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STUDIES ON SMOOTH MUSCLE PLASMA MEMBRANE

I. ISOLATION AND CHARACTERIZATION OF PLASMA MEMBRANE FROM RAT MYOMETRIUM

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SUMMARY

1. The plasma membrane has been isolated from rat myometrium using a single step density gradient centrifugation without any salt extraction. A new kind of continuous sucrose density gradient was prepared using an Instrumentation Specialities Co.(ISCO) density gradient former. Other subcellular fractions were also obtained by this technique.

2. Electron microscopic studies revealed the homogeneity of the plasma membrane fraction. Also the cytoplasmic membranes and mitochondria were relatively free of detectable contamination by other particles. The plasma membrane fraction was mainly vesicular in nature and a unit membrane structure was evident in some sections.

3. The cholesterol:phospholipid ratio of plasma membrane was 0.82. A small amount of RNA-P was found to be associated with the plasma membrane. DNA-P was mainly associated with the nuclear fraction.

4. The following enzymes were found to be concentrated in the plasma membrane: 5′-nucleotidase (EC 3.1.3.5), L-leucyl-β-naphthylamidase (EC 3.4.1.1), *p*-nitrophenylphosphatase, [acid phosphatase (EC 3.1.3.1) and alkaline phosphatase (EC 3.1.3.2)]; phosphodiesterase (EC 3.1.4.1) and ATPase (EC 3.6.1.4). Cytochrome *c* oxidase (EC 1.9.3.1) and NADH–cytochrome *c* reductase (EC 1.6.2.1) were concentrated in a fraction which contained mitochondria and cytoplasmic membranes (not separated in this case).

5. 14–27 % of the total protein and 59 % of the total 5′-nucleotidase activity were found to be present in the plasma membrane fraction.

6. We conclude that high purity and good yield of the plasma membrane from smooth muscle and recovery of other fractions at the same time was achieved by the method presented in this article. This material should be suitable for a variety of pharmacological studies.

INTRODUCTION

To study transport and binding or interaction of drugs in smooth muscle plasma membrane we have developed a method of isolation of plasma membrane from rat

myometrium. Methods have been reported for isolation of plasma membrane from liver[1–3], mouse fibroblast[4], Ehrlich ascites carcinoma[5], kidney[6,7], intestinal mucosa[8], fat cells[9], mammary gland[10], skeletal[11,12] and smooth muscle[13]. The present method has the advantage of a single step centrifugation (density gradient) and good yield of plasma membrane in spite of drastic homogenization conditions. We have avoided the use of salts during the isolation procedure to study the plasma membrane as to its ionic contents. We evaluated the purity of our preparation by a number of criteria: morphological, chemical and enzymatic characteristics of plasma membrane and other subcellular fractions. To our knowledge this is the first detailed method of isolation and characterization of plasma membrane from smooth muscle reported in the literature. A preliminary account of this work has been published elsewhere[14].

MATERIALS AND METHODS

Adult female Wistar rats were injected with 100 μg stilbestrol daily for at least 2 days then killed by a blow at the head.

Chemicals

Tris, NADH, RNA, DNA, bovine serum albumin and enzyme substrates were from Sigma Chemical Co. Stilbestrol was from B.D.H., 4-acetamido-4′-isothiocyanate stilbene-2,2′-disulfonic acid was from N.B.C., Cleveland, Ohio. Sucrose and other reagents were from Fisher Scientific Company.

Density measurements

Pycnometers were constructed from 100-μl pipettes (Fisher Brand). The density was measured after 0.5-ml fractions of the gradient were collected using an ISCO density gradient fractionator or using Micule density markers from Sondell Scientific Instruments, Calif. (Supplied by Beckman, Palo Alto, Calif.).

Tris salt of *p*-nitrophenylphosphate was prepared according to EMMELOT AND BOS[15].

Centrifugation

All centrifugations were carried out in a Beckman L2-65B Ultracentifuge using rotor SW-40 or rotor 65, between 0–4°.

Density gradient

Sucrose density gradient was prepared using 68.4 % (*d* 1.269) and 8.5 % (*d* 1.04) sucrose in the ISCO Model 570 gradient former. The left-hand syringe was adjusted so as to deliver twice as much sucrose solution by volume as the right-side syringe. The heavy solution was in the left-side syringe and lighter solution in the right side. Relative delivery speed was always kept between 1 and 5, higher delivery speeds tend to disturb the gradients. The gradient tubes were left in the cold for 16 h before use.

Density gradient fractionator Model D (ISCO) was used to collect fractions. Absorbance was monitored at 280 mμ.

Chemical determinations

Protein was measured according to LOWRY *et al.*[16] using bovine serum albumin as standard. P_i was measured by the method of FISKE AND SUBBAROW[17]. Phospholipid

was measured according to CHEN *et al.*[18], cholesterol by the method of GLICK *et al.*[19]. A factor of 25 was used to convert μg phosphorus to μg phospholipid. Determination of RNA was by the orcinol method[20] and DNA by the method of BURTON[21].

Enzymatic determinations

The following enzyme activities were measured: cytochrome *c* oxidase[22] (EC 1.9.3.1), NADH–cytochrome *c* reductase[23] (EC 1.6.2.1), 5'-nucleotidase[24] (EC 3.1.3.5), L-leucyl-β-naphtylamidase[25] (EC 3.4.1.1), acid and alkaline phosphomonoesterases[26] [(EC 3.1.3.1) and (EC 3.1.3.2)] (pH 5.5 and 9.5), phosphodiesterase[26] (EC 3.1.4.1) (pH 8.9) using bis-(*p*-nitrophenyl)phosphate, ATPase (EC 3.6.1.4) was measured using 3 mM ATP, 3 mM $MgCl_2$ in 50 mM Tris–HCl buffer (pH 7.5), P_i liberated was measured after 10 min of incubation at 37° by the method of FISKE AND SUBBAROW[17]. K^+-activated *p*-nitrophenylphosphatase activity was measured in presence of 50 mM imidazole buffer (pH 7.8), 5 mM $MgSO_4$, 10 mM KCl, 5 mM *p*-nitrophenylphosphate incubated at 37° for 30 min. The reaction was stopped by adding an equal volume of 1 M NaOH, the mixture was centrifuged and absorbance of the supernatant measured at 400 mμ. Glucose-6-phosphatase (EC 3.1.3.9) was estimated according to HUBSCHER AND WEST[27].

Cytochrome *c* oxidase and NADH–cytochrome *c* reductase activities were determined with a Gilford spectrophotometer (Model 2400), within 6 h after killing the animals.

Electron microscopy

Pellets were fixed in glutaraldehyde buffer (pH 7.1) post fixed in OsO_4 embedded in epon, sectioned on Porter Blum ultramicrotome and mounted on a 400-mesh grid without a supporting film, stained with uranyl acetate and lead citrate and observed on JEM 7A electron microscope.

RESULTS

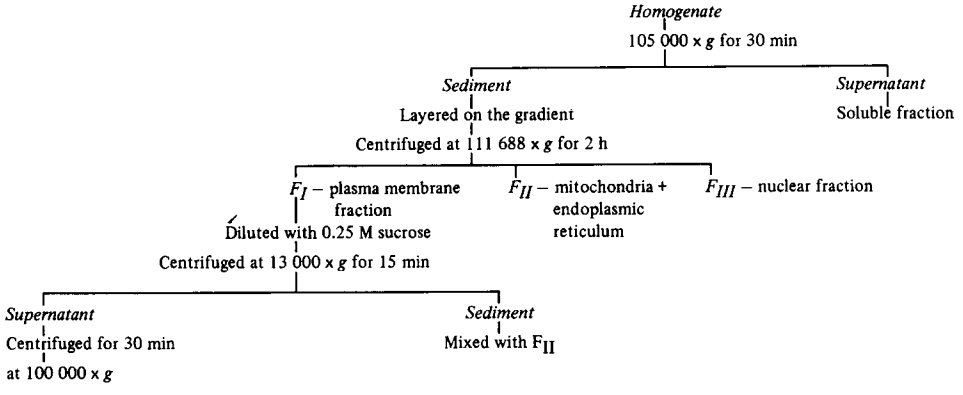
Preparation of the material

Myometrium from the uterine horns of 12 rats was separated from endometrium, the myometrium was cut into I-mm pieces with a set of razor blades and homogenized in a loose fitting teflon–glass homogenizer (thickness of the teflon pestel was 0.586 inch instead of 0.598 inch) by Talboys Instrument Corp. Emerson, N. J. Transi-stir for 2 min at full speed or in a Polytron Type PT 20 (Kinematica GmbH, Luzern-Schweiz) for approx. 4 sec at near maximum speed. The homogenate was filtered through a gauze cloth. Unbroken tissue was re-homogenized, filtered and the final homogenate was made 20% with respect to the 0.25 M mannitol solution (1 mM Tris–EDTA buffer (pH 7.1)). All the steps were carried out between 0–5° unless otherwise stated.

Outline of the procedure (*Scheme* 1)

The homogenate was centrifuged at 105000 $\times$ *g* for 30 min. The sediment was resuspended in a small volume of mannitol–EDTA solution and layered on the top of the gradient (volume 1–1.5 ml) and centrifuged for 2 h at 111688 $\times$ *g* (SW-40 rotor) in a Spinco Model L2 centrifuge. At the end of the run the gradient was collected in

the fractionator and the appearance of various fractions was monitored by following the absorbance at 280 mμ. Three major peaks were observed and were diluted to bring the sucrose concentration to approx. 8 % and recentrifuged at 100000 × *g* for



30 min to remove sucrose. Fraction I was first centrifuged for 15 min at 13000 × *g* to remove any trapped mitochondria and the supernatant fluid was then centrifuged at 100000 × *g* as described. The plasma membrane fraction was that from the top of the gradient (Fraction F_I) which was at the interphase of the loading medium (mannitol-EDTA) and the sucrose gradient. The density of the 0.5 ml sucrose at this site was 1.13. The second major peak was more dispersed in sucrose at a density of 1.15. It was designated as F_{II}. The third peak designated F_{III} was even more dispersed as it contained debris as well as clumped nuclei. The density at this region was approx. 1.20.

The pellets from the last centrifugation were either fixed in glutaraldehyde buffer for electron microscopic observation or resuspended in 0.25 M sucrose and kept frozen until used for chemical and enzymatic analysis.

Preliminary studies were carried out using 4-acetamido-4′-isothiocyanate stilbene-2,2′-disulfonic acid as a plasma membrane marker. Small pieces of tissue (I mm) were incubated in Krebs–Ringer solution in presence and absence of I mM 4-acetamido-4′-isothiocyanate-stilbene 2,2′-disulfonic acid for 1 h at 37° in the presence of O_2. After 1 h the tissue was washed in Krebs–Ringer solution for 1 h, changing the medium every 10 min. The tissue was homogenized, fractionated as described above, and analyzed by the procedure of MARINETTI AND GRAY[28]. It was observed that most of the dye was located in Fraction F_I and a small proportion of the total dye was in Fraction F_{III}. Presence of the dye in Fraction F_{III} can be explained by the presence there of unbroken cells. Fraction F_I was mainly vesicles as revealed by electron micrographs. Fraction F_{III} contained unbroken cells, collagen and nuclei.

Electron microscopy

Fig. 1 represents a typical electron microscopic picture of the fractions. Fig. 1a shows Fraction F_I which is mainly vesicles of varying sizes apparently from plasma membrane fragments. Figs. 1b and 1c are from Fraction F_{II} after this fraction was differentially centrifuged at 13000 × *g* for 15 min to separate the heavy and lighter components of Fraction F_{II}. Earlier electron microscopic studies on this fraction

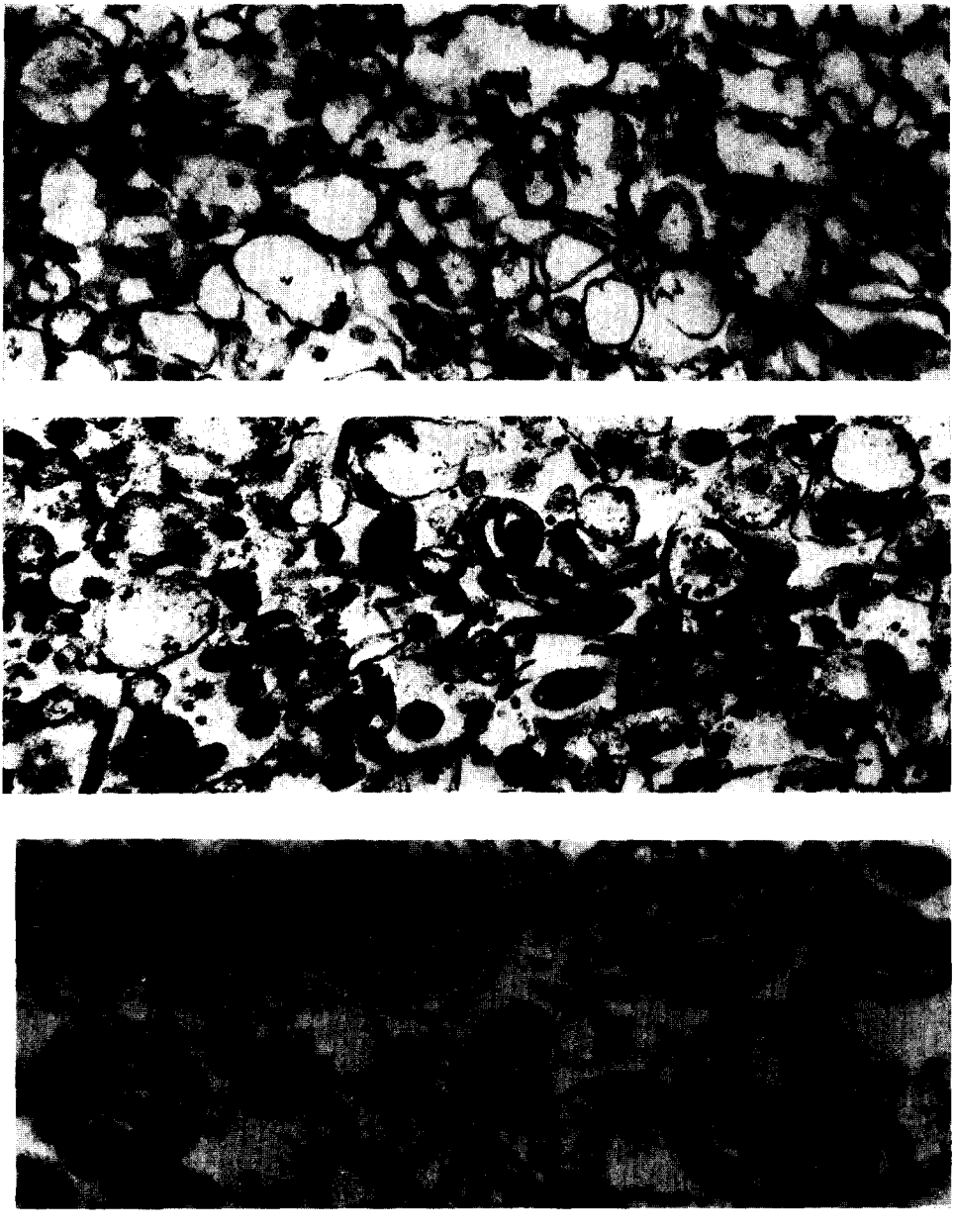


Fig. 1. a. The electron microscopic picture of thin sections prepared from the pellets of Fraction F_I (plasma membrane); mainly vesicles of various sizes and shapes. b. Lighter component of Fraction F_{II} endoplasmic reticulum, Golgi apparatus, ribosomes, *etc.* c. Heavy component of Fraction F_{II} mainly mitochondria. × 40000.

resulted in different appearances of the components depending upon the position of the section passing through the pellet, therefore, we differentially centrifuged Fraction F_{II} before fixing the pellet. Fig. 1b is the lighter component and 1c is the heavier one.

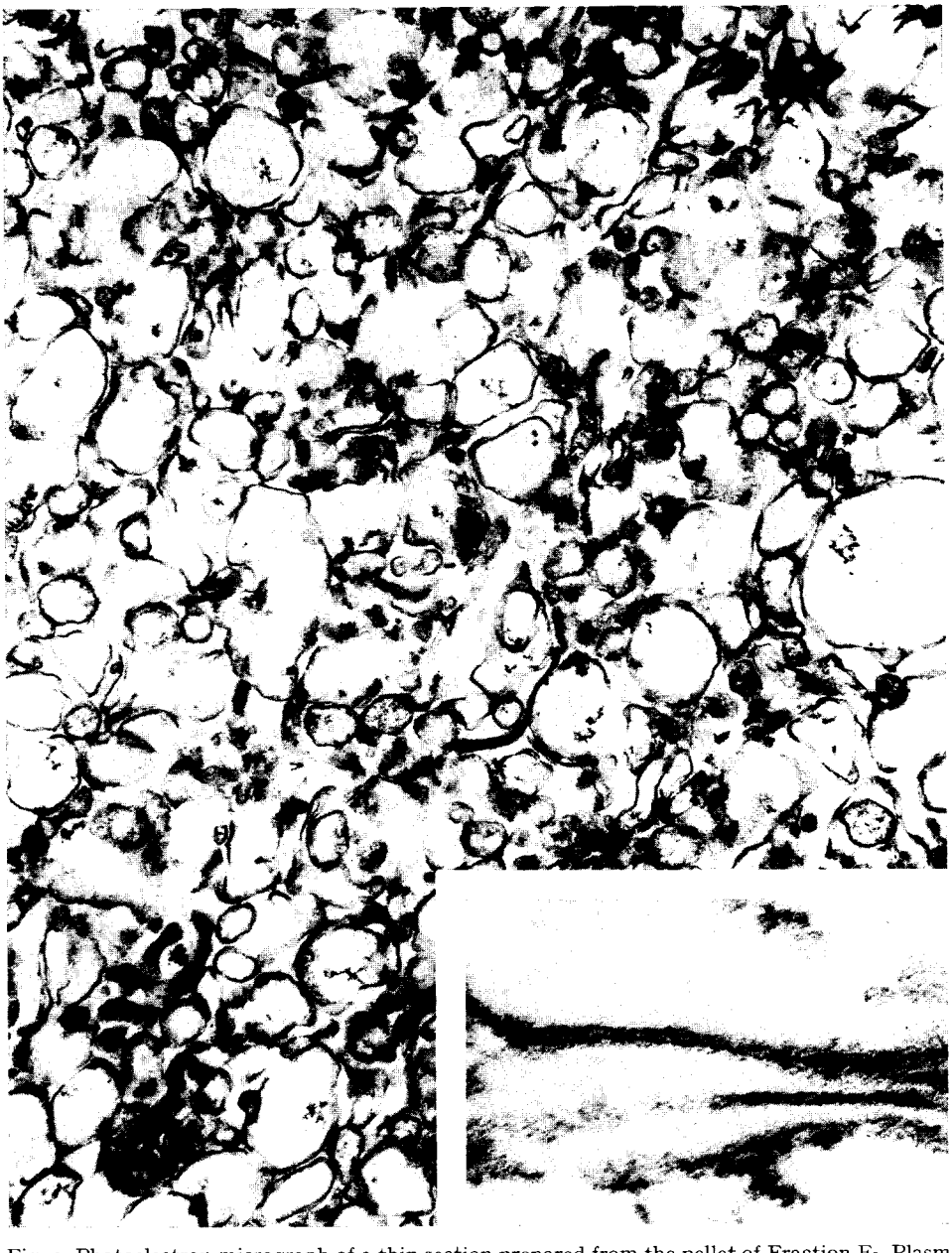


Fig. 2. Photoelectron micrograph of a thin section prepared from the pellet of Fraction F_I. Plasma membrane in the form of vesicles × 40000. Inset shows the triple layer structure × 300000.

Fig. 1b contained mainly fragments of intracellular membranes (Golgi apparatus, ribosomes and vesicles of various sizes and shape) and Fig. 1c contained mainly broken and intact mitochondria. Fraction F_{III} contained clumped nuclei, unbroken cells and few mitochondria (picture not included here).

Fig. 2 represents a low magnification of plasma membrane Fraction F_I and the typical triple-layered structure is evident in the insert at high magnification.

Yield of plasma membrane (Table I)

14–27 % of the total protein was in Fraction F_I and was considered to be all plasma membrane. Of the total 5'-nucleotidase activity in the homogenate, 59 % was in Fraction F_I. The rest was distributed in various fractions. Therefore most of the plasma membrane was recovered in our Fraction F_I, assuming 5'-nucleotidase activity to occur exclusively in the plasma membrane.

TABLE I

YIELD OF PLASMA MEMBRANE

Expressed as percentage of the total. F_I, plasma membrane: F_{II}, mitochondria and cytoplasmic membranes: F_{III}, nuclei and unbroken cells; soluble, soluble fraction (105000 × *g* supernatant). Numbers in parentheses are number of experiments.

Subcellular fractions	*Distribution of protein* (%)	*5'-Nucleotidase activity* (%)
F_I	14–27 (16)	59.0 (5)
F_{II}	11–26 (16)	9.1 (5)
F_{III}	9–38 (16)	17.3 (5)
Soluble	40–62 (16)	15.0 (5)

Density of plasma membrane

As explained above, the density of 0.5 ml of sucrose from the top of the gradient was 1.13. On the other hand the dark green (*d* 1.1) Micule density marker bead corresponded with the plasma membrane. The next band was at a density of 1.15 while the nuclear fraction was at a density of 1.2. In Fig. 3 the Fraction F_I is partly in the gradient and partly in the suspending medium due to the heterogenous composition of the membrane vesicles. This also accounts for the discrepancy between two methods of density measurements.

Plasma membrane when equilibrated on a discontinuous gradient (densities 1.072, 1.108, 1.136, 1.160 and 1.172) was mainly concentrated at the interphase of

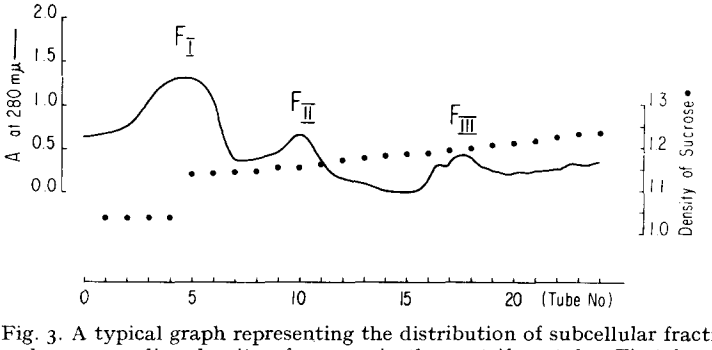


Fig. 3. A typical graph representing the distribution of subcellular fractions measured at 280 mμ and corresponding density of sucrose in the centrifuge tube. First four points on density graph represent the loading medium of the pellet. F_I, plasma membrane; F_{II}, mitochondria and cytoplasmic membranes; F_{III}, nuclear fraction.

d 1.108/*d* 1.136; a lesser amount of protein was found at the interphase of *d* 1.072/*d* 1.108 and *d* 1.136/*d* 1.160. All the three fractions were homogenous and vesicular in electron micrographs. There was no sediment at the bottom of the tube indicating absence of components heavier than *d* 1.172 provided the plasma membrane fraction had previously been centrifuged at low speed for a short time so that trapped mitochondria could be settled to the bottom prior to discontinuous density gradient.

Saline-soluble proteins

The absence of a soluble fraction of plasma membrane was established by suspending 2 mg of plasma membrane protein in 0.9 % NaCl and was allowed to stand at room temperature for 1 h with occasional shaking and then centrifuged at 100000 $\times$ *g* for 1 h. Protein was determined in the sediment and the supernatant. The saline (0.9 % NaCl) supernatant was found to be free of any detectable protein by the method of LOWRY *et al.*[16].

Chemical composition

Table II illustrates the total phosphate, lipid phosphate and cholesterol analysis of all the fractions obtained during the isolation of plasma membrane. Total cholesterol was 22 times as concentrated in the plasma membrane fraction compared to its level in the homogenate; phospholipid was not concentrated as much as cholesterol in the plasma membrane fraction. The molar ratio of cholesterol to phospholipid was found to be 0.82, a much higher value than in other fractions. Table III illustrated the nucleic

TABLE II

CHOLESTEROL–PHOSPHOLIPID COMPOSITION

The numbers in parentheses represent number of experiments.

*Fractions**	*mg phosphate* / *mg protein*	*mg cholesterol* / *mg protein*	*mg phospholipid* / *mg protein*	*μmoles cholesterol* / *μmoles phospholipid*
Homogenate	0.064 (3)	0.015 (3)	1.25 (3)	0.02 (3)
F_I	0.147 (3)	0.340 (3)	1.1 (3)	0.82 (3)
F_{II}	0.102 (3)	0.420 (3)	3.0 (3)	0.26 (3)
F_{III}	0.067 (3)	0.800 (3)	2.67 (3)	0.32 (3)
Soluble	0.068 (3)	0.008 (3)	2.0 (3)	0.005 (3)

* See legend of Table I for explanation.

TABLE III

NUCLEIC ACID DISTRIBUTION

The numbers in parentheses represent number of experiments.

*Fractions**	*μmoles RNA-P* / *mg protein*	*μmoles DNA-P* / *mg protein*
Homogenate	0.12 (4)	0.070 (4)
F_I	0.07 (4)	0.021 (4)
F_{II}	0.059 (4)	0.106 (4)
F_{III}	0.061 (4)	0.400 (4)
Soluble	0.07 (4)	0.009 (3)

* See legend of Table I for explanation.

acid content of various fractions; RNA-P was 0.07 μmoles per mg protein in plasma membrane. None of the fractions had higher specific activity, however, the soluble fraction had the maximum percentage of the total RNA-P.

DNA-P was low in Fraction F_I and F_{II} while Fraction F_{III} had the maximum DNA-P/mg protein ratio as expected from the microscopic observation which showed Fraction F_{III} to be mainly nuclei and unbroken cells.

TABLE IV

ENZYMATIC ACTIVITIES OF THE FRACTIONS

Specific activities expressed as μmoles substrate broken down by 1 mg protein at 37° in 60 min except in case of cytochrome *c* oxidase and NADH–cytochrome *c* reductase. The activities expressed are ΔE/min per mg protein. Numbers in parentheses are the number of determinations each from a separate preparation.

Enzymes	*Fractions*				
	F_I	F_{II}	F_{III}	*Soluble*	*Homogenate*
5′-Nucleotidase	54.8 (11)	17.3 (9)	9.1 (11)	3.2 (6)	11.8 (9)
p-Nitrophenylphosphatase					
Alkaline (pH 9.5)	0.92 (6)	0.4 (5)	0.12 (5)	0.12 (3)	0.12 (5)
Acid (pH 5.5)	7.65 (7)	4.26 (5)	3.57 (5)	1.37 (2)	3.5 (3)
K^+-activated phosphatase	2.0 (7)	0.6 (2)	0.13 (2)	0.0 (2)	0.24 (2)
ATPase (Mg^{2+})	520 (3)	182 (2)	42.5 (2)	0.0 (2)	42 (2)
Leucyl-β-naphthylamidase	1.46 (4)	0.87 (4)	0.4 (4)	0.10 (4)	0.13 (4)
Phosphodiesterase (pH 8.9)	0.57 (5)	0.24 (5)	0.1 (3)	0.0 (3)	0.14 (3)
Cytochrome *c* oxidase	0.5 (3)	3.0 (3)	0.2 (3)	0.0 (3)	0.2 (3)
NADH–cytochrome *c* reductase	0.09 (3)	2.5 (3)	2.5 (3)	0.0 (3)	0.032 (3)

Enzymatic activities of the subcellular fractions

Table IV shows the enzyme distribution among various fractions. 5′-nucleotidase specific activity was 5 times higher in Fraction F_I as compared to the homogenate, indicating an enrichment of this activity in plasma membrane fraction. Other fractions either had specific activities lower or slightly higher than the homogenate.

Alkaline phosphatase activity using *p*-nitrophenylphosphate as the substrate at alkaline pH was 8 times higher in plasma membrane fractions than in the homogenate. Some enrichment of this activity was found in Fraction F_{II} as well. At acid pH the phosphatase activity was only twice that of the homogenate. K^+-activated phosphatase activity was also 8 times higher than in the homogenate. Mg^{2+}-activated ATPase activity was also concentrated in Fraction F_I but other fractions also showed considerable activity of ATPase.

Leucyl-β-naphthylamidase and phosphodiesterase activities were much higher in Fraction F_I, while other fractions had either no activity or lower activities.

Cytochrome *c* oxidase activity was maximum in Fraction F_{II}. So was NADH–cytochrome *c* reductase activity. (These activities determined within 6 h of killing the animal.)

DISCUSSION

Isolation of plasma membrane from smooth muscle is difficult because the tissue is tough and no gentle method of homogenization can break the majority of the cells.

A drastic homogenization procedure results in very small fragments of plasma membrane and most of the membrane is lost at the first step of low speed centrifugation[1]. We tried to overcome this difficulty by using the whole homogenate. After filtration, the homogenate was centrifuged at high speed to suspend the particulate fraction in a small volume for subsequent density gradient centrifugation and to permit recovery of the soluble fraction for other studies.

The density gradient used in our method has an added advantage of grossly separating other subcellular components as well. By adding a low-speed centrifugation step to differentially centrifuged Fraction F_{II} we were able to separate mitochondria from cytoplasmic membrane (Fig. 1). Using this technique we have studied the transport and binding of drugs to myametrium subcellular components (Miss R. Murthy *et al.*, unpublished data), namely plasma membrane, cytoplasmic membranes, mitochondria and nuclei.

MADDY[29] and others[7,28] utilized 4-acetamido-4′-isothiocyanate stilbene-2,2′-disulfonic acid, a fluorescent chemical marker, for cell surfaces. Our findings are consistent with their observation that the dye (4-acetamido-4′-isothiocyanate stilbene-2,2′-disulfonic acid) interacts with the plasma membranes. The dye was found to be concentrated in plasma membrane Fraction F_I.

Histochemical[30,31] evidence for the presence of 5′-nucleotidase in plasma membrane has prompted many[32] to use this enzyme as a plasma membrane marker enzyme. Although it is doubtful that the 5′-nucleotidase is exclusively present in the plasma membrane[33] it is reported to be considerably enriched in plasma membrane fractions isolated from different sources[8,32]. Our plasma membrane fraction had 5 times higher specific activity of 5′-nucleotidase as compared to the homogenate. Some activity was present in other fractions as well which could be due either to the presence of plasma membrane as a contaminant or to the localization of 5′-nucleotidase in other subcellular structures.

BENEDETTI AND EMMELOT[32] have shown the presence of leucyl-β-naphthylamidase activity to be present in the knobs isolated from rat liver plasma membrane. In our preparation β-naphthylamidase activity was found to be 10 times as concentrated in plasma membrane fraction as compared to that of total homogenate. Other enzyme activities which were found to be concentrated in plasma membrane of rat liver and other plasma membrane preparations are *p*-nitrophenylphosphatase (pH 9.5 and pH 5.5); K^+-activated *p*-nitrophenylphosphatase, ATPase (Ca_2^+ or Mg^{2+}) and phosphodiesterase. In our preparation alkaline *p*-nitrophenylphosphatase was found to be 8 times as concentrated in plasma membrane Fraction F_I while other fractions had much lower activities. Acid phosphatase activity using *p*-nitrophenylphosphate as substrate (pH 5.5) was also higher in our plasma membrane preparations. β-Glycerophosphate was not hydrolysed at all at either pH values. K^+-activated *p*-nitrophenylphosphatase activity was found to be 10 times as concentrated in Fraction F_I as compared to the homogenate. The phosphodiesterase (pH 8.9) specific activity was also 5 times higher in the plasma membrane fraction.

The above enzymes have been shown to be concentrated in plasma membrane of rat and mouse liver[32], intestinal epithelium[9] and other tissues. Our data are consistent with others in that the above enzymes were found to be concentrated in plasma membrane Fraction F_I.

Light and electron microscopic observation revealed that the Fraction F_I was

a homogenous vesicular fraction. Fraction F_{II} was found to contain mitochondria and cytoplasmic membranes and Fraction F_{III} was mainly nuclei and cell debri. Electron micrographs of Fraction F_I showed it nearly free of any other subcellular components which are readily recognizable such as mitochondria. No structures other than various size vesicles were observed. At high magnifications a characteristic triple layer of membrane was evident in vesicles sectioned in the appropriate plane. Fraction F_{II} was mainly mitochondria and cytomembranes. This was further confirmed by high concentration of cytochrome *c* oxidase and NADH–cytochrome *c* reductase activities. No appreciable glucose-6-phosphatase activity was found in the homogenate and all other fractions as well and therefore cannot be used as a marker for endoplasmic reticulum in rat uterus. Cytochrome *c* oxidase and NADH–cytochrome *c* reductase activities were measured within 6 h of killing the animals since these activities were found to be labile, and freezing–thawing reduced these activities to a very low level. Cholesterol:phospholipid ratio was high in Fraction F_I and close to the reported values for liver. Other fractions also have high cholesterol values but the ratio of cholesterol to lipid-P is not as high as for plasma membrane fraction.

Presence of a very small amount of RNA in plasma membrane is consistent with the observations of BENEDETTI AND EMMELOT[32]. Most of the RNA was either in the soluble fraction or other fractions. DNA was mainly confined to Fraction F_{III} which contained the nuclei.

The density of plasma membrane fraction under our conditions was around 1.13 which is lower than reported for liver but similar to rat bladder epithelium[34]. This could be due to different homogenizing conditions, *i.e.* ionic content of the homogenizing medium and the method of homogenization. Our homogenizing conditions were necessarily more drastic than those used for liver[32].

Yield of plasma membrane by our method was 14–27 % of the total protein and 59 % of the total 5'-nucleotidase activity. Our plasma membrane preparations seem to be fairly pure under electron microscope. The presence of mitochondrial and endoplasmic membrane marker enzyme in plasma membrane fraction suggests some contamination of mitochondrial fragments but in electron microscope sections there were no mitochondria present.

Saline-soluble proteins are reported to be present in liver plasma membrane[2] preparations which are presumed to originate from the cytoplasm of the cell during homogenization. We were unable to demonstrate the presence of any saline soluble fraction in our plasma membrane preparation.

Since all the subcellular fractions were obtained in one step and in a very short period of time (3 h) without any drastic extractions, and high yield, this technique may prave very useful in the studies on drug-receptor interactions, biosynthetic, enzymatic and chemical studies of plasma membrane and other subcellular components.

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