SUBFRACTIONATION OF RAT LIVER PLASMA MEMBRANES

W.H.EVANS
National Institute for Medical Research, Mill Hill, London N.W.7, United Kingdom

Received 7 May 1969

1. Introduction

Rat liver plasma membranes prepared from crude nuclear fractions by the method of Neville [1] as modified by Emmelot et al. [2] are finally isolated by isopycnic banding at the interface of sucrose densities 1.16 and 1.18 g/cm^3. The plasma membrane fraction has been extensively studied morphologically, enzymically and chemically [3]. The preparation of liver plasma membranes as large membrane fragments depends on carefully controlling the conditions of tissue dispersion, a loose-fitting Dounce homogeniser being utilised throughout the preparative procedure.

In this communication, it is shown that plasma membrane fractions dispersed in a tight-fitting Dounce homogeniser can be resolved in continuous sucrose gradients into two major subfractions of densities 1.12 and 1.21. The lighter subfraction is shown by electron microscopy to be vesicular in nature, and to be enriched in lipid and those enzymic activities generally recognised as plasma membrane markers. The heavier subfraction retains the membrane sheets and structures characteristic of the plasma membrane fraction, and is deficient in plasma membrane marker enzymes with the notable exception of the cation sensitive adenosine triphosphatase.

2. Experimental procedure

Plasma membranes were prepared from the livers of Sprague Dawley rats 150 g weight as described by Emmelot et al. [2] with the following modification: separation of membranes from nuclei and mitochondria by repeated low speed centrifugation was replaced by centrifugation through a sucrose gradient using the low-speed M.S.E. 'A' Zonal rotor [4]. For isopycnic density gradient centrifugation, linear sucrose gradients containing 1 mM NaH CO_3 pH 7.4 were prepared from 12 ml 55% sucrose and 12 ml 35% sucrose. The membranes were suspended in 8 ml 0.25 M sucrose – 1 mM NaH CO_3, either gently using a loose Dounce homogeniser or by 25 strokes of a tight fitting pestle (Blaessig Glass Co., Rochester, New York; stated clearances: 005″ and 003″) and layered onto the gradients. The samples were centrifuged for 5 hr at 63,000 *g* using a Spinco rotor SW 25.1, and the gradients resolved using an Isco density gradient fractionator with concurrent recording of E_{280}. The yield of unfractionated plasma membranes was approximately 3–5 mg protein per 10 g wet liver weight. Sucrose densities were measured at 0° by refractometry.

The following enzymic activities were measured on freshly prepared samples suspended in 0.25 M sucrose: 5′-nucleotidase [2], succinate dehydrogenase [5], glucose-6-phosphatase [6] leucyl β-napthylamidase [7], inosine diphosphatase [8], adenosine triphosphatase [9]. Protein was estimated by the method of Lowry [10]. Membrane subfractions were dialysed against distilled water to remove sucrose and centrifuged to give a pellet which was then extracted three times at room temperature during 24 hr with chloroform methanol 2/1 (v/v). The extract was used for cholesterol estimation [11] and a perchloric acid digest of it was used for phospholipid phosphorus estimation [12]. Electron micrographs were made on glutaraldehyde fixed, osmium tetroxide stained sections.

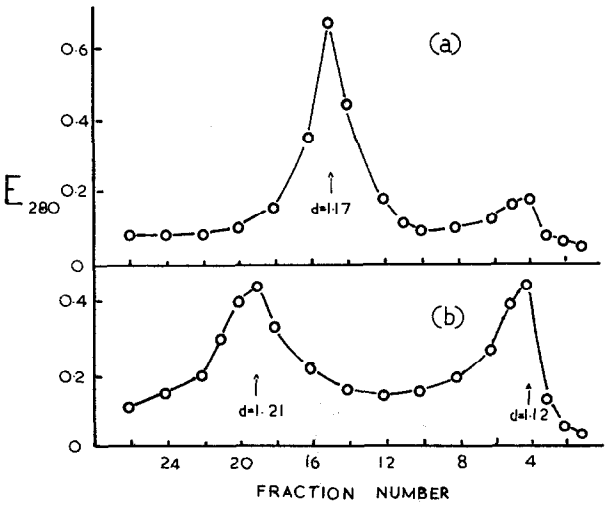

Fig. 1. Density gradient fractionation of rat liver plasma membranes in sucrose. Membranes were dispersed (a) in a loose fitting (b) a tight-fitting Dounce homogeniser. A flow cell of optical path length 2 mm, and approximately 8 mg membrane protein per tube were used. Fractions of 1 ml were collected.

3. Results

Plasma membranes, as usually prepared, have an isopycnic density in sucrose of about 1.17 (fig. 1a). Membranes dispersed in a tight fitting Dounce homogeniser are resolved in sucrose gradients into two major subfractions of buoyant density 1.12 (subfraction A) and 1.21 (subfraction B) (fig. 1b). Enzyme determinations on unfractionated and subfractionated membranes are shown in table 1. Three enzymes – 5′ nucleotidase, inosine diphosphatase and leucine aminopeptidase – are found in subfraction A at approximately four- to six-fold the activity of the enzymes in subfraction B. The total amount of enzymes recovered is also higher in subfraction A. However, another plasma membrane marker enzyme, sodium stimulated adenosine triphosphatase, has a higher specific activity in the subfraction B, whilst enzyme not requiring added sodium ions, measured simultaneously, is present at higher specific activity in subfraction A. Measurement of glucose-6-phosphatase, acid phosphatase, and succinic dehydrogenase activities indicate the low degree of contamination by microsomes, lysosomes and mitochondria respectively.

Electron microscopy (fig. 2a) indicates the plasma membrane fraction to be composed of a population of sheets of membranes frequently connected by des-

Table 1
Enzyme activities of rat liver plasma membrane subfractions.

Enzyme activities	Preparation 1			Preparation 2	
	Unfractionated membranes 8 mg protein	Subfraction A 1.7 mg protein	Subfraction B 5.0 mg protein	Subfraction A 2.1 mg protein	Subfraction B 5.2 mg protein
5′ nucleotidase	16.7	45.8	10.3	104.5	18.7
Inosine diphosphatase	9.0	31.0	6.1	28.4	6.0
Leucine aminopeptidase	7.6	30.6	4.0	18.8	4.6
Na, K, Mg adenosine triphosphatase	8.6	8.7	13.6	4.3	6.5
K, Mg adenosine triphosphatase	60.0	126.0	31.4	80.1	30.1
Glucose-6-phosphatase	0.91	1.70	0.84	1.38	0.90
Acid phosphatase	0	0	0	1.3	0.4
Succinic dehydrogenase	0.08	0	0.09	0	0.17

Enzyme activities are expressed as μmoles product liberated per mg protein per hr at 37°.

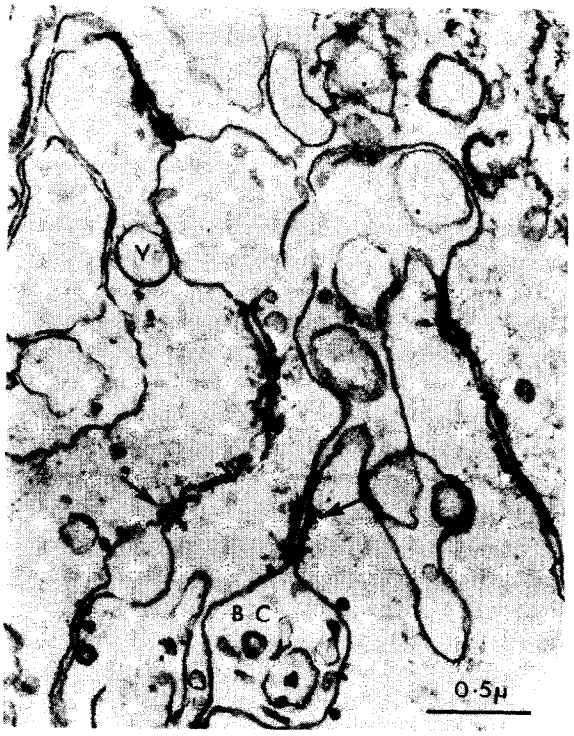

Fig. 2a.

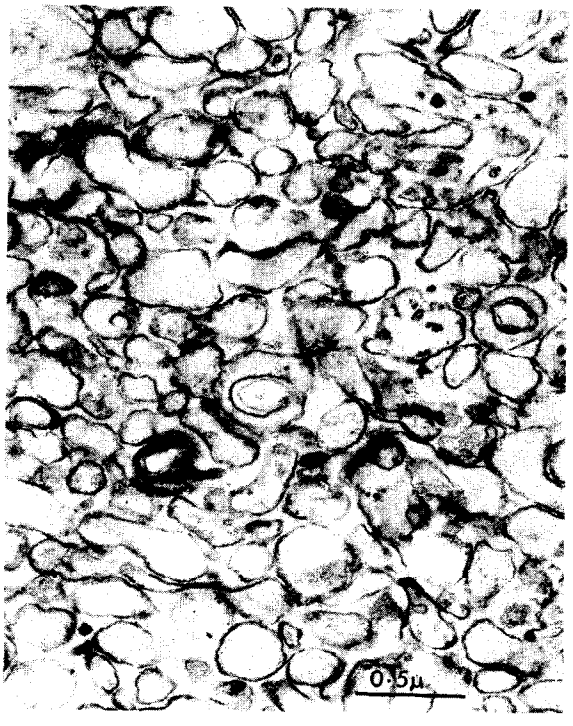

Fig. 2b.

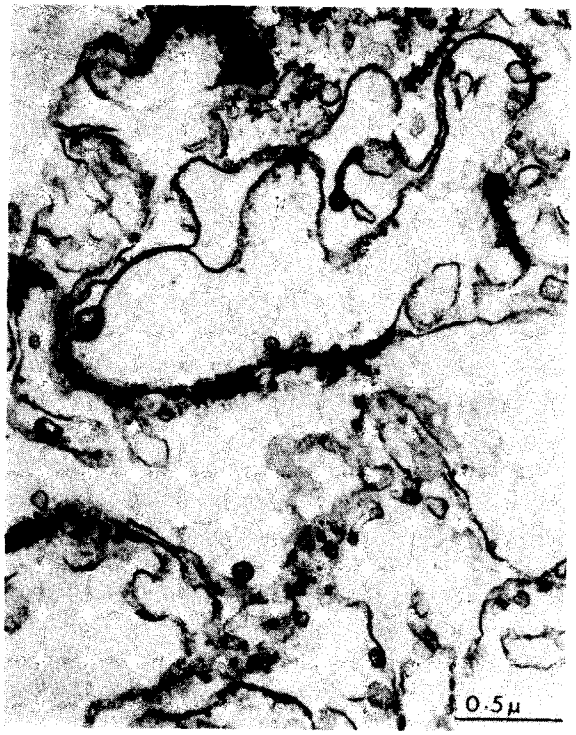

Fig. 2c.

Fig. 2. Electron micrographs X 28,190 of rat liver plasma membranes. (a) Unfractionated membranes; v = smooth vesicle, bc = bile canaliculus, arrows point to desmosomes. (b) Subfraction A. (c) Subfraction B.

Table 2
Phospholipid and cholesterol content of liver plasma membrane subfractions.

	Subfraction A	Subfraction B
mg phospholipid/ mg protein	1.30	0.62
mg colesterol/ mg protein	0.186	0.075

Values represent duplicates on two preparations. Phospholipid weight was calculated as μg phospholipid $P \times 25$.

mosomes and tight junctions, smooth membrane vesicles, and bile canaliculi. Fig. 2b shows subfraction A to be composed predominantly of large and small smooth vesicles. Subfraction B (fig. 2c) consists mainly of sheets of membranes, and occasional desmosomes and membrane vesicles.

Examination of the phospholipid, sterol and protein content of the subfractions provides an explanation of their widely differing buoyant densities. Table 2 indicates that with reference to unit weight of protein subfraction A contains twice as much phospholipid and cholesterol as subfraction B. Both subfractions contain a similar ratio of phospholipid to cholesterol.

4. Discussion

The liver plasma membrane fraction is undoubtedly a complex subcellular fraction. The subfractionation achieved can be regarded as a contribution to resolving the plasma membrane fraction into its components. Electron microscopy indicates that the subfractionation has achieved the removal into subfraction A of the vesicular components of the plasma membrane fraction. The enzyme activities measured strongly suggest that the vesicles are the main subcellular location of the 5′-nucleotidase, inosine diphosphatase, and leucine aminopeptidase enzymes. The low glucose-6-phosphatase activity of this fraction indicates that the vesicles are not derived from the endoplasmic reticulum. The activities of these enzymes present in subfraction B may be due to the persistence of attached vesicles as indicated by electron microscopy. The results suggest that sodium stimulated adenosine triphosphatase activity is probably fairly evenly distributed in the various components of the plasma membrane fraction, but the high activity of adenosine triphosphatase in the absence of sodium, found in subfraction A points to the separate location of the two adenosine triphosphatase enzyme systems.

Besides the relatively high specific activity of certain enzymes in subfraction A, its high lipid content also deserves note, especially when compared with other mammalian membrane systems [13]. The high lipid content, because of its effect upon surface tension, probably accounts for the vesicular nature of subfraction A and poses questions about the molecular arrangement of lipid and protein in this membrane system.

The origin of the vesicles in subfraction A with respect to possible biochemically specialized regions of the plasma membrane of rat liver remains to be elucidated.

Acknowledgements

I wish to thank Dr. H.R.Perkins for his advice and encouragement, Dr. J.A.Armstrong for the electron micrographs, and Mr. R.Fordham for excellent technical assistance.

References

[1] D.M.Neville, J. Biophys. Biochem. Cytol. 8 (1960) 413.
[2] P.Emmelot, C.J.Bos, E.Benedetti and Phe.Rumke, Biochim. Biophys. Acta 90 (1964) 126.
[3] E.L.Benedetti and P.Emmelot, in: The Membranes, eds. A.J.Dalton and F.Haguenau (Academic Press, New York, 1968) p. 33.
[4] W.H.Evans, in preparation.
[5] D.C.N.Earl and A.Korner, Biochem. J. 94 (1965) 721.
[6] M.A.Swansen, in: Methods in Enzymology, Vol. 2, eds. S.P.Colowick and N.O.Kaplan (Academic Press, New York, 1955) p 514.
[7] J.A.Goldberg and A.M.Rutenburg, Cancer 11 (1958) 283.
[8] G.W.E.Plaut, in: Methods in Enzymology, Vol. 6, eds. S.P.Colowick and N.O.Kaplan (Academic Press, New York, 1962) p. 231.
[9] P.D.Swanson, H.F.Bradford and H.McIlwain, Biochem. J. 92 (1964) 235.

[10] O.H.Lowry, N.J.Rosebrough, A.L.Farr and R.J.Randall, J. Biol. Chem. 193 (1951) 265.
[11] E.J.King and I.D.P.Wootton, Micro-analysis in Medical Biochemistry (J. and A.Churchill, London, 1956) p. 42.
[12] G.R.Bartlett, J. Biol. Chem. 234 (1959) 466.
[13] J.S.O'Brien, J. Theoret. Biol. 15 (1967) 307.