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ISOLATION AND CHARACTERIZATION OF LYMPHOCYTE PLASMA MEMBRANES

E. FERBER, K. RESCH, D. F. H. WALLACH AND W. IMM*

Max-Planck-Institut für Immunbiologie, D-78 Freiburg i. Br. (Germany)

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SUMMARY

We describe a method for the isolation of plasma membranes and other organelles from the lymphocytes of pig mesenteric lymph nodes, as well as from calf mediastinal nodes. The cells were disrupted by nitrogen cavitation leaving the nuclei intact. Nuclei, the large granule fraction and microsomes consisting of vesicles derived from plasma membrane and endoplasmic reticulum were separated by differential centrifugation. Plasma membranes were separated from the fragmented endoplasmic reticulum by equilibrium density ultracentrifugation in buffered dextran solutions.

All fractions were characterized using marker enzymes and by their chemical composition. The microsomal fraction seemed to be free of cell constituents other than plasma membranes and endoplasmic reticulum. The yield of plasma membranes was 1.7 % (protein content relative to whole cell homogenate). Specific activities of 5'-nucleotidase (5'-ribonucleotide phosphohydrolase, EC 3.1.3.5), a widely used plasma membrane marker, were 125 nmoles·(mg protein)$^{-1}$·min^{-1} being enriched by a factor of 25 as compared with whole cell homogenate. The cholesterol content of plasma membranes was 751 nmoles/mg protein (homogenate = 104 nmoles/mg protein) and the phospholipid content 727 nmoles/mg protein (homogenate = 90.3 nmoles/mg protein). The cholesterol: phospholipid molar ratio was 1.03.

Lysolecithin acyltransferase (acyl-CoA: 1-acyl-*sn*-glycero-3-phosphorylcholine-*O*-acyltransferase, EC 2.3.1.?.) showed specific activities between 10 to 12 nmoles·(mg protein)$^{-1}$·min^{-1} in the plasma membrane compared to 3–4 nmoles·(mg protein)$^{-1}$·min^{-1} in endoplasmic reticulum and thus appears to be a plasma membrane component in lymphocytes.

INTRODUCTION

The participation of lymphocytes in immune reactions intimately involves their outer membrane, whether one is dealing with antigen recognition by membrane-bound immunoglobulins[1,2] or expression of antigenic individuality[3]. Moreover,

Abbreviations: DTNB, 5,5-dithio-bis-(2-nitrobenzoate); HEPES, 4-(hydroxyethyl)-1-piperazinyl-ethane-2-sulfonic acid.

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among the first events during lymphocyte activation are changes of membrane permeability[4-6]. However, there have been few efforts to isolate plasma membranes — or other lymphocyte organelles — in pure and characterized form[7,8] whereas well characterized plasma membranes from many other mammalian cells have been isolated successfully[9].

During our studies on the phospholipid metabolism of stimulated lymphocytes[10] we came face to face with the problem of isolating plasma membranes as well as other cell organelles in order to clarify the dynamics of membrane-bound enzymes involved in the metabolism of phospholipids. We, therefore, extended the techniques of Wallach and Kamat[11,12] to achieve a mild isolation procedure preserving structural components and enzymatic activities optimally and at the same time providing a high and reproducible yield of the desired organelles. We herein describe the application of these new methods to lymphocytes from calf mediastinal lymph nodes and the mesenteric lymph nodes of pigs.

MATERIALS AND METHODS

Preparation of lymphocyte suspensions

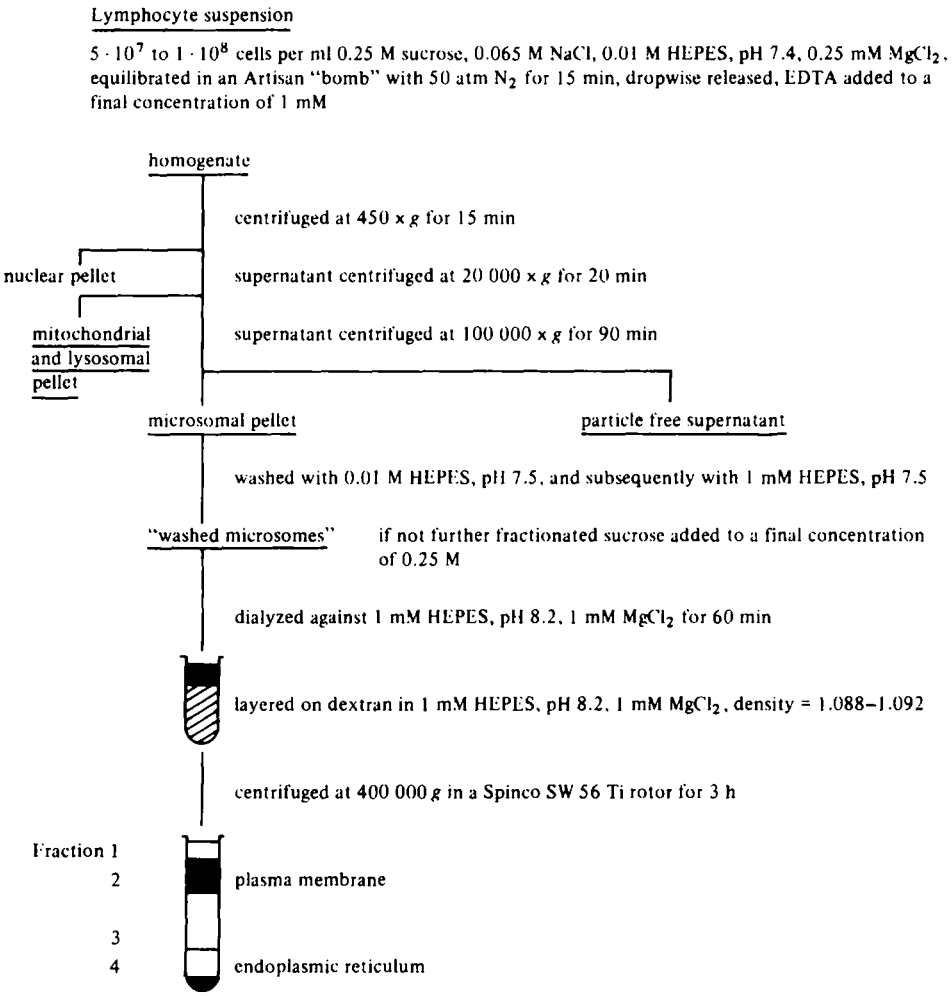
Calf mediastinal, as well as pig mesenteric lymph nodes, were removed within 10–20 min after slaughtering. The lymph nodes were put into cold, isotonic phosphate-buffered saline (pH 7.2), cleaned of adherent connective tissue, cut into small pieces, and macerated gently in a glass Tenbroek tissue grinder (Bellco, clearance of 0.004–0.006 inch), to yield the lymphocytes. Tissue remnants were removed by filtering through a small column of Perlon wool (Farbwerke Hoechst, Frankfurt/M). The cells were washed in phosphate-buffered saline (5 min at 300 × *g* in an International PR 6 centrifuge), resuspended in phosphate-buffered saline and incubated for 30 min at 37 °C in a column of Perlon wool to remove phagocytizing and dead cells. The lymphocyces were eluted from the column with phosphate-buffered saline in a volume about four times that placed on the column. Not more than about 20 % of the cells were damaged (trypan blue positive) before column treatment and nearly none after. A Coulter counter was used for cell counts. Except for the column incubation all steps were at 0 °C.

Cell disruption

The lymphocytes were suspended at a concentration of $5 \cdot 10^7$–$1 \cdot 10^8$ cells per ml in 0.02 M 4-(hydroxyethyl)-1-piperazinyl-ethane-2-sulfonic acid (HEPES), pH 7.4 (Serva, Heidelberg, Germany), 0.13 M NaCl, 0.5 mM $MgCl_2$. An equal volume of 0.5 M sucrose was then added with constant gentle stirring. Under these conditions uniform suspensions of lymphocytes were obtained (but instantaneous suspension in sucrose-containing media caused irreversible clumping). Cell disruption was by the "nitrogen cavitation method"[11,12]: the cell suspensions were equilibrated in a Artisan "bomb" (Artisan Metal Products, Waltham, Mass., U.S.A.) with 50 atm of N_2 for 15 min (or 30 atm for 20 min) at 0 °C and with constant gentle stirring. Cell disruption occurred after dropwise release of the suspension from the "bomb". Shortly after the release EDTA was added to a final concentration of 1 mM.

Isolation of subcellular components

This proceeded according to the following flow chart:



Various modifications of the method (disruption media, conditions, *etc.*) were investigated to achieve a procedure optimal for the special case of lymphocytes.

Nuclei. Using the above solutions the nuclei could be fully pelleted at 450 × *g* for 15 min, but sometimes the sediment proved difficult to resuspend. This could be avoided by raising the Mg^{2+} concentration during cell disruption from 0.25 to 2 mM. This modification gave a good suspension of nuclei but a lesser yield of microsomal material, which tends to aggregate at these Mg^{2+} levels and to pellet partly in the "large granule fraction". The nuclei were resuspended in 0.02 M HEPES, pH 7.4, 0.13 M NaCl, 4 mM $MgCl_2$. An equal volume of 0.5 M sucrose was then mixed in. At 0 °C, this medium preserves the nuclei for at least 3 days.

"Large granule fraction" (mitochondria plus lysosomes). After removal of nuclei

these particles were pelleted at 20 000 × *g* for 15 min (Sorvall, Centrifuge RC 2B, Rotor SS 34), resuspended in 0.25 M sucrose 1 mM HEPES, pH 7.5, and stored at −70 °C.

"*Microsomal fraction*". These particles were sedimented at 100000 × *g* for 60 min at 0 °C (Spinco L2 Ultracentrifuge, Rotor 50.1) washed once with 0.01 M HEPES, pH 7.5, and subsequently with 1 mM HEPES, pH 7.5. If the procedure was stopped at this stage, the particles were suspended in 0.25 M sucrose, 1 mM HEPES, pH 7.5, and frozen at −70 °C.

Separation of plasma membrane and endoplasmic reticulum. The washed microsomes were dialyzed for 1 h *vs* 1 mM HEPES 1 mM $MgCl_2$, pH 8.2. They were then layered upon 26% dextran (Dextran-150-, Pharmacia Inc. Frankfurt, Germany) in 1 mM HEPES, pH 8.2, 1 mM $MgCl_2$, d = 1.088–1.092.

When using a SW 56 Spinco rotor, 1.0 ml microsomal suspension containing about 1 mg protein was layered on top of 3 ml of dextran solution. Better separations were obtained when the interface between buffer and dextran was disturbed by gentle stirring with a glass rod or best by depositing 2.0 ml dextran solution of d = 1.088–1.092 as above, building up a short continuous gradient (d= 1.09–1.00) on top of this cushion and layering 1.0 ml of microsomal suspension on top of this gradient. (The buffer throughout was 1 mM HEPES, pH 8.2, 1 mM $MgCl_2$). After centrifugation at 400000 × *g* for 3 h two fractions have separated: the upper, at the buffer–dextran interface, designated as plasma membrane (Fraction 2) and a pellet, designated as endoplasmic reticulum (Fraction 4). In some experiments the upper layer (Fraction 1), containing buffer and soluble protein, and the zone between plasma membranes and endoplasmic reticulum (Fraction 3) were also analyzed.

The fractions were isolated using a syringe and needle. For storage fractions were mixed with equal volumes of 0.25 M sucrose, 0.02 M HEPES, pH 7.5, 5 mM EDTA and then maintained at —70 °C.

Analytical methods

Chemical determinations. Protein was determined either by the ninhydrin method[13] or fluorimetrically[14] and inorganic phosphate according to Fiske and SubbaRow[15]. DNA and RNA were extracted according to the method of Munro and Fleck[16]. For the estimation of RNA the absorbance of extracts was measured at 260 nm. DNA was determined using the diphenylamine reaction according to the method of Dische[17] and Seibert[18] with calf thymus DNA as standard. For determination of phospholipids and cholesterol aliquots of subcellular fractions were extracted twice with total volumes of 7 ml methanol and 10 ml chloroform. The extracts were washed once with 3 ml 0.1 M KCl. Cholesterol content of these extracts were measured according to the method of Zlatkis *et al.*[19] and total phospholipids by phosphorus determination by the method of Lowry *et al.*[20].

Marker enzymes. Among the markers employed in establishing purity, membrane-bound enzymes are used most widely for reasons of sensitivity, precision and quantitation. Moreover, they link *in vitro* fractionations to *in situ* histochemical evidence.

5'-Nucleotidase (5'-ribonucleotide phosphohydrolase, EC 3.1.3.5) was used as a marker for the plasma membrane[21], since this enzyme seems to be an ubiquitous

plasma membrane function. No equally suitable enzyme has been reported for endoplasmic reticulum. We used NADH oxidoreductase (EC 1.6.4.3), which seems to be a reliable marker[12], especially if glucose-6-phosphatase is low. Succinate dehydrogenase (succinate: (acceptor)oxidoreductase, EC 1.3.99.1) was a marker for mitochondria[22] and acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) or β-glucuronidase (β-D-glucuronide glucuronohydrolase, EC 3.2.1.31) for lysosomes (de Duve[23]).

The reagents for our enzymatic assays were as follows: 5,5-dithio-bis-(2-nitrobenzoate) (DTNB), cytochrome *c*, succinate, AMP and 4-nitrophenyl-β-D-glucuronide were obtained from Boehringer and Söhne, (Mannheim, Germany) and oleoyl-CoA from General Biochemicals (Chagrin Falls, Ohio, U.S.A.). [^{14}C]lysophosphatidylcholine (1-palmitoyl-[1'-^{14}C]glycerophosphorylcholine) was prepared by a modification of the method of Portman and Alexander[24].

Succinate dehydrogenase was measured according to the method of de Duve *et al.*[25], 5'-nucleotidase according to the method of Weaver and Boyle[21], acid phosphatase with *p*-nitrophenylphosphate as substrate according to the method of Fishman and Lerner[26] and NADH oxidoreductase according to the method of Wallach and Kamat[12].

β-Glucuronidase was followed using 4-nitrophenyl-β-D-glucuronide as substrate. The reaction mixture contained, in a total volume of 1.25 ml, 10 μmoles nitrophenyl glucuronide, 60–600 μg enzyme protein and 0.2 M acetate buffer, pH 4.6. After a 60-min incubation at 37 °C the reaction was stopped by addition of 0.25 ml 1 M NaOH. Liberated nitrophenol in supernatants was measured at 400 nm and calculated using a molar extinction coefficient of 18200 $l \cdot mole^{-1} \cdot cm^{-1}$.

For determination of the lysolecithin acyl-transferase (acyl-CoA:1-acyl-*sn*-glycero-3-phosphorylcholine-*O*-acyltransferase, EC 2.3.1.?) we utilized (1) the photometric assay of Lands and Hart[27], measuring CoASH released from oleoyl-CoA, and (2) the incorporation of oleoyl-CoA into labelled lysolecithin. In the former method the reaction mixture (1 ml 0.1 M phosphate, pH 7.5) contained 0.5 μmole DTNB, 8–40 μg enzyme protein, 30 nmoles oleoyl-CoA, and 50 nmoles lysolecithin. The reaction of the SH-groups of the liberated CoASH with DTNB was measured continuously at 405 nm at 37 °C.

To follow the conversion of labelled lysolecithin we used a reaction mixture (1 ml 0.1 M phosphate, pH 7.5) containing 50 nmoles 1-palmitoyl-[1'-^{14}C]glycerophosphorylcholine, 30 nmoles oleoyl-CoA and 8–40 μg enzyme protein. After 10 min at 37 °C the phospholipids were extracted and separated as described elsewhere[28].

RESULTS

Nuclei, large granules and microsomes

Tables I and II show the yield of the subcellular fractions nuclei, large granule fraction and microsomes in pig lymphocytes from a representative experiment. The percentage of the total cell protein found in these fractions was similar to those reported by others[8]. About 30 % of the protein remained in the supernatant after centrifugation at 100000 × *g* for 90 min. The nuclear fraction obtained during the differential centrifugation represented 20 % of the total protein. It contained 70 % of total DNA and 33 % of total RNA. Less than 10 % of the total activities

TABLE I

CHEMICAL COMPOSITION OF SUBCELLULAR FRACTIONS FROM PIG LYMPHOCYTES

Total amounts refer to fractions obtained from $1 \cdot 10^{10}$ cells. Values are given as means from triplicates.

Subcellular fraction	*Protein (mg total)*	*DNA*		*RNA*		*Cholesterol*		*Phospholipid*	
		μg/mg of protein	*mg total*	*μg/mg of protein*	*mg total*	*nmoles/mg protein*	*μmoles total*	*nmoles/mg of protein*	*μmoles total*
Homogenate	165.2	142.0	23.5	41.3	6.9	104.0	17.3	90.3	15.0
Nuclei	32.4	504.6	16.3	70.1	2.3	130.4	4.2	171.8	5.5
Large granules	22.2	270.7	6.0	37.7	0.8	155.0	3.4	210.8	4.6
Microsomes	7.7	70.2	0.5	56.5	0.4	227.2	1.7	211.0	1.6
Supernatant	50.0	7.3	0.4	67.3	3.4	137.4	6.9	44.6	2.2
Recovery (%)	68.0*		98.6		101.3		93.9		92.9

* The low protein recovery is probably due to low values of the supernatant because the amount of cytoplasmic protein trapped within the microsomes was not determined. The value given for microsomes is that of shocked vesicles containing no soluble protein. According to Wallach and Kamat[12] microsomes trap about 3 times the amount of soluble protein compared to protein of microsomal membranes.

TABLE II

ENZYMIC ACTIVITIES OF SUBCELLULAR FRACTIONS FROM PIG LYMPHOCYTES

Total activities (given as nmoles·min^{-1}) are present in fractions obtained from $1 \cdot 10^{10}$ cells. Specific activities are expressed as nmoles·(mg protein)$^{-1}$·min^{-1}. Values are given as means from triplicates.

Subcellular fraction	*5'-Nucleotidase*		*β-Glucuronidase*		*Succinate dehydrogenase*		*Lysolecithin acyltransferase*	
	Specific activity	*Total activity*	*Specific activity*	*Total activity*	*Specific activity*	*Total activity*	*Specific activity*	*Total activity*
Homogenate	5.0	828.9	0.7	114.4	8.3	1375.9	2.9	480.7
Nuclei	2.4	77.1	0.6	19.0	3.8	122.3	6.2	199.8
Large granules	6.7	149.7	1.5	34.0	48.0	1067.0	6.2	137.8
Microsomes	60.2	466.6	0.2	1.4	0	0	8.0	62.0
Supernatant	2.3	116.2	0.8	40.4	0	0	0.3	14.1
Recovery (%)		97.5		94.9		86.4		85.9

of 5'-nucleotidase and succinate dehydrogenase and about 17 % of β-glucuronidase sedimented within this fraction.

About 13 % of total protein pelleted at 20000 × *g* (large granule fraction). DNA content in this fraction was 26 % and RNA 12 % of total amounts. Most of the activity of succinate dehydrogenase sedimented with this fraction and 30 % of total β-glucuronidase activity.

About 5 % of total protein were found in the microsomal fraction, only trace amounts of DNA and 6 % of RNA. Cholesterol content was 227 nmoles/mg protein (homogenate = 104) and phospholipid was 211 nmoles/mg protein (homogenate = 90.3). Specific activity of 5'-nucleotidase, the most specific marker enzyme for plasma membranes so far discovered was 60 nmoles·(mg protein)$^{-1}$·min^{-1} being enriched 12-fold over the homogenate. The total activity of this enzyme in microsomes accounted for 56 % of the activity of the homogenate. Only 1.2 % of β-glucuronidase and no activity of succinate dehydrogenase could be detected. This indicates that plasma membrane vesicles sedimenting with other small particles constitutes the microsomal fraction which proved to be not contaminated with nuclei, mitochondria or lysosomes. A similar distribution of subcellular fractions was obtained with calf lymphocytes (Table III).

TABLE III

PROTEIN CONTENT AND ENZYMIC ACTIVITIES OF SUBCELLULAR FRACTIONS FROM CALF LYMPHOCYTES

Protein content (mg) in each fraction refers to a total amount of protein present in the respective fraction obtained from 3.1·10^9 cells. Specific enzyme activities are given as nmoles·(mg protein)$^{-1}$·min^{-1} and total activities as nmoles·min^{-1}. Values are given as means from triplicates.

Subcellular fraction	*Protein (mg total)*	*Succinate dehydrogenase*		*Acid phosphatase*		*5'-Nucleotidase*	
		Specific activity	*Total activity*	*Specific activity*	*Total activity*	*Specific activity*	*Total activity*
Nuclei	11.2	0	0	0.673	7.53	2.4	26.8
Large granules	6.8	33	224	8.060	54.8	9.6	65.3
Microsomes	0.72	0	0	0	0	64.0	46.1

Plasma membrane and endoplasmic reticulum

Microsomal membranes consist of small vesicles (500–3000 Å diameter) derived from plasma membrane and endoplasmic reticulum, which can be separated on dextran gradients. Table IV summarizes the results of a representative separation of the microsomal membranes from pig lymphocytes. Two major bands appeared after centrifugation: Fraction 2 and the pellet (Fraction 4), which together contained most of the protein. Fraction 1 contained soluble protein which exhibited none (or trace amounts) of the marker enzyme activities. Fraction 3 located in the dextran layer between Fraction 2 and the pellet contained material, which was presumably a mixture. Fraction 2 was designated as plasma membranes: it contained 1.7 % of total cell protein and exhibited a specific activity of 5'-nucleotidase of 125.4 nmoles·(mg protein)$^{-1}$·min^{-1} (enrichment of 2 compared with microsomes and of 25 compared with homogenate). Cholesterol and phospholipid content per mg protein in this fraction was 7 times higher than in total cell homogenates.

TABLE IV

PREPARATION OF PLASMA MEMBRANES AND ENDOPLASMIC RETICULUM BY SUBFRACTIONATION OF MICROSOMES FROM PIG LYMPHOCYTES

Total enzyme activities are given as nmoles·min^{-1} and specific activities as nmoles·(mg protein)$^{-1}$·min^{-1}. Values are given as means from triplicates.

Subcellular fraction	*Protein (mg total)*	*5′-Nucleotidase*		*NADH oxido-reductase*		*Lysolecithin acyltransferase*		*Cholesterol*		*Phospholipid*	
		Specific activity	*Total activity*	*Specific activity*	*Total activity*	*Specific activity*	*Total activity*	*nmoles/mg protein*	*μmoles total*	*nmoles/mg protein*	*μmoles total*
Microsomes	5.62	58.5	328.9	282.8	1589.9	8.5	47.8	468	2.63	479	2.693
Fraction 1	0.54	8.7	4.8	0	0	0.2	0.11	170	0.092	124	0.067
Fraction 2 (Plasma membrane)	2.09	125.4	261.4	687.2	1432.8	10.0	20.85	751	1.57	727	1.515
Fraction 3	0.96	22.2	21.2	140.2	134.6	5.1	4.89	131	0.126	102	0.097
Fraction 4 (Endoplasmatic reticulum)	1.07	25.9	27.6	336.4	358.2	4.2	4.47	314	0.334	199	0.212

TABLE V

PROPERTIES OF PIG LYMPHOCYTE PLASMA MEMBRANES

Values are given ± standard deviation with numbers of determinations of different preparations in parentheses. Enzymic activities are expressed as nmoles·(mg protein)$^{-1}$·min^{-1}.

Protein (mg/10^{10} cells)	2.87 ± 0.18	(10)
DNA (μg/mg protein)	10 ± 2.0	(2)
RNA (μg/mg protein)	25 ± 2.1	(2)
Cholesterol (nmoles/mg protein)	751 ± 20	(6)
Phospholipid (nmoles/mg protein)	727 ± 30	(6)
Cholesterol/phospholipid molar ratio	1.03 ± 0.04	(6)
Lysolecithin acyltransferase	10.0 ± 1.5	(8)
β-Glucuronidase	0	(3)
Succinate dehydrogenase	0	(3)
5′-Nucleotidase	125.4 ± 10.5	(8)

In cells containing large amounts of endoplasmic reticulum NADH oxidoreductase is enriched in this fraction[12]. In lymphocytes, however, we found no consistent distribution pattern. This was also the case with glucose-6-phosphatase thought to be a marker of endoplasmic reticulum. The characterization of Fraction 4 designated as endoplasmic reticulum is therefore not sufficient.

Lysolecithin acyltransferase has been reported by Eibl *et al.*[29] to be mainly associated with endoplasmic reticulum in liver. In lymphocytes, however, this enzyme showed a strikingly different subcellular localization: in all species tested we found the highest specific activities within the plasma membrane. Thus this enzyme turned out to be a component of lymphocyte plasma membranes.

Table V summarizes some characteristic data of plasma membranes from lymphocytes. The dominant features are the low content of DNA and RNA, the high cholesterol content and a molar ratio of cholesterol/phospholipid of about 1.0. This and the high specific activities of 5'-nucleotidase and the absence of β-glucuronidase and succinate dehydrogenase activity suggest that these plasma membrane preparations are not contaminated with other subcellular constituents.

DISCUSSION

Recently Allen and Crumpton[8] have published for the first time a suitable method for the preparation of plasma membranes from lymphocytes. They minced whole pig lymph nodes by passing them through a perforated plate with holes of 1 mm diameter. By this procedure a cell homogenate was obtained consisting of fairly intact nuclei and large granules, and rather large fragments of plasma membranes and endoplasmic reticulum. The plasma membrane fragments were pelleted at 20000 $\times$ *g* and then purified using a sucrose gradient. The plasma membrane vesicles varied largely in size and tended to fragment further. They had trapped cytoplasma during the disruption of the cells.

In contrast our method for the isolation of plasma membranes and other cellular organelles from lymphocytes starts with lymphocytes in suspension which were purified on a nylon column to get rid of damaged cells and especially of macrophages. This provides that the same well defined lymphocyte suspension may be used for functional, *e.g.* immunological, and biochemical studies. The isolation procedure is an extention of the techniques developed by Wallach and Kamat[11,12] for other mammalian cells. This method has major advantages, especially for cells in suspension: the forces effecting cell disruption by cavitation with an inert gas are the same for each cell and, for a given cell type, depend only on the equilibration time, pressure and temperature and are highly reproducible. Disruption occurs under iso-osmotic conditions and without danger of local heating. Plasma membrane and endoplasmic reticulum fragment into small, semipermeable vesicles which sediment principally in the "microsomal fraction". Furthermore the orientation of the vesicles is such that the outer parts of the intact membrane form the outer surface of the vesicle. Other cell organelles, especially the nuclei, remain intact.

Nuclei and the large granules can be separated by conventional sedimentation procedures. Endoplasmic reticulum and plasma membrane, however, are separated by density centrifugation, making use of the different charge densities on the internal surface of the two types of membrane vesicle.

Certain special steps are required with lymphocytes to work effectively: (1) Abrupt suspension of lymphocytes in sucrose solutions causes irreversible cell aggregation, as also observed by Allan and Crumpton[8]. Therefore, we add sucrose to the lymphocytes suspended in an isotonic salt solution. The final medium is deliberately kept hypertonic to minimize lysosome damage after cell rupture. (2) Since lymphocytes contain only a small cytoplasmic space the conditions of equilibration with nitrogen are critical. Using 50 atm we found that after 15 min, nearly all cells are disrupted but with the nuclei intact, whereas after 20 min, some nuclei are damaged. The same was achieved using 30 atm for 20 min. (3) A certain amount of Mg^{2+} is necessary to stabilize nuclei during cell disruption but higher amounts aggregate smaller organelles indiscriminately. The Mg^{2+} level we used (0.25 mM) effects an optimal yield of intact nuclei and of microsomal membranes. (4) Substitution of HEPES for Tris as buffer yields higher activities of membrane-associated enzymes.

About 1.7 % of the total cell protein is recovered in purified plasma membrane fractions. Since our plasma membrane isolates do not contain soluble proteins trapped with the membrane vesicles this is a good yield as compared with other methods[8]. Microsomal vesicles obtained after disruption of cells contain up to 60 % cytoplasmic protein[12]. This soluble protein was removed by two hypotonic shocks as proved by lactate dehydrogenase determinations. According to enzymatic criteria and to chemical composition our plasma membrane preparations appear to be highly purified.

The procedure developed is sufficiently mild and reproducible to give good yields of stable membrane fragments which can be stored for prolonged periods at −70 °C without detectable alterations. Thus we are using the method for our current analyses of plasma membrane metabolism during the early stages of lymphocyte stimulation.

The preparation of plasma membranes described for pig mesenteric lymph node cells was applied also to lymphocytes from other species. Thus plasma membranes with the same purity could be isolated from calf mediastinal lymph node cells, rabbit lymph node and thymus lymphocytes.

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