secondly, although the ambiguity involved in setting an appropriate baseline makes it impossible to determine accurately the amount of stain in the high-molecular-weight bands from scans of the nuclear membrane gels, the measured amount of stain agrees with the amount of stain expected from plasma membrane contamination (as determined by (Na^+,K^+)ATPase assay). Finally, when gel scans of nuclear membrane samples prepared in the presence and absence of protease inhibitors are compared (Figure 9, traces A and B), it can be seen that the faint high-molecular-weight bands corresponding in mobility

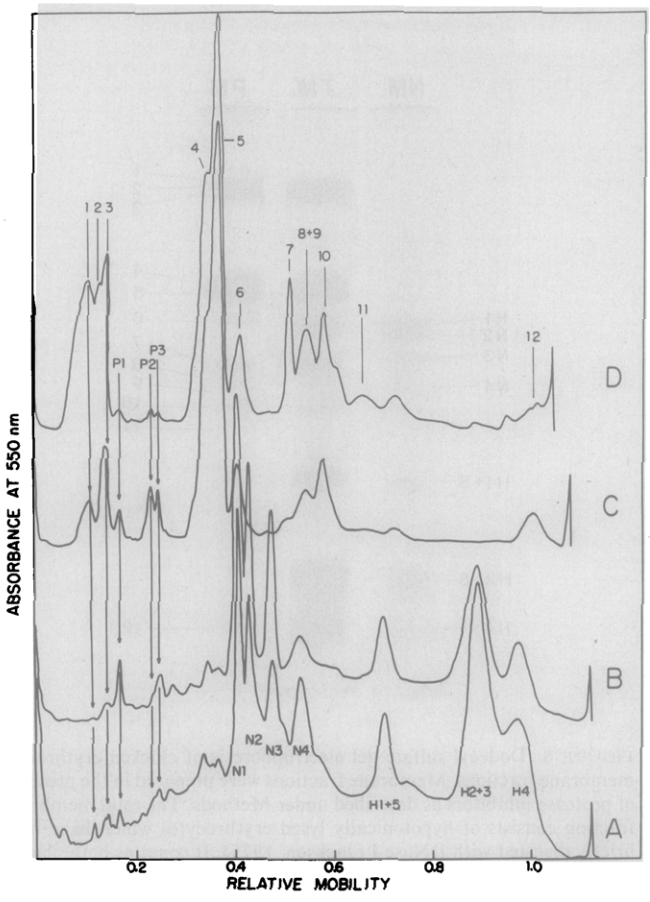

FIGURE 9: Effect of protease inhibitors on chicken erythrocyte plasma and nuclear membrane proteins. Chicken erythrocyte plasma and nuclear membranes were prepared in the presence and absence of the protease inhibitors, PMSF and sodium tetrathionate. Membrane polypeptides (20 μg) were separated by gel electrophoresis in dodecyl sulfate, stained with Coomassie brilliant blue, and scanned at 550 nm on a Gilford spectrophotometer. Trace A: nuclear membrane, with protease inhibitors; trace B: nuclear membrane, no protease inhibitors; trace C: plasma membrane, no protease inhibitors; trace D: plasma membrane, with protease inhibitors. Arrows indicate corresponding high-molecular-weight polypeptides of the plasma and nuclear membrane preparations. High-molecular-weight polypeptides of the nuclear membrane preparations are attributed to plasma membrane contamination (see text).

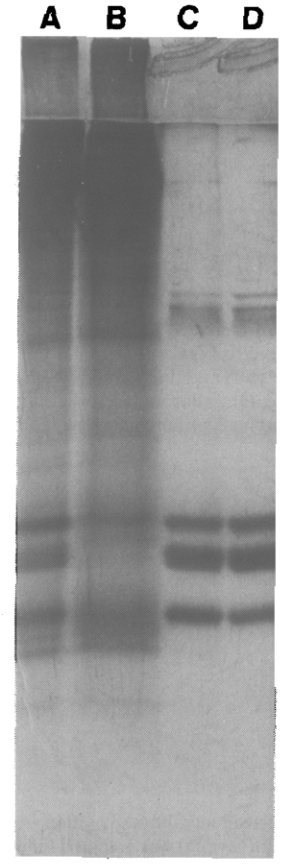

FIGURE 10: Dodecyl sulfate slab gel of H_2SO_4 extracted erythrocyte nuclear membrane. A sample of erythrocyte nuclear membrane was extracted with 0.2 M H_2SO_4 for 30 min at 4 °C and centrifuged at 27 000g for 15 min. The supernatant fraction was dialyzed against 0.9 M acetic acid, lyophilized, resuspended in dodecyl sulfate gel buffer, and subjected to electrophoresis on a 20% acrylamide gel, as described by Studier (1973). Slot A: erythrocyte nuclear membrane, unextracted, 21 μg; slot B: H_2SO_4 extracted nuclear membrane, 40 μg; slot C: H_2SO_4 extract of nuclear membrane, 3.3 μg; slot D: authentic erythrocyte histones, 2.5 μg.

to plasma membrane bands 1 and 2 disappear and bands corresponding to plasma membrane protease fragments P1–P3 appear in the nuclear membrane fraction not containing protease inhibitors. These three lines of evidence, the correspondence in molecular weight, approximate amount of stain, and proteolytic pattern in the absence of protease inhibitors, all favor the conclusion that the faint bands with apparent molecular weight greater than 80 000 in the dodecyl sulfate gel of the nuclear membrane fraction are primarily the result of plasma membrane contamination.

Attempts to further reduce the level of plasma membrane contamination by isopycnic centrifugation were disappointing. Samples of the erythrocyte nuclear membrane fraction were centrifuged to equilibrium in 20–50% sucrose gradients prepared either in 2 M NaCl or in 10 mM Tris-HCl, pH 7.5 buffer. In both cases, a membrane band collected at a density of 1.21 ± 0.01 g/cm^3. Analysis of the material by gel electrophoresis in dodecyl sulfate indicated a reduction in the amount of histone, but otherwise the polypeptide composition was identical to that of the original nuclear membrane sample. In particular, there was no reduction in the high-molecular-weight plasma membrane specific bands.

Bands H1–H5 have been identified as histones 1–5, respectively, on the basis of their electrophoretic mobility in two gel systems: Studier's high-resolution dodecyl sulfate slab gel system (Studier, 1973) and the low pH urea gel system of Paynim and Chalkley (1969). A sample of erythrocyte nuclear membrane was extracted with ice-cold 0.2 M H_2SO_4 for 30 min. After centrifugation, the pellet and supernatant fractions were assayed for protein and subjected to slab gel electrophoresis in dodecyl sulfate (Figure 10). The majority of the solubilized polypeptides derived from bands H1–H5 of the nuclear membrane fraction and comigrated with a sample of authentic erythrocyte histones (Figure 10). The H1–H5 region of the H_2SO_4 extracted membrane sample (Figure 10, slot B) contains some polypeptides which are not extracted by this procedure. Another aliquot of the H_2SO_4 extract was subjected to electrophoresis in the low pH urea gel system of Paynim and Chalkley (1969). The sample contained polypeptides with electrophoretic mobilities identical to those of a sample of erythrocyte histones (data not shown). The H_2SO_4 extraction procedure solubilized 6% of the protein of the erythrocyte nuclear membrane fraction. Whether the histones and DNA which copurify with nuclear membrane should be considered to be membrane associated is problematic. Electron micrographs of intact nuclei (Figures 1–4) indicate a close association of chromatin and the nuclear envelope. Furthermore, the

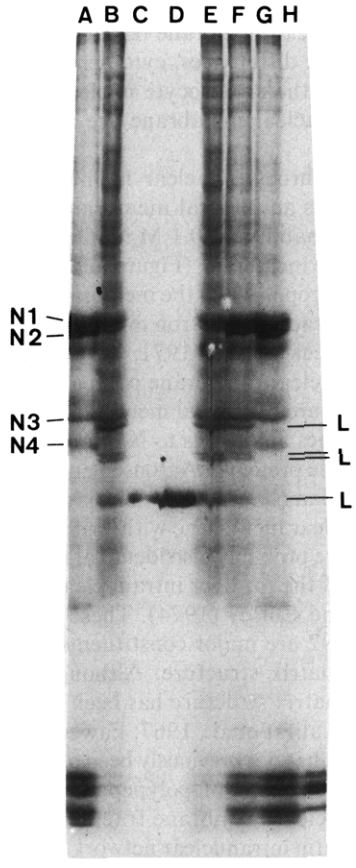

FIGURE 11: Comparison of chicken liver and erythrocyte nuclear membrane fractions. The polypeptides of the liver and erythrocyte nuclear membrane fractions were separated by electrophoresis on a 22-cm (7.5–15% polyacrylamide gradient) slab gel in dodecyl sulfate and stained with Coomassie blue. Slot A: chicken erythrocyte nuclear membrane fraction, ca. 56 μg; slot B: chicken liver nuclear membrane fraction, 57 μg; slot C: rabbit muscle actin, ca. 2 μg; slot D: rabbit muscle actin, ca. 4 μg; slot E: chicken liver nuclear membrane fraction, ca. 57 μg; slot F: liver and erythrocyte nuclear membrane fractions mixed prior to electrophoresis, 56 μg total; slot G: erythrocyte nuclear membrane, ca. 56 μg; slot H: erythrocyte histones, ca. 3 μg. The position of the major liver nuclear membrane polypeptides which have no erythrocyte nuclear membrane analogues is designated L.

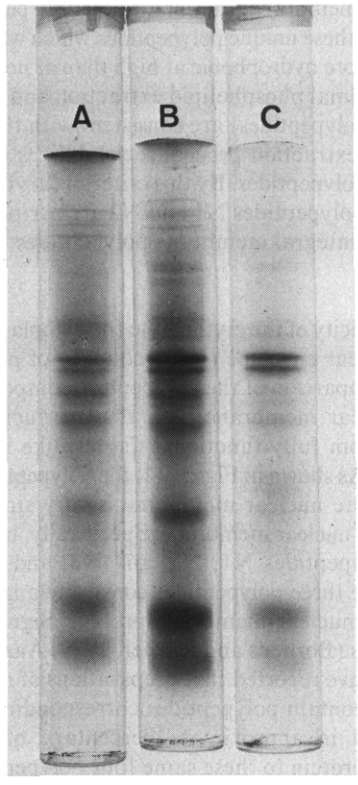

FIGURE 12: NaOH subfractionation of nuclear membrane proteins. Chicken erythrocyte nuclear membrane was extracted with 0.1 M NaOH for 40 min, as described under Methods. Polypeptides were separated by gel electrophoresis in dodecyl sulfate and stained with Coomassie blue. Gel A: NaOH-extracted membrane fraction (ca. 14 μg); gel B: unextracted nuclear membrane (ca. 30 μg); gel C: proteins released from the membrane by the NaOH extraction procedure (ca. 15 μg).

small fraction of DNA which is always found in nuclear membrane fractions has been shown to be enriched in repetitive DNA sequences (Franke et al., 1973). Nevertheless, such associations may be simply fortuitous.

Polypeptide Composition of Liver Nuclear Membrane. Although the polypeptide composition of the chicken liver nuclear membrane fraction is more complex than that of the erythrocyte nuclear membrane fraction (Figure 11), both preparations contain common polypeptide bands. Specifically, major polypeptides with apparent molecular weights of 80 000, 74 000, and 62 000 are found in both preparations. In addition, the liver nuclear membrane fraction contains four major polypeptides (designated by an "L" in Figure 11) with molecular weights of approximately 58 000, 49 000, 48 000, and 43 000 which do not comigrate with major erythrocyte nuclear membrane polypeptides. It is noteworthy that disc gels and short slab gels (10 cm) do not resolve the 58 000-dalton liver nuclear membrane polypeptide from erythrocyte band N3, nor do they resolve the 48 000- and 49 000-dalton polypeptides from erythrocyte band N4. The 43 000-dalton liver nuclear membrane polypeptide is either peripherally associated with the membranes or represents a contaminant, since it can be removed by sonication and sucrose gradient flotation of the liver nuclear membrane fraction. The liver nuclear membrane fraction contains no major bands which correspond in molecular weight to erythrocyte nuclear membrane band N4 (50 000 daltons). Comparison of the polypeptide composition of the chicken liver nuclear membrane fraction with those of mitochondrial, microsomal, and postmicrosomal supernatant fractions (not shown) supports the conclusion, drawn from enzymatic and microscopic data above, that the liver nuclear membrane fraction is free from contamination from these sources.

Separation of Peripheral and Integral Erythrocyte Nuclear Membrane Polypeptides. Recent reports (Steck and Yu, 1973; Smith and Verpoorte, 1970) have demonstrated the efficacy of high pH extraction in the subfractionation of the proteins of the human erythrocyte ghost. This procedure seems to extract nonselectively those membrane polypeptides which are not firmly embedded in the lipid bilayer. In order to gain some insight into the arrangement of nuclear membrane polypeptides with respect to the phospholipid bilayer, samples of erythrocyte nuclear membrane were extracted with 0.1 M NaOH as described under Methods. The procedure solubilized 43 ± 5% of the erythrocyte nuclear membrane protein, but less than 5% of the membrane phospholipid. Analysis of the extract and membrane fractions by gel electrophoresis in dodecyl sulfate (Figure 12) revealed that a significant proportion of bands N1 and N2 (40 and 51%, respectively, as determined from gel scans) had been solubilized by the extraction procedure, while the bulk of bands N3 (95%) and N4 (88%) remained membrane bound. Histones were observed in both the

extract and membrane fractions, reflecting, perhaps, the basic character of these unique polypeptides which would cause them to be even more hydrophobic at high than at neutral pH. These results (minimal phospholipid extraction and differential extraction of polypeptides) are consistent with the proposal that the NaOH extraction procedure solubilizes only peripheral membrane polypeptides. By this criterion, erythrocyte nuclear membrane polypeptides N1 and N2 are peripheral, while N3 and N4 are integral membrane polypeptides.

Discussion

The simplicity of the erythrocyte (no cytoplasmic organelles) and its nuclear envelope (low frequency of pore complexes) makes a comparison of the polypeptide composition of erythrocyte nuclear membrane with that of nuclear membrane prepared from fully functional tissues, like liver, especially interesting. As shown in Figure 11, the polypeptide composition of erythrocyte nuclear membrane is very similar to that of chicken liver nuclear membrane. Specifically, both membranes contain polypeptides N1, N2, and N3, and, in both membranes, these three polypeptides constitute a large percentage of the total nuclear membrane protein. Significantly, other investigators (Bornens and Kasper, 1973; Aaronson and Blobel, 1975) have reported that preparations of rat liver nuclear membrane contain polypeptides corresponding in molecular weight[2] and in approximate percentage of total nuclear membrane protein to these same four polypeptides.

The chicken liver nuclear membrane fraction and the rat liver nuclear membrane fractions obtained by other investigators (Bornens and Kasper, 1973; Aaronson and Blobel, 1975) contain a considerable proportion of high-molecular-weight (>80 000 daltons) polypeptides. While the erythrocyte nuclear membrane fraction also contains high-molecular-weight polypeptides, there is good reason to believe that the bulk of these polypeptides derive from plasma membrane contamination of the nuclear membrane fraction (see Figure 9). Bornens and Kasper also observed two high-molecular-weight glycopeptides, in their rat liver nuclear membrane fraction. Analysis of the erythrocyte nuclear membrane by the periodate–Schiff staining technique revealed no glycopeptides. Whether these differences are an expression of the increased nuclear activity of liver as compared to erythrocytes, or, as in the case of the faint high-molecular-weight erythrocyte nuclear membrane polypeptides, an expression of cross-contamination with cytoplasmic membranes, remains to be determined.

Since the number of pore complexes per square micron of surface area of the erythrocyte nuclear envelope is approximately one-third that of the liver nuclear envelope (Franke, 1967; Kartenbeck et al., 1971), one would expect to see a substantial increase in the proportion of any nuclear pore complex polypeptides in liver as compared to erythrocyte nuclear membrane. Nevertheless, the most prominent erythrocyte nuclear membrane polypeptides (N1, N2, and N3) are equally prominent in both chicken and rat liver nuclear membrane preparations. These results suggest that none of the major polypeptides common to both erythrocyte and liver nuclear membrane are elements of the nuclear pore complex. Differences between the polypeptide compositions of the liver and erythrocyte nuclear membrane fractions are more difficult to interpret. Tissue differences, cytomembrane contamination, and the bias of the erythrocyte nuclear membrane fraction toward inner nuclear membrane are all possible sources of variation.

Chicken erythrocyte nuclear membrane polypeptide N3 (and also N4) is an integral membrane polypeptide, as evidenced by the inability of 0.1 M NaOH to extract this polypeptide from the membrane (Figure 12). It probably comprises a considerable proportion of the membrane-associated particles seen in freeze-fracture electron micrographs of nuclear envelope (Kartenbeck et al., 1971; Monneron et al., 1972). Erythrocyte nuclear membrane polypeptides N1 and N2, on the other hand, are peripheral membrane polypeptides (Figure 12). Polypeptides analogous to N1 and N2 comprise a large percentage of the protein of Aaronson and Blobel's (1975) pore complex-dense lamella fraction which is prepared by extraction of rat liver nuclear membrane with Triton X-100. These same polypeptides are probably also identical to the most prominent polypeptides of the rat liver intranuclear matrix preparation of Berezney and Coffey (1974). These observations suggest that N1 and N2 are major constituents of a dense lamella–intranuclear matrix structure. Although a dense lamella–intranuclear matrix structure has been observed in a variety of cell types (Kalifat et al., 1967; Fawcett, 1966; Patrizi and Poger, 1967), it has not previously been observed in erythrocyte nuclei. The occurrence of polypeptides N1 and N2 in the erythrocyte nuclear membrane fraction and the observation of a nonchromatin intranuclear network with extensions to the nuclear periphery (Figure 3) indicate that erythrocyte nuclei do indeed contain a dense lamella–intranuclear matrix structure. The existence of a dense lamella–intranuclear matrix in this, perhaps the simplest of all nuclei, suggests that this structure may be a ubiquitous feature of all eukaryotic cells.

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[2] Bornens and Kasper (1973) report apparent polypeptide molecular weights of 74 000, 70 000, and 60 000 for these three bands, while Aaronson and Blobel (1975) report apparent molecular weights of 69 000, 68 000, and 50 000. My findings, 80 000, 74 000, and 62 000, agree more closely with those of Bornens and Kasper (1973). These discrepancies in molecular weight are probably attributable to the use of different gel electrophoresis systems and molecular weight standards.

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