2 components and fused with 2 bands formed between AS and M-2/S (II). These 2 bands, in turn, fused with 2 similar bands formed between AS and sodium deoxycholate-solubilized microsomal membranes (III). Likewise, Band D split into at least 3 components and fused with 3 similar bands between AS and sodium deoxycholate-solubilized membranes (III).

Dissociation of a single antigenic material into smaller units that contain different antigenic determinants has been shown[8] to result in the splitting of precipitin bands in double diffusion studies in an Ouchterlony plate. The band-splitting noted in our studies indicate that ultrasonic irradiation of M-1 resulted in the dissociation of membrane antigens into several antigenic subunits. That these precipitin bands are indeed due to membrane antigens is shown by their reaction of identity with bands formed between AS and sodium deoxycholate-solubilized microsomal membranes.

AS reacted with 105000 × *g* supernatant of rat-liver homogenate (IV) to yield 4 precipitin bands in addition to the Band A. These 4 bands, however, showed no interaction with any of the bands formed between AS and various microsomal preparations. The M-2/S preparation used for immunization may have contained a trace of cell sap, enough to produce an antibody response in the rabbits but not enough to form precipitin bands on the Ouchterlony plate.

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The isolation of rat liver plasma membrane fragments

Preparations of liver plasma membranes may be obtained from crude nuclear fractions by modifications of the method of NEVILLE[1] (*e.g.* ref 2). Such preparations have been extensively studied both in the electron microscope and by analysis of gross composition and enzymic content[2–5]. KAMAT AND WALLACH[6] have shown that

plasma membrane preparations of Ehrlich ascites carcinoma cells can be isolated from microsomal pellets. This method has been applied to liver and a fraction obtained which corresponds to the plasma membrane of the carcinoma cells[7]. Proof has now been obtained that this fraction is, in fact, derived from the plasma membrane.

Livers were removed from eight normal rats killed by exsanguination under light chloroform anaesthesia and each lobe chopped up and divided into two equal portions on an ice-cold surface. One portion was transferred to 0.25 M sucrose, 0.0002 M $MgSO_4$ and the other to 0.001 M $NaHCO_3$ and portions were combined to give two equal masses of chopped liver. One was fractionated according to EMMELOT *et al.*[2], the other according to KAMAT AND WALLACH[6], a sample of the original homogenate being retained in each case. The only modification was the use of a Potter–Elvehjem homogeniser instead of a nitrogen pressure vessel in the KAMAT AND WALLACH method. A comparison was then made of the level of certain enzymes in the original homogenate and in the two plasma membrane preparations.

The enzymes measured were: glucose-6-phosphatase (EC 3.1.3.9) and succinate–cytochrome *c* reductase (EC 1.3.99.1)[4,8] to assess contamination by endoplasmic reticulum and mitochondria, respectively; 5′-nucleotidase (EC 3.1.3.5), L-leucyl-β-naphthylamidase (EC 3.4.1.1) and alkaline phosphatase (EC 3.1.3.1)[2,4,9]. The last three are all known to be concentrated in the rat liver parenchymal cell plasma membrane[2,4,5]. Protein was determined using the Folin–Ciocalteu reagent.

The results of the analyses are shown in Table I. Each figure is the mean of three determinations. These agreed to within 20% in all cases except for the 5′-nucleotidase of the preparation made according to EMMELOT *et al.* which showed quite wide variation (5.7–10.4 μmoles/mg protein per h). Thus the differences in the content of this enzyme between the two preparations may not be as great as the results suggest. The detection of alkaline phosphatase depends on the substrate used. As we have confirmed, phenylphosphatases are present in liver plasma membranes[2,5] but glycerolphosphatases are not[2,4].

The results establish that the preparations made according to KAMAT AND WALLACH do consist of plasma membrane with negligible contamination by mitochondria or endoplasmic reticulum. Examination of these in the electron microscope shows that they consist of closed vesicles as is the case with the ascites cell prepa-

TABLE I

ENZYMIC CONTENTS OF PLASMA MEMBRANE PREPARATIONS

The activities of all enzymes are expressed as μmoles of substrate hydrolysed per mg of protein per h at 37° except for the succinate–cytochrome *c* reductase which is given in arbitrary units/mg of protein. The substrate used in the determination of alkaline phosphatase was disodium phenyl phosphate.

	Yield (mg protein per g liver protein)	*Enzyme activity*				
		5′-Nucleotidase	*Alkaline phosphatase*	*L-Leucyl-β-naphthylamidase*	*Glucose-6-phosphatase*	*Succinate–cytochrome c reductase*
Whole homogenate	—	<0.6	<2.5	0.9	19.6	25.2
Plasma membranes[2]	2.2	7.7	13.1	11.1	<0.3	2.5
Plasma membranes[6]	1.1	11.3	16.0	12.9	<0.3	<0.5

rations[10] whereas the preparations made according to EMMELOT *et al.* consist largely of planar sheets[2,4].

These results confirm that the earlier conclusions[11] based on the use of KAMAT AND WALLACH preparations were valid. The method appears to be of value in two important respects. Firstly, if as seems probable from their similar properties, the vesicles form in the same way as those from ascites cells with the original outer surface of the membrane still to the outside[9] they would be more useful for certain binding and permeability studies than the open sheets obtained by the alternative method. Secondly, much interest is now being shown in the use of isolated parenchymal cells for metabolic studies[12,13]. The method of EMMELOT *et al.* does not work with these cells because the junctional complexes are broken[14,*] but the KAMAT AND WALLACH method still gives plasma membrane vesicles*. The work of EL-AASER *et al.*[15] indicates that the application of zonal ultracentrifugation will greatly improve the yield and the speed of the isolation process.

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