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Plasma membranes from isolated liver cells

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SUMMARY

Plasma membranes have been prepared from isolated rat or guinea pig liver cells by a method established for whole liver. However, plasma membranes of liver cells isolated by disruption of the tissue with collagenase and hyaluronidase are in some detail dissimilar to those obtained from whole liver. This was indicated by lower cholesterol content and low specific activities of some marker enzymes.

Isolation of plasma membranes from liver by the method of Neville[1,2] or by the modifications of Emmelot *et al.*[3] and Ray[4] is considered to yield mostly, if not exclusively, membrane fragments of parenchymal cells. In the case of the intact liver tissue the adjacent hepatocytes are tightly connected especially around the bile canaliculi ("tight junctions") and the rigidity thereby accorded to these segments of the plasma membranes might be important for their preparative isolation. Attempts to prepare plasma membranes from isolated parenchymal cells, using the above methods, have been reported to have been unsuccessful[5,6]. By the application of the method of Kamat and Wallach[7], however, Graham *et al.*[6] could obtain plasma membrane vesicles from isolated liver cells. Wright and Green[8] reported that the method of Warren *et al.*[9], based on a different principle again, is applicable to isolated liver cells.

We report here the preparation of plasma membranes from isolated liver cells from rat and guinea pig livers using the method of Ray[4].

The procedure for the isolation of liver cells was a modification of the method of Berry and Friend[10]. The livers (two at a time in the case of guinea pig, four in the case of rat) were submerged in the medium which was recirculated through the portal vein and allowed to drain through the hepatic vein. The perfusion medium consisted of 500 ml Ca^{2+}-free Hanks' solution with 0.05% collagenase (Type I, Sigma) and 0.1% hyaluronidase

(Type I, Sigma) in 10 mM phosphate buffer, pH 7.4. The perfusion lasted 20 min at 37 °C at a rate of 100 ml/min. The air phase consisted of O_2 which was flushed through the closed container at a rate of 5 l/min. The pH drop was not more than 0.1–0.2. The livers were then perfused with ice-cold Ca^{2+}- and Mg^{2+}-free Hanks' solution containing 2 mM EDTA. The disrupted tissue was gently stirred for 30 min with a teflon-coated magnetic stirrer in a siliconized beaker containing ice-cold Ca^{2+}-free Hanks' solution before filtering through four layers of cheese cloth. The diluted cell suspension was washed twice by centrifugation at 50 × *g* for 2 min. The preparation was checked morphologically by phase contrast microscopy (magnification from 500 × to 1250 ×) in the native state, and after fixation in 1% formalin in Hanks' solution. The samples were then stained with Giemsa's stain or embedded and stained with hematoxylin and eosin.

The yield of cells varied greatly from preparation to preparation, from 11% to 53% of wet liver weight (average 23%). A satisfactory preparation consisted of 85–90% intact and viable (Trypan blue test), almost exclusively parenchymal cells. Morphological examination, as well as the determination of lactate dehydrogenase and glutamate–pyruvate transaminase activities in the supernate of cell suspensions and in the homogenate indicated no significant disruption of the cells. A number of experiments indicated, however, that during incubation of cell suspensions at 0, 25 or 37 °C for 60 min with [^{14}C] AMP or [γ-^{32}P] ATP or blue dextran (approximate molecular weight, 250 000) the cells were permeable to these molecules which are supposed to be excluded. At the same time, the O_2 consumption of the cell suspension in Tris or Hanks' media, without additions, ranged between 0.52–1.00 μl/mg protein per h which are values comparable to those reported by others[11,12]. Furthermore, the isolated cells utilized [1-^{14}C] palmitic acid, at rates similar to intact liver, for the synthesis of neutral glycerides and phospholipids.

The isolation of plasma membranes was carried out according to the method of Ray[4] with the addition of a 26% sucrose layer to the density gradient. (All sucrose solutions are expressed on w/w basis.) The cells were pelleted by centrifugation at 10 000 rev./min for 10 min in Ray's medium[4], then homogenized in a loose-fitting Dounce homogenizer (clearance, 0.10–0.15 mm) with 2.5 times their wet weight of medium. 24–30 strokes were necessary for the disruption of the total population of cells in order to avoid contamination of the membrane fraction with whole cells. The low-speed pellet from 2 g or less of packed liver cells was added in each centrifuge tube. Membranes were collected at the 26%/37% and 37%/41% sucrose interphases. In case of whole liver 9–10 strokes were sufficient to obtain comparable homogenate under otherwise identical conditions. The isolated plasma membranes were characterized by their lipid composition and by the activities of two marker enzymes. The total lipid content was determined according to the method of Bragdon[13], total cholesterol according to that of Zlatkis and Zak[14], and total phospholipid according to that of Bartlett[15]. 5'-Nucleotidase (EC 3.1.3.5), leucyl-β-naphthylamidase (EC 3.4.1.1), nucleotide pyrophosphatase (EC 3.6.1.9) and glucose-6-phosphatase (EC 3.1.3.9) were assayed as described previously[16]

Table I summarizes the data on lipid composition. The increased lipid to protein

TABLE I

LIPID CONTENT OF PLASMA MEMBRANES FROM RAT AND GUINEA PIG LIVERS*

Species	*Fraction*	*Tissue*	*Total lipid (mg)* / *Protein (mg)*	*Phospholipid (mg)* / *Protein (mg)*	*Cholesterol (mg)* / *Protein (mg)*	*Phospholipid (mole)* / *Cholesterol (mole)*
Rat	Homogenate	Whole liver	0.24, 0.25	0.18, 0.16	0.016, 0.017	5.7, 4.6
		Isolated cells	0.31, 0.32	0.26, 0.28	0.018, 0.018	7.2, 7.7
	Plasma membrane	Whole liver	0.44, 0.52	0.37, 0.41	0.081, 0.10	2.3, 2.0
		Isolated cells	0.70, 0.52	0.62, 0.48	0.051, 0.025	6.1, 9.7
Guinea pig	Homogenate	Whole liver	0.18, 0.23 0.23	0.16, 0.19, 0.18	0.007, 0.008, 0.010	11.1, 11.6, 9.0
		Isolated cells	0.31, 0.33	0.31, 0.25	0.021, 0.014	7.3, 9.0
	Plasma membrane	Whole liver	0.49, 0.53, 0.45	0.36, 0.33, 0.35	0.094, 0.13, 0.10	1.9, 1.2, 1.7
		Isolated cells	0.39, 0.54, 0.54	0.33, 0.37 0.38	0.070, 0.089, 0.085	2.3, 2.1, 2.2

*The values represent separate experiments.

ratio of isolated cell homogenates, as compared to whole livers, is probably due to the removal of intercellular protein components during cell isolation. In the membranes derived from isolated cells there is a markedly lower cholesterol content than in those of whole liver. This is reflected in higher phospholipid to cholesterol molar ratios. In addition, in the membranes of isolated rat liver cells the total lipid to protein and the phospholipid to protein ratios are higher than in those from whole liver. This suggests possible ablations of some protein components.

Table II presents data on 5′-nucleotidase and leucyl-β-naphthylamidase activities in rat plasma membranes. In the homogenate of isolated cells the specific activity of 5′-nucleotidase is lower and that of leucyl-β-naphthylamidase is higher than in the whole liver homogenate. However, there is a significantly lower specific activity of both enzymes in the membranes obtained from isolated cells than in those from whole liver.

TABLE II

MARKER ENZYME ACTIVITIES IN RAT LIVER PLASMA MEMBRANES*

Specific acitvity is expressed in μmoles of substrate metabolized per mg protein per h.
Total activity is expressed in μmoles of substrate metabolized per total protein per h.

Fraction	*Tissue*	*Total protein (mg)*	*5′-Nucleotidase*		*Leucyl-β-naphthylamidase*	
			Spec. act.	*Total act.*	*Spec. act.*	*Total act.*
Homogenate	Whole liver	1520	3.8	5780	0.33	502
		1710	3.8	6500	0.34	581
	Isolated cells	1880	2.5	4700	0.43	808
		475	2.0	950	0.46	219
Plasma membrane	Whole liver	8.7	38.9	338	4.3	37
		16.7	37.3	623	4.5	75
	Isolated cells	6.6	8.7	57	1.1	7.3
		4.3	2.2	9.5	0.33	1.4
Yield in plasma membrane	Whole liver	0.57%		5.8%		7.4%
		0.98%		9.6%		12.9%
	Isolated cells	0.35%		1.2%		0.90%
		0.91%		1.0%		0.64%

*Each value represents a separate experiment.

Table III presents data on 5′-nucleotidase, leucyl-β-naphthylamidase, nucleotide pyrophosphatase and glucose-6-phosphatase activities in guinea pig plasma membranes. The results were similar to those obtained with the rat. The specific activities of the marker enzymes were only 3–4-fold higher than in the homogenate. This is less

TABLE III

ENZYME ACTIVITIES IN GUINEA PIG LIVER PLASMA MEMBRANES*

Specific activity is expressed in μmoles of substrate metabolized per mg protein/h.
Total activity is expressed in μmoles of substrate metabolized per total protein/h.

Fraction	*Tissue*	*Total protein (mg)*	*5'-Nucleotidase*		*Leucyl-β-naphthylamidase*		*Nucleotide pyrophosphatase*		*Glucose-6-phosphatase*	
			Spec. act.	*Total act.*	*Spec. act.*	*Total act.*	*Spec. act.*	*Total act.*	*Spec. act.*	*Total act.*
Homogenate	Whole liver	1880	0.38	714	0.42	790	0.03	56	3.8	7140
		1880	0.34	639	0.41	771	–	–	4.0	7520
	Isolated cells	648	0.35	227	0.87	564	0.08	52	9.4	6090
		351	0.20	70	0.68	239	0.07	25	10.9	3830
Plasma membrane	Whole liver	2.00	2.56	5.1	5.1	10.2	0.64	1.3	4.9	9.8
		2.00	3.14	6.3	4.2	8.4	0.53	1.1	6.5	13
	Isolated cells	4.4	1.10	4.8	3.00	13.2	0.33	1.5	4.1	18
		5.4	0.53	2.9	2.53	13.6	0.19	1.0	3.7	20
Yield in plasma membrane	Whole liver	0.11%		0.71%		1.3%		2.3%		0.14%
		0.10%		0.99%		1.1%		–		0.17%
	Isolated cells	0.67%		2.1%		2.3%		2.9%		0.3%
		1.53%		4.1%		5.7%		4.0%		0.52%

*Each value represents a separate experiment.

than the increase found in plasma membranes from whole liver. The specific acivity of the microsomal marker enzyme glucose-6-phosphatase was lower in the plasma membranes than in the homogenate of isolated cells. The yield of glucose-6-phosphatase in the plasma membranes was significantly lower than that of the marker enzymes. This suggests that the differences observed between plasma membranes from isolated liver cells and from whole liver were not due to contamination with microsomes.

Experiments were carried out in order to evaluate whether the collagenase and hyaluronidase preparations might have a direct effect on the membranes. Plasma membranes isolated from whole liver were incubated in the perfusion medium for 20 min and washed using high-speed centrifugations; control experiments consisted of incubation in Ca^{2+}-free Hanks' solution devoid of enzymes. Specific activities of marker enzymes did not decrease as the result of collagenase and hyaluronidase treatment. Collagenase and hyaluronidase had no marker enzyme activities on their own. Neither has the enzyme treatment caused changes in the lipid composition of the membrane preparation.

A greater number of strokes was required for the homogenization of isolated cells than for the whole liver. This may result in different fragmentation of the plasma membranes, and may be reflected in different enzymic properties of the preparations due to heterogenous composition of plasma membranes. However, differential fragmentation could occur even if the number of strokes were the same because the consistency of the isolated cell suspension is not the same as that of the intact liver tissue. Such factors undoubtedly complicate the sole use of marker enzyme activities in the characterization of plasma membrane preparations[16, 17].

An apparently selective reduction in cholesterol content associated with lower 5′-nucleotidase activity was reported for liver plasma membranes of essential fatty acid deficient rats[18]. Such findings together with our results suggest a possible role for cholesterol in plasma membrane 5′-nucleotidase activity. In the present study, however, the loss of some enzyme proteins has also to be considered. Phospholipids have been reported to be essential for the integrity of Mg^{2+}-ATPase and of adenylate cyclase activities, but not for plasma membrane 5′-nucleotidase[19].

These studies show that plasma membranes can be prepared from isolated rat or guinea pig liver cells by the methods established for whole liver. The membrane fragments behave similarly in the sucrose gradient, appear comparable under phase contrast microscope and have similar total lipid content. However, a reduction of cholesterol content and of some marker enzyme activities indicate that the plasma membranes obtained from liver cells isolated by disruption of the tissue with collagenase and hyaluronidase are in some details dissimilar to those obtained from whole liver.

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