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NUCLEOTIDE PHOSPHOHYDROLASE ACTIVITIES OF THE PLASMA MEMBRANES OF EMBRYONIC CHICK LIVER CELLS

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SUMMARY

Plasma membrane fractions of embryonic chick liver cells have been isolated by the techniques of homogenization, differential centrifugation in isotonic, low ionic strength medium, and zonal centrifugation. The purity of the fractions has been determined by electron microscopy and the absence of enzymatic markers for contaminating subcellular fractions. CTPase (EC 3.6.1.4) specific activities are high for plasma membranes isolated from embryonic chick livers. Other nucleoside triphosphatase and nucleoside diphosphatase activities are associated with the plasma membranes. It has not been determined if all nucleotide phosphohydrolase activities are due to a single enzyme or group of enzymes.

INTRODUCTION

Studies of embryonic cell plasma membranes are desirable in elucidating the roles of plasma membrane in cell morphogenesis and development. The embryonic chick has been one of the subjects of classical investigations in embryology. An organ highly useful for developmental studies is the liver because it attains a large size in terms of tissue mass early in development and is easy to identify and remove in very young embryos, thus assuring homogeneous tissue for isolation of plasma membranes. Also, many embryos of the same age and strain may be processed in a short period of time and their livers may be pooled to provide a sufficient quantity for subcellular fractionation.

With exception of the investigations of Rosenberg[1] and Perdue and Sneider[2], no procedures for the isolation of plasma membranes from embryonic chick cells have been reported. Rosenberg[1] reported the isolation of plasma membranes from pooled embryonic chick livers while Perdue and Sneider[2] isolated plasma membranes from primary cell cultures of embryonic chick fibroblasts. Both investigators relied upon swinging bucket isopycnic sucrose-gradient centrifugation of homogenates thus limiting yields of plasma membranes from a single preparation.

Several investigators have reported excellent results in applications of zonal centrifugation for isolation of plasma membranes from rat liver[3–5]. Zonal centrifugation is desirable since it allows isolation of milligram quantities of plasma membranes in a single step of centrifugation. The procedure is rapid and avoids the repetitive steps of isopycnic centrifugation needed for isolation of comparable yields of

membranes. The ability to isolate milligram quantities of plasma membranes for morphological and biochemical characterization in a single step of centrifugation is desirable. The purposes of this investigation are to establish a large scale isolation procedure for embryonic chick liver plasma membranes and to assess enzymes associated with the plasma membrane fraction.

MATERIALS AND METHODS

Equipment

Rate-isopycnic zonal centrifugation was performed in a modified Beckman Model L ultracentrifuge using a Ti-15 zonal rotor. Linear with respect to volume sucrose gradients were programmed using a Beckman 141 high capacity gradient pump.

The products of the reaction catalysed by nucleotide phosphohydrolases were analyzed by means of high-pressure, high temperature liquid column chromatography. The apparatus consisted of a stainless steel column (max. pressure 750 lb/inch^2) operated at 47 °C and 400 lb/inch^2. Zipax SAX resin was used as the stationary phase; 50 mM phosphate buffer (pH 6.55) was the mobile phase. Column dimensions were 90 cm × 1.8 mm. Effluents were monitored with a 254-nm ultraviolet monitor equipped with an 8-μl flowcell.

A Zeiss Photomicroscope, equipped with phase contrast and Nomarski differential interference contrast optics, was employed for light microscopy of homogenate fractions.

Transmission electron microscopy was performed on a Hitachi HU-11C microscope. Sections were obtained with a Porter–Blum ultramicrotome.

Analytical procedures

Nucleotide phosphohydrolase determinations were conducted in 3 mM nucleoside diphosphate or triphosphate, 3 mM $MgCl_2$, 100 mM KCl, 12.5 mM Tris–HCl buffer (pH 8.0) and 15–20 μg of plasma membranes. Choline chloride was added so that the ionic strength, $I = 0.25$. The final volume of the reaction mixture was 1.0 ml. Reactions were allowed to proceed for 30 min at 37 °C and stopped by the addition of 0.25 ml of 25 % (w/v) cold trichloroacetic acid. Tubes were centrifuged at 1000 × g for 15 min; orthophosphate was determined in the supernatant by the method of Ames[6]. Nucleotide phosphohydrolase determinations on sucrose-gradient fractions were accomplished by diluting 1:2 with 5 mM Tris–HCl buffer (pH 7.4) and centrifuging for 15 min at 1000 × g. Pellets, suspended in the buffer to one-half the previous volume, were recentrifuged under the identical conditions. Pellets were resuspended in the buffer, pooled, and stored at 5 °C until assayed.

Marker enzyme determinations for contaminating cellular organelles were performed. Unless otherwise indicated, the procedure was identical to the reference listed below. NADH–cytochrome c reductase (E.C 1.6.2.1) activity was determined by the method of Sottocasa *et al.*[7] with 50 mM phosphate buffer (pH 7.6). Glucose-6-phosphatase (EC 3.1.3.9) activity was measured by the method of Swanson[8] modified by the addition of 4 mM EDTA and 2 mM KF (ref. 9). Acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) activities were determined by the method of Hubscher and West[9]. Succinate dehydrogenase (EC 1.3.99.1) activity was

determined by the method of King[10]. Orthophosphate products from glucose-6-phosphatase, acid phosphatase, and alkaline phosphatase determinations, were measured by the method of Ames[6]. Protein was determined by the method of Lowry *et al.*[11]; bovine serum albumin was used as standard. NADH–cytochrome *c* reductase and succinate dehydrogenase activities were measured at 25 °C. Other marker enzyme activities were determined at 37 °C.

All nucleoside diphosphates and triphosphates, with the exception of ATP, were sodium salts and were purchased from P–L Biochemicals. ATP was the Tris salt and was purchased from Sigma Chemical Co. Other chemicals were reagent grade.

Disc gel electrophoresis

For sodium dodecyl sulfate polyacrylamide gel (7–5 % cross-linked) electrophoresis, plasma membranes in 5 mM Tris–HCl buffer (pH 7.4) were dialyzed for 48 h against 5 mM EDTA–5 mM 2-mercaptoethanol (pH 7.5) at 5 °C as described by Lenard[12]. Following the procedure of Laemmli[13], the dialysate was adjusted to a concentration of 1 % (w/v) in sodium docedyl sulfate and heated at 100 °C for 3 min. 50–100 μg of protein were electrophoresed at a current of 2.5 mA/tube. Gels were removed from tubes by squirting water between the gel and the wall of the tube with a hypodermic needle and fixed in 50 % (w/v) trichloroacetic acid for 12 h prior to staining with 0.1 % (w/v) Coomassie brilliant blue in 20 % trichloroacetic acid for 2 h. Destaining was accomplished by repeated changes of 7 % (w/v) acetic acid.

Electron microscopy

Following isolation, plasma membranes were centrifuged at 10800 × *g* for 15 min. Pellets were fixed in 3.5 % glutaraldehyde and postfixed in 1 % (w/v) OsO_4. Fixed plasma membranes were dehydrated in an acetone series and embedded in Epon. Sections on 200 mesh, carbon-coated grids were stained with uranyl acetate and lead citrate preceding examination with the electron microscope.

Isolation of plasma membranes

Fertilized white leghorn eggs were obtained from the Poultry Science Department, University of Minnesota or Ghostley Enterprises, Anoka, Minn. Fresh fertilized eggs were incubated at 39 °C for the desired period of time before removal of the embryo liver for isolation of plasma membrane. Livers were homogenized at 4 °C in a loose fitting Dounce homogenizer (maximum radial clearance 300 μm). 15 g of livers were pooled in a preweighted beaker containing homogenization medium (0.25 M sucrose–5 mM Tris–HCl buffer (pH 7.4)). The livers were gently minced with scissors, then submitted to several washes of homogenization medium for removal of red blood cells. Homogenization medium was decanted from the mince; fresh medium was added to equal a 1:4 dilution (w/v) of the wet liver weight. The mince was passed through a garlic press, with washing, for removal of vascular and capsular elements. Homogenization of the suspension in a Dounce homogenizer (4–5 even strikes by hand without foaming) was followed by filtering through a double layer of cheesecloth.

10-ml volumes of the homogenate were layered over 25-ml volumes of 43 % (w/w) sucrose and centrifuged at 10400 × *g* for 15 min. This procedure resulted in pelleting of red blood cells and nuclei. Plasma membrane, mitochondria, endoplasmic reticulum,

lysosomes and the soluble fractions remained at or above the homogenate–43 % sucrose interface. After reaming the tubes with wooden applicator sticks, supernatant fractions were decanted so as not to disturb the pellets. The pooled supernatant fractions were dispersed with one stroke of the Dounce homogenizer and homogeni-

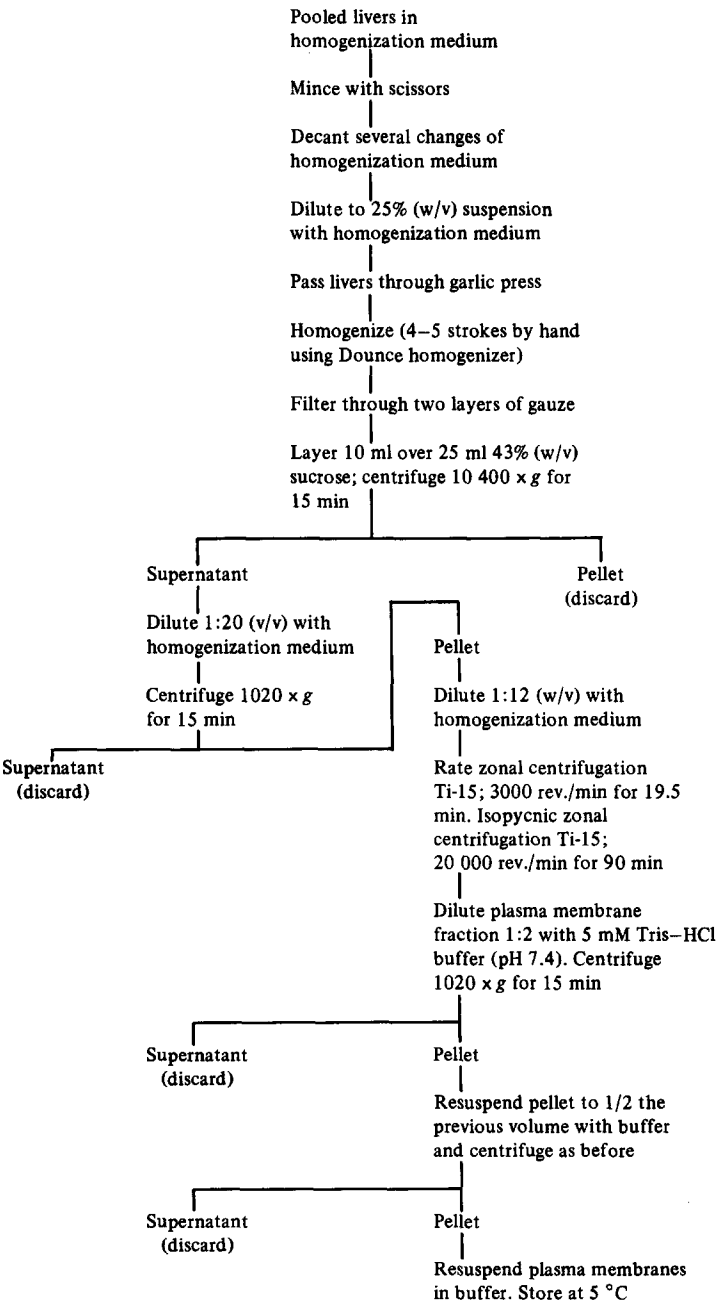

Fig. 1. Flow chart for isolation of plasma membranes.

zation medium was added to equal a 1:100 dilution of the wet weight of liver. Pellets were discarded.

The dilute homogenate was centrifuged in a GSA rotor at 1020 × *g* for 15 min. Pellets were resuspended in a sufficient homogenization medium to equal a 1:12 (w/v) dilution of the wet weight of liver and dispersed with one stroke of the Dounce homogenizer. Final volume of the homogenate was 180 ml.

The rate–isopycnic zonal centrifugation procedure detailed below is a modification of that described by Pfleger *et al.*[3]. The Ti-15 rotor, spinning at 3000 rev./min in the Beckman Model L ultracentrifuge, was programmed with a 500 ml 20–35 % (w/w), linear with respect to volume, sucrose gradient. Sufficient 45 % (w/w) sucrose was added to complete fill the rotor. Filling was accomplished from the rotor edge, displacing the sucrose gradient to the rotor core. The 180 ml of homogenate, in homogenization medium, was added to the top of the gradient using a 50-ml Luer-lok syringe. An overlay, 200 ml of 5 mM Tris–HCl buffer (pH 7.4) was added. Equal volumes of 45 % sucrose were displaced from the rotor edge with addition of the homogenate and overlay. After loading, the rotor was allowed to spin at 3000 rev./min for 19.5 min.

Following centrifugation, twenty 40-ml fractions were displaced by the introduction of 45 % sucrose from the rotor edge. A second overlay, 50 ml of the buffer previously described, was added and the rotor accelerated to 20000 rev./min for 90 min. An additional ten 40-ml fractions were collected by displacement with 50 % (w/w) sucrose following deceleration to 3000 rev./min. Specific gravities of fractions were determined with a gravimeter. The fraction having a density (*d*) of 1.18–1.19 and peak 280-nm absorbance was pooled with two additional fractions, one from either side of this tube in the gradient. These pooled fractions were diluted 1:2 with 5 mM Tris–HCl buffer (pH 7.4) and centrifuged at 1020 × *g* for 15 min. The resulting pellets were suspended in one-half the previous volume with buffer and recentrifuged under identical conditions. Washed plasma membrane pellets were again resuspended in buffer and stored at 4 °C for nucleotide phosphohydrolase and marker enzyme determinations. Fig. 1 summarizes the isolation procedure.

RESULTS

Isolation of plasma membrane

Electron micrographs of plasma membrane preparations revealed little contamination with rough endoplasmic reticulum and no contamination with nuclei or mitochondria (Fig. 2). Plasma membranes of 15-day-old embryonic chick livers demonstrate gap junctions and "unit membrane" structure (Fig. 3). The average yield of plasma membrane for three preparations of 15-day-old embryo chick liver was 221 μg of plasma membrane protein per g of wet liver weight; protein averaged 45 % of plasma membrane dry weight.

Several modifications of the final isolation procedure were performed in attempts to maximize both plasma membrane yield and enzymatic activity of plasma membranes. Ray[14] reported increased rat plasma membrane 5'-nucleotidase (EC 3.6.1.5) activities in preparations isolated in 10^{-3} M Ca^{2+}. In the embryonic chick liver system, inclusion of 10^{-3} M Ca^{2+} in the basic 0.25 M sucrose–5 mM Tris–HCl buffer (pH 7.4) homogenization medium resulted in both clumping of the homogenate and contami-

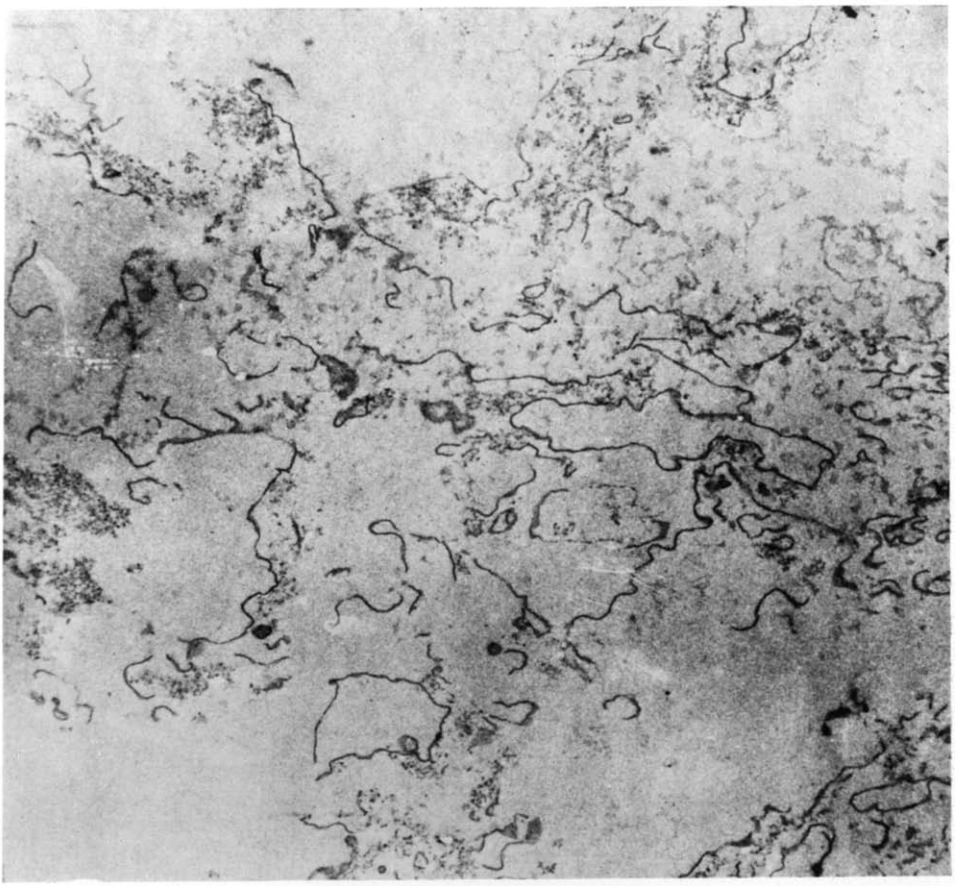

Fig. 2. Low-magnification electron micrograph of 15-day-old embryonic chick liver plasma membranes. Membranes were prepared as described in Materials and Methods. Magnification 17680 ×.

nation of the plasma membrane with adhered mitochondria, presumably due to reduction of the electrical double layer. Clumping and adhesion also occurred when embryonic chick liver was homogenized in Hank's balanced salt solution and isotonic saline for isolation of plasma membranes. An attempt was made to reduce adherence of mitochondria by suspending the 1000 × *g* pellet in medium containing 10^{-3} M EDTA prior to zonal centrifugation. However, this treatment resulted in the release of nucleohistone and gelling of the homogenate. Isolation of embryonic chick liver plasma membranes by isopycnic sucrose gradient centrifugation following homogenization in high ionic strength medium may be attributed to high centrifugal forces and high dilutions[2].

In isolating plasma membrane from embryonic chick liver, two conflicting factors must be taken into account. The addition of divalent cations will reduce the release of nucleohistone but enhance clumping of subcellular fractions. This difficulty can be partially overcome by using high dilutions and high *g* forces for long periods of centrifugation, but a very large number of repetitive centrifugations are required for useful yields. The zonal procedure described allows for minimal dilution of the liver

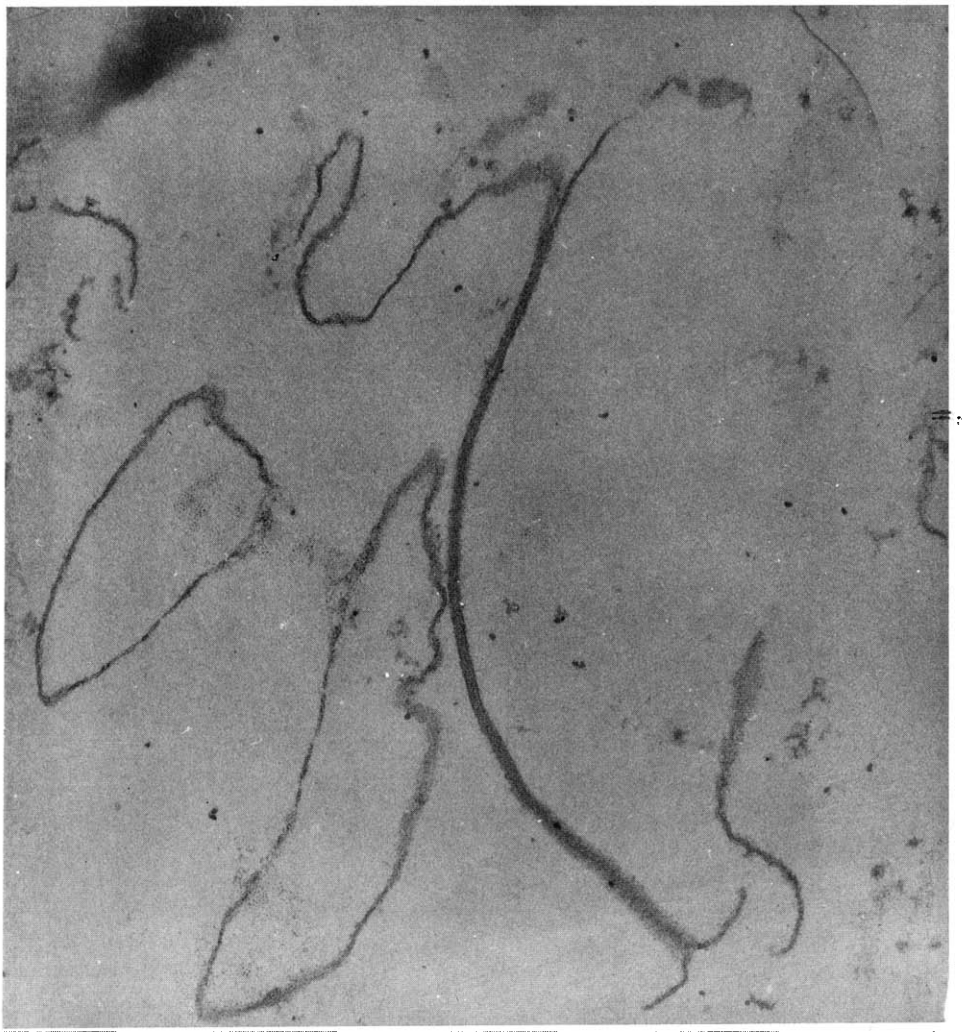

Fig. 3. High-magnification electron micrograph of 15-day-old embryonic chick liver plasma membrane gap junction. Note "unit membrane" structure. Magnification 82500 ×.

with homogenizing medium without addition of exogenous cations. Endogenous cations are sufficient to prevent nucleohistone release and many nuclei are separated from the homogenate in a very early step. Clumping of the homogenate is greatly reduced and yield of plasma membrane is high.

Embryonic chick liver is rich in red blood cells; for this reason use of a hypotonic homogenization medium is not advised. Removal of red blood cells early in the isolation is desirable to prevent contamination of the plasma membrane preparation with red blood cell ghosts due to mechanical lysis. Many red blood cells are removed by washing the livers several times with homogenization medium after mincing. Layering the liver homogenate over 43% (w/w) sucrose and centrifugation at 10000 × g for 15 min yields a pellet containing red blood cells and many nuclei. The few re-

maining red blood cells and nuclei are centrifuged to the rotor wall during zonal centrifugation.

Sucrose gradient and $A_{280\,nm}$ profiles of a typical 15-day-old embryonic chick liver plasma membrane preparation are presented in Fig. 4. Tubes 1–20 contain the 800 ml collected following rate-centrifugation at 3000 rev./min for 19.5 min. Tubes 21–30 contain the 400 ml collected following isopycnic centrifugation. Maximum $A_{280\,nm}$ in the isopycnic fractions is found in Tube 25 corresponding to a d of 1.188.

CTPase (EC 3.6.1.4) determinations were performed on the total homogenate and on Fractions 7, 11 and 25 of the sucrose gradient. These fractions corresponded to $A_{280\,nm}$ maxima of Tubes 1–30. In addition, CTPase determinations were performed on microsomal and mitochondrial fractions prepared as previously reported[15]. Specific activities are given in Table I. All specific activities are reported as units

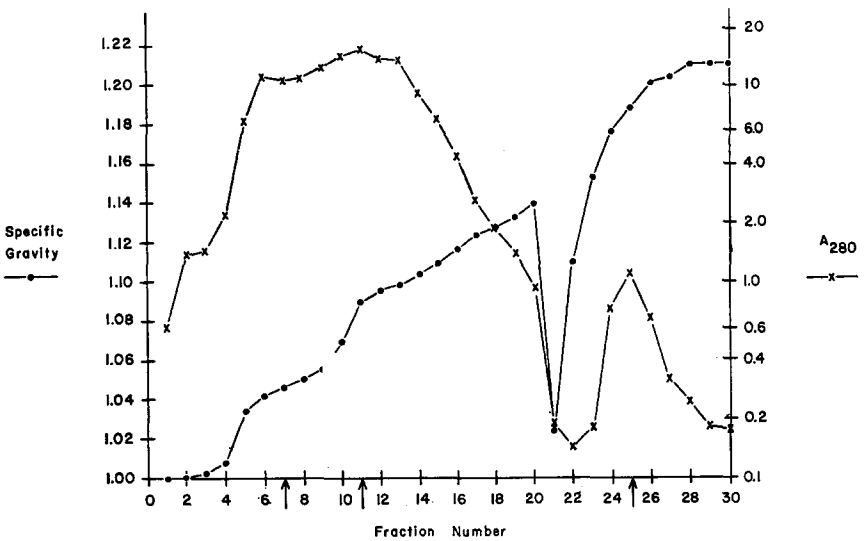

Fig. 4. Sucrose gradient and $A_{280\,nm}$ profiles of rate-isopycnic zonal centrifugation of 15-day-old embryonic chick liver homogenate. Arrows indicate $A_{280\,nm}$ maxima.

TABLE I

COMPARISON OF CTPASE SPECIFIC ACTIVITIES IN SELECTED FRACTIONS OF RATE–ISOPYCNIC ZONAL CENTRIFUGATION AND SUBCELLULAR FRACTIONS OF 15-DAY-OLD EMBRYONIC CHICK LIVER HOMOGENATE

Fraction	*Specific activity (units/mg)* *
0 (total homogenate)	0.300
7	0.400
11	0.147
25	3.100
Mitochondria	0.174
Microsome	0.175

* Unit: 1 μmole P_i liberated per min.

per mg of plasma membrane protein; a unit is defined as 1 μmole of the substrate catalyzed or phosphate hydrolyzed per min under the assay conditions. Fraction 25 ($d = 1.188$), which contains plasma membranes, exhibits a 7-fold increase in activity over the next most active fraction and a 10-fold increase in activity over the total homogenate. In agreement with the data of Perdue and Sneider[2] for fibroblast plasma

TABLE II

NUCLEOSIDE TRIPHOSPHATE HYDROLASE SPECIFIC ACTIVITIES FOR THREE PLASMA MEMBRANE PREPARATIONS FROM 15-DAY-OLD EMBRYONIC CHICK LIVERS

Substrate	*Specific activity (units/mg)* *		
	x	$\bar{x}$	*S.E.*
ATP	2.37		
	2.70	2.42	0.152
	2.18		
CTP	4.23		
	4.38	4.08	0.232
	3.62		
GTP	2.43		
	2.75	2.64	0.108
	2.75		
UTP	1.73		
	1.55	1.69	0.070
	1.78		

* Unit: 1 μmole P_i liberated per min.

TABLE III

NUCLEOSIDE DIPHOSPHATE HYDROLASE SPECIFIC ACTIVITIES FOR THREE PLASMA MEMBRANE PREPARATIONS FROM 15-DAY-OLD EMBRYONIC CHICK LIVERS

Substrate	*Specific activity (units/mg)* *		
	x	$\bar{x}$	*S.E.*
ADP	1.11		
	0.84	0.95	0.082
	0.90		
CDP	0.07		
	0.10	0.08	0.007
	0.08		
GDP	0.45		
	0.29	0.37	0.047
	0.38		
UDP	0.28		
	0.20	0.24	0.023
	0.24		

* Unit: 1 μmole P_i liberated per min.

membrane, high specific activity of CTPase is associated with the plasma membrane fraction of embryonic chick liver homogenates.

Nucleoside diphosphate hydrolase and triphosphate hydrolase specific activities for three plasma membrane preparations from 15-day-old embryonic chick livers are given in Tables II and III. No significant nucleoside monophosphate hydrolase (5'-nucleotidase) activity was detected while nucleoside diphosphate hydrolase (EC 3.6.1.6) activities were one-tenth those of the nucleoside triphosphate hydrolases.

TABLE IV

MISCELLANEOUS NON-PLASMA MEMBRANE MARKER ENZYME SPECIFIC ACTIVITIES FOR THREE PLASMA MEMBRANE PREPARATIONS FROM 15-DAY-OLD EMBRYONIC LIVERS

Enzyme	*Specific activity (μmoles/mg protein per min)*	
Acid phosphatase	0.026	
	0.030	
	0.020	
Alkaline phosphatase	0.000	
	0.005	
	0.000	
Glucose-6-phosphatase	0.007	0.813 (embryonic chick liver microsomes)
	0.000	
	0.003	
Succinate dehydrogenase	0.000	0.420 (embryonic chick liver mitochondria)
	0.000	
	0.000	
NADH–cytochrome *c* reductase	0.028	0.252 (embryonic chick liver microsomes)
	0.036	
	0.014	

TABLE V

NUCLEOTIDE PHOSPHOHYDROLASE AND PHOSPHATASE ACTIVITIES FOR TOTAL HOMOGENATE AND PLASMA MEMBRANE PREPARATIONS FROM 15-DAY-OLD EMBRYONIC LIVERS

Nucleotide substrate or enzyme	*Total homogenate*		*Plasma membrane fraction*		*Enrichment*	*% recovery*
	*Units/mg**	*Total units**	*Units/mg**	*Total units**		
ATP	0.155	178	2.42	8.03	15.6	4.5
CTP	0.300	343	4.08	13.6	13.5	4.0
GTP	0.297	340	2.64	8.50	8.9	2.5
UTP	0.349	399	1.69	5.61	4.8	1.4
ADP	0.133	152	0.95	3.15	7.1	2.1
CDP	0.018	20.6	0.08	0.27	4.4	1.3
GDP	0.113	130	0.37	1.23	3.3	0.9
UDP	0.066	75.6	0.24	0.08	3.6	1.1
Acid phosphatase	0.015	22.0	0.02	0.06	1.3	0.0
Alkaline phosphatase	0.028	40.0	0.00	0.00	0.0	0.0
Glucose-6-phosphatase	0.120	173	0.00	0.00	0.0	0.0

* Unit: 1 μmole P_i liberated per min.

Marker enzyme specific activities for possible contaminating fractions are given in Table IV. Marker enzyme activities for microsomal and mitochondrial fractions are also reported in Table IV. Table V presents specific activities, total activities, enrichments and recoveries for the phosphate hydrolases assayed. Recoveries for nucleotide phosphohydrolases are predicated on the assumption that all hydrolysis of a nucleotide substrate by the total homogenate fraction is due to the nucleotide phosphohydrolase. The most active plasma membrane nucleotide phosphohydrolases investigated are the nucleoside triphosphate hydrolases.

Both Rosenberg[1] and Perdue and Sneider[2] report detecting $(Na^+ + K^+)$-stimulated Mg^{2+}-ATPase (EC 3.6.1.3) in plasma membrane preparations of embryonic chick cells. However, $(Na^+ + K^+)$-stimulated Mg^{2+}-ATPase, inhibited by cardiac glycosides and stimulated in the presence of 100 mM Na^+ and 10 mM K^+, was not detected in embryonic chick liver plasma membranes prepared by the method described.

TABLE VI

DIVALENT CATION REQUIREMENTS FOR CTPASE ACTIVITY OF PLASMA MEMBRANES PREPARED FROM 15-DAY-OLD EMBRYONIC CHICK LIVERS

Cation (3 mM)	*% of maximum activity*
Mg^{2+}	100
Ca^{2+}	66
Mn^{2+}	44
$Mg^{2+} + Ca^{2+}$	75
No Mg^{2+}	7

In this system, nucleoside triphosphate hydrolase requires divalent cations for activity and has a pH optimum of 8.0–8.5 (Table VI). The apparent lack of marked substrate specificity could indicate that the enzyme is a nucleoside triphosphate pyrophosphohydrolase as described by Lieberman *et al.*[16] for rat liver plasma membranes. Nucleoside triphosphate pyrophosphohydrolase catalyzes the following generalized reaction:

$$\text{Nucleoside 5'-triphosphate} \xrightarrow{Mg^{2+}} \text{nucleoside 5'-monophosphate} + PP_i$$

Inorganic pyrophosphate is the hydrolysis product of the nucleoside triphosphate pyrophosphohydrolase, and ubiquitous inorganic pyrophosphohydrolases could catalyze the formation of an orthophosphate which would react in the Ames phosphate determination. To exclude this possibility the following experiments were conducted. $Na_4P_2O_7$ was substituted for nucleoside triphosphate in equimolar quantities in the standard reaction mixture. This substrate was not hydrolyzed by the plasma membrane preparation. As an additional test, yeast inorganic pyrophosphate hydrolase was added to the reaction mixture. There was no increase in the concentration of orthophosphate when a nucleoside triphosphate was used as substrate. From these experiments, we have concluded that orthophosphate is the hydrolysis product of the membrane associated enzyme and that the enzyme is not a nucleoside triphosphate pyrophosphohydrolase nor an inorganic pyrophosphohydrolase.

A direct assessment of the nucleotide products of nucleoside triphosphate hydrolase reactions was afforded by the technique of high-pressure, high-temperature liquid column chromatography. The corresponding nucleoside diphosphate was the major hydrolysis product when ATP, CTP, GTP, or UTP was used as substrate. These observations further support our proposal that the plasma membrane bound enzyme is a nucleoside triphosphate hydrolase.

Disc gel electrophoresis

When membranes were solubilized with sodium dodecyl sulfate for disc gel electrophoresis as described in Materials and Methods, more than 20 bands were observed in the polyacrylamide separating gel following electrophoresis and staining with Coomassie brilliant blue. The gels were calibrated by plotting the distance of migration of similarly treated proteins of known molecular weight as a function of the log of their molecular weight[12]. Fig. 5 is a photograph of a Coomassie brilliant

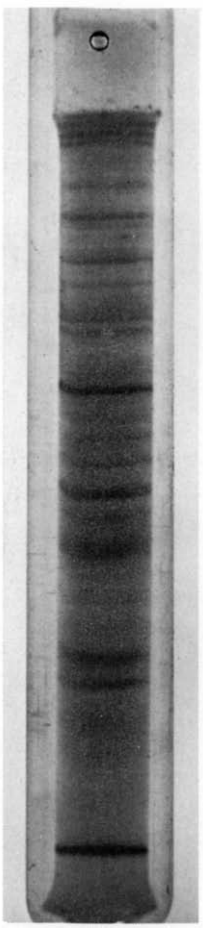

Fig. 5. Polyacrylamide gel electrophoresis of sodium dodecyl sulfate solubilized 15-day-old embryonic chick liver plasma membranes. Membranes were solubilized, electrophoresed and stained as described in Materials and Methods.

blue stained plasma membrane polyacrylamide gel. Five "major" bands of the solubilized membrane corresponded to "molecular weights" of 74000, 54000, 46000, 29000 and 28000.

CONCLUSION

Perdue and Sneider[2] found high specific activities of CTPase in plasma membranes prepared from primary cultures of embryo chick fibroblasts. Specific activities of embryonic chick liver plasma membranes isolated by the procedure described in this paper are on the order of 20 times greater than in plasma membranes isolated by the method of Perdue and Sneider. The relatively low activity of the enzyme may be intrinsic to fibroblasts.

Perdue and Sneider[2] reported Mg^{2+}-ATPase (EC 3.6.1.4), $(Na^+ + K^+)$-stimulated Mg^{2+}-ATPase, ADPase (EC 3.6.1.6), AMPase (EC 3.6.1.5), CDPase (EC 3.6.1.6) and UTPase (EC 3.6.1.4) activities in the plasma membranes of embryonic chick fibroblasts. With the exceptions of AMPase and $(Na^+ + K^+)$-stimulated Mg^{2+}-ATPase, these activities were detected in plasma membranes prepared from embryonic chick liver. GTPase (EC 3.6.1.4), GDPase (EC 3.6.1.6) and UDPase (EC 3.6.1.6) activities were also detected in membranes prepared as described. We do not as yet know whether all nucleotide phosphohydrolase activities are due to a single enzyme or several enzymes.

High specific activity of 5'-nucleoside monophosphatase is found in rat liver plasma membrane preparations. Many investigators also observe $(Na^+ + K^+)$-stimulated Mg^{2+}-ATPase[4,5,17,18]. Other investigators seeking a source of $(Na^+ + K^+)$-stimulated Mg^{2+}-ATPase have routinely prepared membranes from brain and kidney[19,20]. Meldolesi *et al.*[21] were not able to detect $(Na^+ + K^+)$-stimulated Mg^{2+}-ATPase in plasma membranes isolated from pancreatic cells. Whether or not our failure to detect $(Na^+ + K^+)$-stimulated Mg^{2+}-ATPase results from (a) the isolation procedure, (b) accurately reflects enzymatic activity of the embryonic chick liver plasma membrane, or (c) is due to masking by high activities of Mg^{2+}-ATPase, remains to be determined.

Warren and Glick[22] have estimated that 4.1 % of the protein of a total homogenate of rat liver is plasma membrane protein. If we assume that this figure is applicable to embryonic chick liver homogenates, we calculate a recovery of 7 % of plasma membrane. On this basis, from total homogenate activities, we can assign 65 % of the ATPase, 57 % of the CTPase, 36 % of the GTPase and 20 % of the UTPase to plasma membrane.

In summation, this report describes a procedure for large scale isolation of embryonic chick liver plasma membranes and demonstrates that high specific activities of CTPase may be a putative marker for the plasma membrane fraction. A definitive assignment of the enzyme awaits the results of additional biochemical and cytochemical studies now underway in our laboratories. The "purity" of the plasma membrane fraction has been defined in terms of its appearance in electron micrographs, and the absence of marker enzymes commonly associated with other subcellular fractions. The plasma membranes do not exhibit significant acid and alkaline phosphatase or pyrophosphatase activities. Investigations into possible

metabolic roles of embryonic chick liver plasma membrane nucleotide phosphohydrolases are continuing.

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