

*Biochimica et Biophysica Acta*, 604 (1980) 27–64  
 © Elsevier/North-Holland Biomedical Press

BBA 85203

## A BIOCHEMICAL DISSECTION OF THE FUNCTIONAL POLARITY OF THE PLASMA MEMBRANE OF THE HEPATOCYTE

W. HOWARD EVANS

*Medical Research Council, National Institute for Medical Research, The Ridgeway, Mill Hill,  
 London NW7 1AA (U.K.)*

(Received September 20th, 1979)

### Contents

I.	Introduction . . . . .	28
II.	Anatomical correlates of liver plasma membrane diversity . . . . .	28
	A. Parenchymal and other liver cells . . . . .	29
	B. Morphology of the hepatocyte cell surface regions . . . . .	31
III.	Devolution of plasma membrane functions . . . . .	31
	A. Cellular aspects . . . . .	31
	B. Regional devolution of plasma membrane functions on hepatocytes . . . . .	33
	C. Hepatocyte functional heterogeneity . . . . .	33
IV.	Preparation of plasma membrane fractions . . . . .	33
V.	Functional characterisation of plasma membrane fractions . . . . .	35
VI.	Hepatocyte intercellular junctional complexes . . . . .	37
VII.	Biochemical properties of the hepatocyte plasma membrane . . . . .	38
	A. Enzyme distribution and properties . . . . .	38
	B. Chemical composition . . . . .	43
VIII.	Plasma membrane pathological modifications . . . . .	45
IX.	Plasma membrane biogenesis and turnover – their role in the generation of functional polarity . . . . .	48
	A. Biogenesis . . . . .	48
	B. Turnover . . . . .	51
X.	Concluding remarks . . . . .	53
	Acknowledgements . . . . .	54
	References . . . . .	54

## I. Introduction

During the last decade the mammalian plasma membrane has emerged as a complex and heterogeneous organelle. Its outer surface may contain a range of sophisticated and highly discriminating, information transducing transmembrane signalling systems that enable the cell to recognise and react promptly to environmental signals. The plasma membrane features in intercellular adhesion and direct communication as well as carrying out the more mundane barrier and transporting functions. A large number of membrane proteins and glycoproteins are arranged transversely across the lipid bilayer in a highly specific configuration and emerge as candidates for functioning as receptors, enzymes and transmembrane molecular links and conduits. A further feature of plasma membrane organisation and cell surface topography concerns the concentration of many classes of membrane components at specific functional regions of the pericellular membrane. This differentiation of the plasma membrane into anatomical domains with functional connotations is most marked in cells comprising tissues and organs. The hepatocyte plasma membrane is one such example of a membrane showing distinct functional polarity and this aspect forms a major theme of this review on its biochemistry and biogenesis.

A wide range of activities occur at the plasma membrane of the hepatocyte reflecting the well established physiological versatility of the cell with respect to classical hepatic and biliary functions. Thus, anatomically, the hepatocyte surface comprises two regions studded with numerous microvilli corresponding to the vascular and biliary poles and these are separated by a smoother region where desmosomes and gap junctions are located. The biliary pole is completely enclosed by tight junctions, that effectively segregate this membrane region from the remainder of the plasma membrane. The tight junction is a membrane specialisation found in epithelia allowing control of transcellular and pericellular permeability and in the hepatocyte it separates the 'apical' biliary region and the 'baso-lateral' regions exposed to the blood sinusoids. Physiologically, the plasma membrane comprising the vascular pole and especially the microvilli at the sinusoidal region feature in the transport of metabolites to and from the blood and for interaction with hormones and numerous blood-borne substances that regulate hepatic functioning. The plasma membrane comprising the biliary pole is involved in the discharge of bile into the canalicular spaces.

Plasma membrane preparations are now used to study an ever-increasing number of hepatic functions. However, the question of how representative of the various cell surface functional domains the isolated fractions are has received relatively little attention. In this review the preparation and properties of liver plasma membranes are discussed giving emphasis to anatomical and physiological correlates. Since liver plasma membranes are used increasingly to study changes underlying disease states, the current status of membrane modifications in hepatobiliary disease, malignancy and after exposure to toxic substances is discussed and assessed. Mechanisms governing the biogenesis and turnover of a functionally polarised plasma membrane are also considered. The literature survey extends mainly from 1968 when the landmark review of Benedetti and Emmelot [1] appeared.

## II. Anatomical correlates of liver plasma membrane diversity

Earlier microscopic investigations of liver [2–5] have been superseded by those using transmission and scanning electron microscopy and a new picture of the inter-relation-

TABLE I

## SURFACE AREA AND VOLUME OF THE FOUR MAJOR LIVER CELL TYPES

Data from a stereological study using rat liver [11]. A recent estimate of the relative surface area of the three major hepatocyte surface regions is: sinusoidal, 72%; lateral, 15%; bile, 13% [175].

	Aggregate plasma membrane surface area in cell type (%)	Percent of parenchymal volume
Hepatocytes	73.5	77.8
Non-hepatocytes	26.6	6.3
Endothelial cells	15.2	2.8
Kupffer cells	4.3	2.1
Fat-storing cells	7.1	1.4
Intercellular spaces		15.9
Disse space		4.9
Sinusoid lumen		10.6
Bile canaliculi		0.4

ships of liver cells and the nature of the sinusoidal spaces and bile channels has now emerged [6]. The application of stereological techniques has helped to define the relative cell surface areas contributed by liver parenchymal and non-parenchymal cells (Table I).

#### *IIA. Parenchymal and other liver cells*

The cellular heterogeneity of liver has been analysed, especially with respect to the functions and morphology of the non-parenchymal cells [7-10]. As the technology of subcellular fractionation of liver tissue becomes more sophisticated, knowledge of the contribution of various cell populations to the isolated fractions becomes increasingly desirable. The relative distribution between hepatocytes and non-parenchymal cells of organelles has now been thoroughly quantified [11,12]. These studies show, for example, that although non-hepatocytes account for only 8% of the total surface area of all liver membranes, they constitute 26.5% of all plasma membranes and 15.1% of the Golgi apparatus [11]. In addition to transient red blood cells, at least four major categories of non-parenchymal cells have been described. The sinusoids are lined with thin fenestrated endothelial cells that demarcate the space of Disse which extends to the sinusoidal plasma membrane of the hepatocyte. Mobile Kupffer cells, characterised by amoeboid protusions and fat (stellate) cells storing vitamin A are found between the endothelial cells and hepatocytes. Endothelial cell arrangement and the permeability of the outer-endothelial fenestrations varies between species, being most marked between herbivores and carnivores [13]. The closely-knit endothelial cell network may function, for example, in controlling entry of chylomicrons into the space of Disse [14] and in protecting hepatocytes from parasitic infection in herbivores. Morphological surface characteristics and functional distinctions (see below) are now being reinforced further by knowledge of the origin of non-parenchymal cells. For example, Kupffer cells (sometimes referred to as hepatic macrophages) are not self-sustaining but originate from the bone marrow [15].

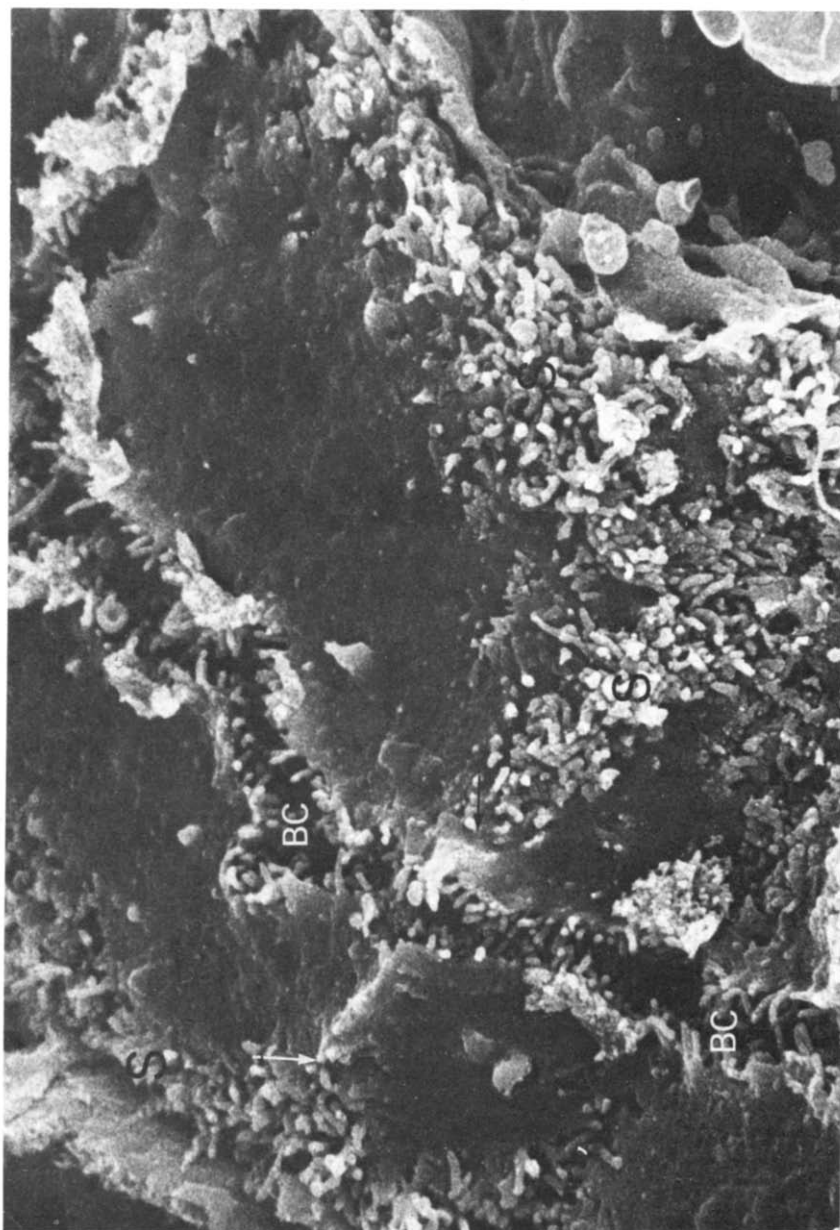


Fig. 1. Scanning electron micrograph showing topographical relationships between domains on the hepatocyte surface among sinusoids (S) and the bile canaliculi (B.C.). A continuous net of bile canaliculi run along the exposed cell surfaces of the liver plates. The perisinusoidal space of Disse sometimes extends into intercellular recesses (arrows). Magnification, approx.  $\times 2500$ . Photograph kindly given by P. Motta.

Bile ductule cells are associated with channels delivering bile away from the liver lobules. Nerve fibres are also found in the space of Disse and between hepatocytes [16].

### *II.B. Morphology of the hepatocyte cell surface regions*

Scanning electron microscopy has provided new details of the configurations and surface topography of hepatocytes. The plate theory [17] which proposed that the adult mammalian liver consisted of interacting polyhedral hepatocytes arranged in plates (laminae) and forming a sponge-like cellular mass perforated by a communicating system of cavities (lacunae) which contain the sinusoids is now generally accepted. The hepatocytes are irregular or elongated polyhedrons about  $25-30\ \mu\text{m}$  by  $20-25\ \mu\text{m}$  in width having six or more faces covered with microvilli, except at the intercellular channel regions. About  $25-50\ \text{microvilli}/\mu\text{m}^2$  of hepatocyte cell surface are found [18]. Scanning electron microscopy (Fig. 1) illustrates the features of the major functional regions [6,19-21]. The bile canaliculi form channels containing numerous microvilli; in many instances the canaliculi carved out of the hepatocyte surface bifurcate into two or more branches. In the context of the isolation of plasma membranes from the functionally distinct surface regions two further important observations emerge from scanning electron microscopy studies. First, branches of the bile canalicular channels may extend across the contiguous surface areas ending blindly near (about  $0.1\ \mu\text{m}$ ) the sinusoidal surface region. Thus, in some instances, the blood sinusoidal and bile canalicular plasma membranes are in close proximity, although the blood and bile spaces are still segregated by tight junctions. Second, the existence of intracellular extensions of the bile canalicular channels postulated by Pflüger in 1869 [22] and Kupffer in 1876 [23] has been confirmed by scanning electron microscopy [6] and this opens up the possibility of a closer anatomical and functional continuity between the bile canalicular plasma membrane and the Golgi apparatus and possibly other intracellular regions.

Integrated stereological and biochemical studies have shown differences in particle densities determined in freeze-fractured areas of lateral, sinusoidal and canalicular plasma membranes [24]. Thus, although particle density was the same in the bilayer half abutting on the external side of the cell, particle density on the cytoplasmic half varied between regions with the canalicular plasma membranes possessing the highest particle density.

Pathological modification to surface membrane topography has frequently been reported, especially at the bile canalicular region under conditions of choleretic infusion and intra- and extra-hepatic cholestasis. Such studies indicate that surface morphology at this region may be regulated by the degree of bile secretion [25-29]. Modifications to microfilaments that insert into the bile canalicular microvilli and the tight junctional complex forming the pericanalicular web have also been reported [27,31].

## **III. Devolution of plasma membrane functions**

### *III.A. Cellular aspects*

Before describing those functions attributed to the sinusoidal, contiguous and biliary regions of the hepatocyte plasma membrane, it is appropriate to categorise liver functions in the context of the cell populations involved. In addition to its role in secretion of plasma proteins, storage, conjugation and detoxication, the liver is involved in the catabolism of

metabolites during plasma clearance. A hepatic glycoprotein recognition system has been identified [32-39] and a role in the uptake and degradation of a wide range of plasma components, including lipoproteins [40,41], chylomicron remnants [42] and enzymes [43] has been demonstrated using liver plasma membranes. The separation of hepatocytes from the sinusoidal cells (mainly endothelial and Kupffer cells) following enzymic dissociation of liver has aided in apportioning the role(s) played by these major cell types. Hepatocytes feature in the recognition and removal of circulating glycoproteins containing a terminal galactose, whereas glycoproteins terminating in *N*-acetylglucosamine or mannose are preferentially taken up by non-parenchymal cells [460]. Carcinoembryonic

**TABLE II**  
**BIOCHEMICAL STUDIES ON LIVER PLASMA MEMBRANE FUNCTIONS CLASSIFIED**  
**ACCORDING TO REGIONAL LOCATION**

Region	Physiological functions examined	Biochemical parameters explored	Refs.
Blood sinusoidal	Recognition, uptake and degradation of metabolites	Asialoglycoproteins	32-34,36,37,39,164
		Glucuronidase	43
		Fatty acids	45
		Bile acids	46,47,477
		Bromosulphonaphthalein	48,468,475
		Plasma lipoproteins	41
		Chylomicron fragments	42
		Guanine nucleotides	49
	Hormone receptors, binding, etc.	Prolactin	50-52, 478
		Proinsulin	53
		Insulin	54-62,30
		Growth-inducing hormones	63,447
		Prostaglandins E <sub>1,2</sub>	64,65
		Adrenalin	66-69,74,75,457
		Glucagon	68-72,459,435
		Angiotensin	73
		Thyroxine	455,484
		Oestrogen	476
	Binding of cations	Calcium	76,77,79,80,482
	Effect of toxins	Cholera toxin	81,471
		Lectins	81-83
		Cytochalasins	84
		Phallotoxins	85-89
		Procaine	79,90
	Antigens	Histocompatibility	91-94
		Immunoglobulin A	95,465
	Transport of metabolites	Amino acids	96
		D-glucose	97
Contiguous	Adhesion	Specificity	98,483
	Cell-cell communication	Gap junctions	99-107
	Tissue permeability	Tight junctions	108,109
	Hormone receptors	Insulin	110,111
Bile canalicular	Bile release	Bile composition and enzymes	112-121

antigen appears to be taken up initially by Kupffer cells and is then transferred to hepatocytes for excretion in bile [165] thus suggesting that some form of metabolic cooperation between liver cells may occur.

The endothelial cells also have a special role in the phagocytosis of vastly supraphysiological concentrations of protein. For example, concentrations of insulin above 5  $\mu\text{M}$  are degraded by endothelial cells, whereas normally, insulin, following induction of its immediate metabolic effects, is degraded by hepatocytes [44].

### *IIIB. Regional devolution of plasma membrane functions on hepatocytes*

The functions carried out at the three major hepatocyte functional regions, and the related biochemical studies are summarised in Table II. The wide range of functions occurring at the blood sinusoidal region and in which the underlying biochemical mechanisms are being studied is striking and underlines the metabolic versatility of the hepatocyte. It is likely that this list of functions will lengthen as other toxins, infectious agents and iron complexes [60] are shown to interact with the sinusoidal plasma membrane of the hepatocyte. It is easy to distinguish between the extensive functions at the blood sinusoidal and the limited number of bile canalicular functions, but more difficult to pinpoint a physiological boundary between the sinusoidal and contiguous plasma membrane regions, since the extent of access of various blood-borne substances to the inter-hepatocyte clefts is variable.

### *IIIC. Hepatocyte functional heterogeneity*

A comment should be made on the apparent heterogeneous distribution between liver lobules of hepatic components and functions including, for example, enzymes [120-124], glycogen metabolism [125] and albumin synthesis [126]. These differences, combined with differences in amounts or distribution of hepatocyte organelles shown by stereological studies [11] and the separation of at least two major populations of hepatocytes [470] have to be borne in mind when using the sub-cellular fractionation approach. Differences in size between centrilobular and periportal cell morphology occur with age [128].

## **IV. Preparation of plasma membrane fractions**

Following the initial description of the morphological and biochemical properties of rodent liver plasma membranes [1], the basic methodology has been modified extensively mainly to improve membrane purity and yield. The major technical modifications used can be summarised as follows: (a) use of various tissue disruption methods [129-131]; (b) changes in ion content and tonicity of media [132-141]; (c) improving fraction purity, especially diminishing mitochondrial contamination, by modified centrifugation conditions [142-145]; (d) the use of rate-zonal centrifugation techniques [146-151]; (e) adaptation of the method to two-phase polymer separations [152-154]; and (f) use of rapid immunological procedures [155-156]. Common to all these techniques of liver plasma membrane preparation is the adoption of a mild tissue homogenisation step to minimise fragmentation of the bile canaliculi, an observation described by Novikoff in 1955 [158]. The plasma membrane fragments sediment at low speed (in a 'nuclear fraction') and are subsequently separated from cell debris, nuclei, mitochondria and vesicles. The plas-

ma membrane fractions prepared by these methods generally account for approx. 1–2 mg membrane protein/g liver wet weight, and have an equilibrium density in sucrose gradients of 1.16–1.18 g/cm<sup>3</sup> (the theoretical yield calculated on the basis of stereological data [5] should be approx. 2–3 mg membrane protein per g liver weight [157]). The fractions consist mainly of membrane sheets, many of which are attached to each other by intercellular junctions, and bile canaliculi profiles together with large numbers of vesicles; intact intracellular organelles are largely absent. About 8–20% of plasma membrane marker enzymes are recovered by these procedures and contamination by nuclear, endoplasmic, lysosomal, mitochondrial and Golgi components assessed using enzymic morphological and chemical markers is low.

Plasma membranes equilibrating at a lower density in sucrose gradients (1.12–1.15 g/cm<sup>3</sup>) that contain very few intercellular junctions but are enriched in the same enzymic and chemical markers have also been prepared from the post-nuclear supernatant normally discarded in the classical methods described above [131,135,159,160]. These fractions consist predominantly of vesicular profiles and can account for up to 30% of the plasma membrane enzymic markers, but in contrast to the plasma membranes isolated at the 1.16–1.18 g/cm<sup>3</sup> sucrose density interface, they contain a higher content of glycosyl transferases, activities usually assigned to the Golgi apparatus and endoplasmic reticulum.

Adding to the heterogeneous nature of the various plasma membrane fractions described above is the demonstration that the standard Neville-Emmelot plasma membrane fraction of sucrose density 1.16–1.18 g/cm<sup>3</sup> can be subfractionated following mechanical disruption to yield 'light' and 'heavy' components equilibrating at sucrose densities 1.12–1.14 and 1.16–1.18 g/cm<sup>3</sup>, respectively [133,134,141,149,160–162, 326]. The 'heavy' subfraction retains the sheets with junctions and large vesicles present in the parent fraction, whereas the 'light' subfraction is vesicular. The 'light' fraction also represents a further increase in specific activity of many plasma membrane marker enzymes; for example, 5'-nucleotidase and alkaline phosphodiesterase.

The anatomical and physiological considerations described earlier in this review provide a basis for resolving the diverse properties shown by the plasma membrane subfractions of differing densities and composition prepared by the various methods. Gentle homogenisation of liver generating low shear forces disrupts hepatocytes releasing large strips of the plasma membrane attached by tight junctions to relatively intact bile canaliculi. The plasma membrane fragments originating predominantly from bile canaliculi, and lateral surface regions, together with small variable amounts of sinusoidal membrane are sedimented at low speed together with nuclei, and heavier mitochondria and are thus separated from the bulk of other intracellular organelles and membrane components. Filtration of tissue homogenates through muslin cloth removes cell debris and many of the non-parenchymal cells undisturbed by the gentle homogenisation, especially endothelial cells [163,164]. The use during tissue dispersion of alkaline low ionic strength media, for example, bicarbonate or borate buffers [136] in combination with low Ca<sup>2+</sup> (less than 1 mM) [132] appears to aid in minimising fragmentation of the plasma membrane, thus enhancing the rapid sedimentation at low speeds of partial membrane 'ghosts' containing large fragments of the pericellular membrane. Procedures that aim to study blood sinusoidal functions using plasma membranes purified from low speed pellets minimise the number of mechanical manipulations involved, e.g. in studies of glucagon activation of adenylate cyclase activity or in the purification of the organic anion transport-protein partially purified liver plasma membrane have been extensively used [70,475]. The microvillar blood-sinusoidal region of the hepatocyte plasma membrane rapidly forms vesicles



predominantly of the right-side-out configuration [166–168] even under mild conditions of cell disruption and these have to be pelleted at higher centrifugal forces.

## V. Functional characterisation of plasma membrane fractions

Topographical assignment of the plasma membrane subfractions to the correct hepatocyte surface regions is an obvious advantage for biochemical studies of functions occurring at specific domains. Four lines of evidence help assign to the appropriate cell surface region the plasma membrane subfractions prepared by the above procedures. The utility of the morphological markers, intercellular junctions and bile canalicular profiles has already been alluded to in assigning components in the classical 'nuclear fraction' derived plasma membrane to a mainly bile front-contiguous surface origin [158]. Second, histochemical evidence [169–171] pinpointing high phosphatase activities at bile canaliculi supports this regional assignment, and also suggest that 'light' vesicular plasma membranes recovered after vigorous homogenisation of the standard fraction and characterised by extremely high phosphatase activities are derived mainly from the microvilli projecting into the bile canalicular spaces (Fig. 2). Third, analysis of glucagon-activated adenylate cyclase activity in plasma membranes prepared from 'nuclear' and 'microsomal' fractions also aids in identifying the microsomally-derived plasma membrane vesicles and the heavy plasma membrane subfraction derived from the 'low speed' pellet as originating mainly from the sinusoidal and contiguous plasma membrane regions respectively. The light subfraction released by high shear forces from plasma membranes prepared from low-speed pellets of hypotonic homogenates contained an adenylate cyclase activity that was not activated by a range of glucagon concentrations, and this can be interpreted as further evidence in support of an origin from a non-hormonally activated region of liver cells, most likely the bile canalicular region [160]. Fourth, direct evidence for plasma membrane fractions containing membranes having access to blood was obtained by use of a domain-specific labelling technique [170,173] (Fig. 2). Perfusion into the portal vein of radio-labelled ligands, e.g. glucagon, wheat germ agglutinin and asialofetuin known to interact with specific receptor sites on the hepatocyte's vascular pole, followed by preparation and analysis of plasma membrane fractions from 'nuclear' and 'microsomal' pellets demonstrated that 'light' plasma membrane vesicles present in microsomal and nuclear pellets of sucrose-dispersed homogenates are the most suitable for study of blood sinusoidal functions [131,160,164,172,173]. Fractions containing intact bile canalicular attached to sheets, or the shear-released vesicles are most suitable for study of biliary functions [112,114,116,117,142,149,174,175].

Knowledge of the major sites of origin on the cell surface of the various plasma membrane fractions described helps to resolve some apparent contradictions in the properties of plasma membranes isolated by different methods. This is illustrated by variations in insulin binding properties of 'light' and 'heavy' plasma membrane fractions. Thus, in studies using liver dispersed in a hypotonic medium, or isotonic sucrose, higher binding was associated with 'heavy' membranes (density in sucrose 1.16–1.18 g/cm<sup>3</sup>) than with 'light' membranes (1.13–1.14 g/cm<sup>3</sup>) [57,176], whereas in another study [133] a light vesicular plasma membrane fraction prepared using a different methodology bound higher amounts of insulin than heavy plasma membranes. The extent of fragmentation of the plasma membrane and the relative amounts of sinusoidal and bile canalicular membranes recovered in the 'light' fractions appear to be factors governing the subcellular distribution of cell surface receptors, since the sinusoidal membranes are a richer source of

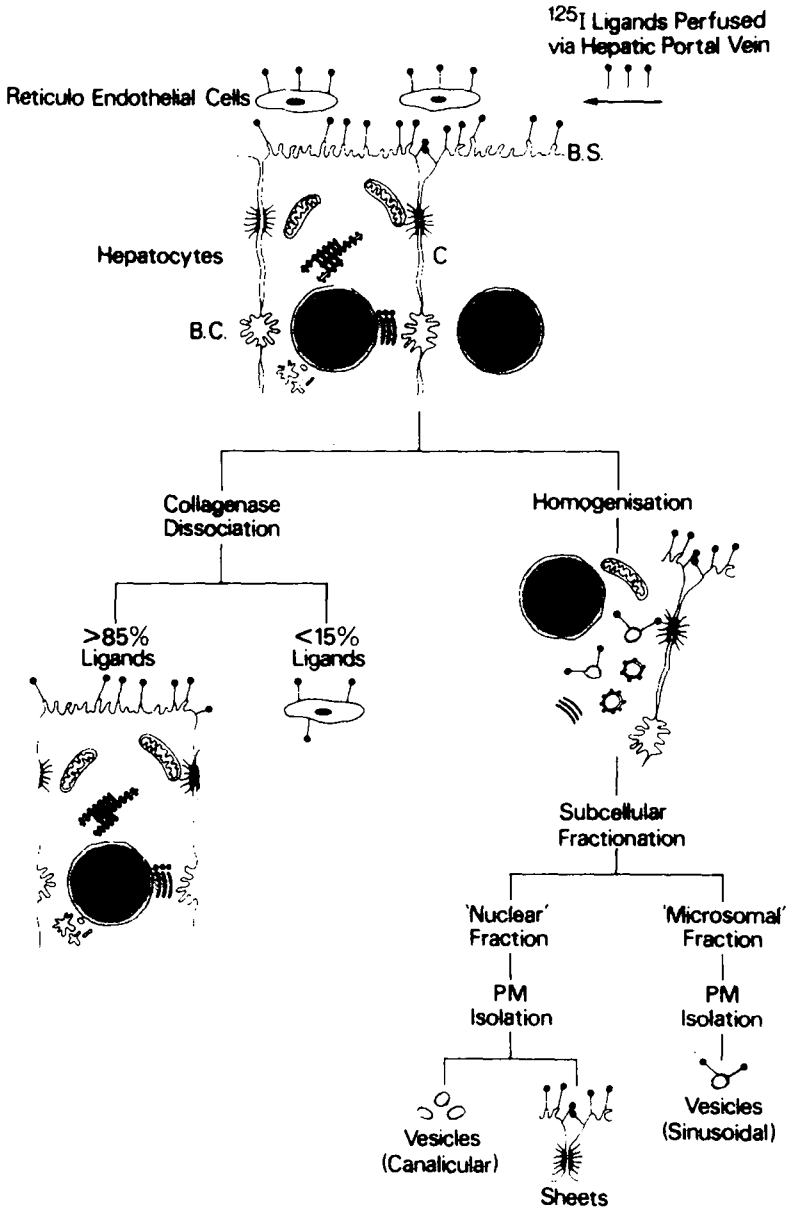


Fig. 2. Diagram showing a domain-specific approach for labelling and identifying in subcellular fractions plasma membrane vesicles derived from the hepatocyte's blood sinusoidal surface [172,173]. Iodinated ligands (e.g. wheat germ agglutinin, glucagon, desialofetuin) were perfused for 2 min into liver and became attached to receptors shown, after tissue dissociation using collagenase, to be located mainly on the hepatocyte's cell surface. After tissue homogenisation and preparation of plasma membrane subfractions from low-speed (nuclear) pellets [149] and microsomal fractions [131,160] radioactivity was recovered mainly in 'heavy' plasma membrane subfraction (density  $1.16\text{--}1.18\text{ g/cm}^3$ ) containing intercellular junctions and vesicles and in the subfraction (density  $1.11\text{--}1.14\text{ g/cm}^3$ ) prepared by flotation from the microsomal fraction and containing vesicles originating from the blood sinusoidal surface. Vesicles (density  $1.12\text{--}1.14\text{ g/cm}^3$ ) released by shearing forces from the plasma membrane prepared by the standard Neville-Emmelot procedure contained lowest radioactivity, thus suggesting a bile canaliculus (B.C.) origin.

hormone binding membranes relative to the contiguous membranes present in the 'heavy' subfraction. In addition, the binding of polypeptide hormones to intracellular membranes, e.g. Golgi [177,179] and nuclei [180] can also influence the hormone-binding capacity when impure fractions are used. Further complicating the resolution on the basis of blood sinusoidal or biliary origin of the various membrane subfractions is the unknown molecular basis of the gradation of the sinusoidal into contiguous plasma membrane region and morphological evidence that the proximity of canalicular and sinusoidal regions on hepatocytes can vary considerably [6]. These considerations, combined with others discussed above demonstrate some difficulties in achieving by current subcellular fractionation procedures the preparation of membrane subfractions fully resolved with respect to the sinusoidal, contiguous and canalicular plasma membrane regions.

## VI. Hepatocyte intercellular junctional complexes

Intercellular junctional complexes that attach adjacent hepatocytes may be considered as discrete membrane functional differentiations located within the contiguous cell surface domain. Three major classes of junctions are described on hepatocytes. The tight junctions (zonulae occludentes) control transepithelial flux between cells [181,182]. They form a continuous belt-like structure around the bile canalicular space [6]. Freeze-cleave studies show tight junctions to be composed of strands of intramembranous particles present in the plasma membrane of each cell [181,182]. Their interdigitation creates the permeability barrier that controls passage of molecules between the blood and bile spaces [183]. Presently, knowledge of the chemical composition of the tight junction region of the plasma membrane is limited [469] owing to the lack of methodology for their preparation. Desmosomes (maculae/facia adherens) are sites of adhesion between adjacent hepatocytes, and although advances in their isolation and characterisation from other tissues are reported [186,187], hepatic desmosomes have not been isolated and studied biochemically. Far greater knowledge of gap junctions has emerged since it was shown that their isolation was possible by taking advantage of their relative insolubility in detergents, especially *N*-dodecyl sarcosinate [99-107]. Gap junctions have been shown ultrastructurally to be composed of aggregates of intramembranous particles in the apposed plasma membranes, paired particle-to-particle across the intercellular space, so constructing a direct channel for exchange of ions and small molecules between cells [188]. The absence of any biochemical or immunological markers has required their purification from plasma membrane fractions to be dependent upon morphological monitoring, especially of the regular polygonal structure seen in negatively stained preparations (Fig. 3B). The insolubility in mild detergents of collagen fibres present in isolated liver plasma membrane fractions and the use of highly impure collagenases to dissolve these fibres, have delayed their molecular characterisation and thus far, even when proteolytic treatment is avoided during preparation, consensus on the nature of the polypeptide(s) believed to construct the intercellular channels and believed to correspond to the intramembranous particles observed by freeze-fracturing techniques has yet to be achieved [100-109,189,190]. Models of gap junction structure based on electron microscopy and X-ray diffraction have been proposed [191,419] (Fig. 3A).

Ultrastructural [192,260] and electrophysiological studies [189,191] indicate that the number and area of gap junctions and the extent of coupling between hepatocytes are reduced after hepatectomy, but the full complement of gap junctions returns within 2 days of the operation.

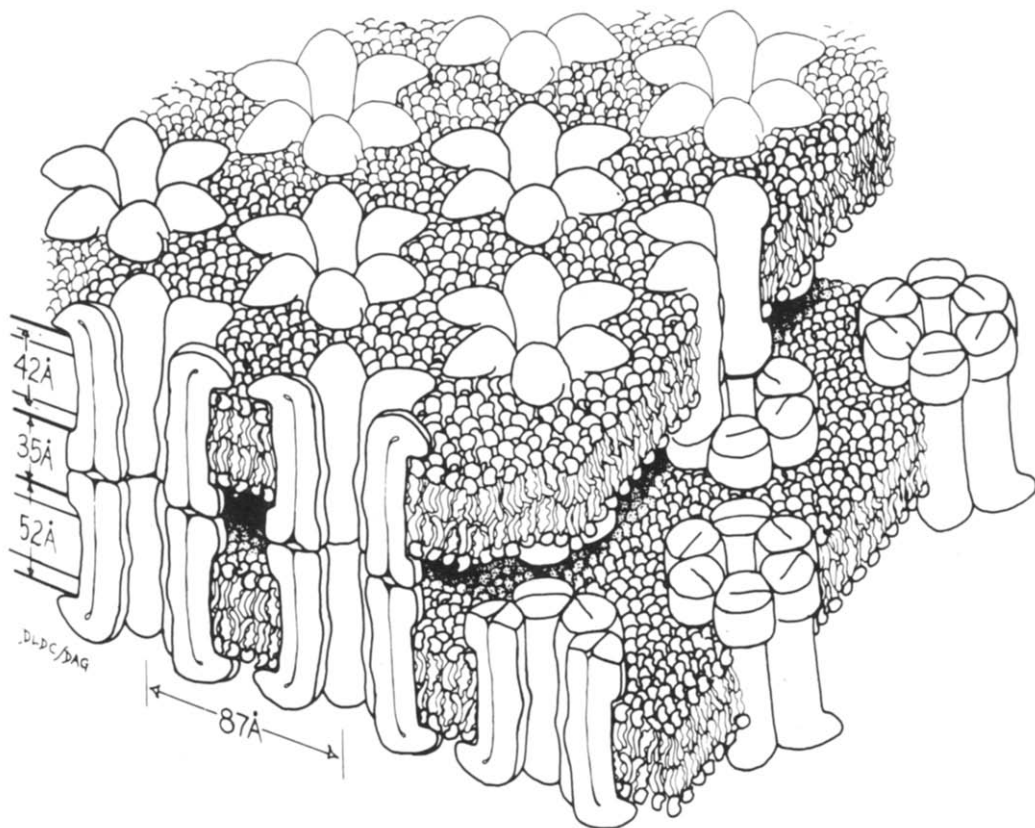


Fig. 3. (A) Diagrammatic representation of the organisation of the hepatic gap junction as inferred from X-ray diffraction and electron microscopical studies. The model proposes hexagonal symmetry and the hydrophilic channel is located between the subunits [191]. An alternative model that proposes that the channels run between twisting subunits has been proposed on the basis of image processing of negatively stained plasma membranes [419].

## VII. Biochemical properties of the hepatocyte plasma membrane

### VIIA. Enzyme distribution and properties

Analytical subcellular fractionation studies have provided much information on the equilibrium densities of liver plasma membrane enzymes [166,194–195]. Preparative studies on the distribution of an increasing number of marker enzymes among plasma membrane subfractions have been interpreted to reflect their uneven distribution on the hepatocyte surface. For example, many of the recognised marker enzymes are present at highest specific activities relative to homogenates in bile-canalicular fractions, although lower levels of enrichment are also measured in blood-sinusoidal fractions [149,160–162]. By far the lowest activities of such marker enzymes are measured in contiguous region fractions. Increasingly, exceptions to this distribution pattern are being noted; thus, CMP-neuraminic acid hydrolase [197], glutamyl transpeptidase [198] and glucagon-activated adenylate cyclase [160] may have primarily a blood-sinusoidal plasma mem-

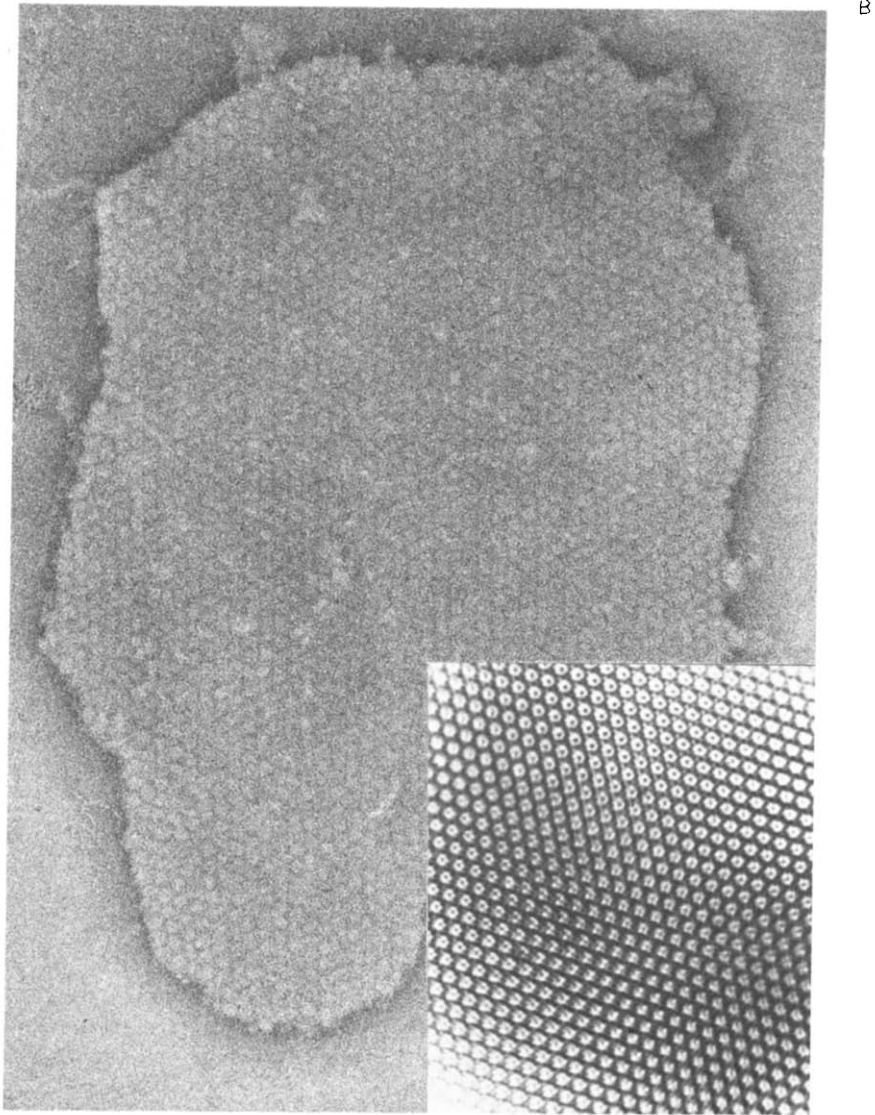


Fig. 3. (B) Isolated hepatic gap junction negatively stained with sodium silicotungstate (magnification,  $\times 250\,000$ ). Inset: after filtration showing the hexagonal packing of the above model with centre-to-centre spacing between units of about 8 nm.

brane location.  $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$  is mainly found in the contiguous plasma membrane fraction [479].

A number of plasma membrane enzymes have been purified, and characterised by molecular and kinetic studies (Table III). Furthermore, in many instances the location in the membrane of the enzyme's active site relative to the exterior and interior of the cell has been determined. Most of the classical plasma membrane marker enzymes are ectoenzymes [199–202] which is a fortuitous location in view of their extensive use in subcellular fractionation studies, since most plasma membrane vesicles produced during tis-

TABLE III

## PROPERTIES OF SOME LIVER PLASMA MEMBRANE ENZYMES

glc, glycoprotein; ecto, ectoenzyme; endo, endoenzyme (enzyme is positioned on the cytoplasmic side of the membrane).

Enzyme	Molecular parameters	Comments
1. 5'-Nucleotidase (EC 3.1.3.5)	$M_r$ 70 000–140 000 [208–211]; glc, ecto [206]	Lipid association [209,214]; actin interaction [215,216]
2. Nucleotide pyrophosphatase (EC 3.6.1.9)	$M_r$ 130 000; glc [217–222] ecto [214,223,224]	Identical to alkaline phosphodiesterase I [217,221,222] $Ca^{2+}$ -dependent [453]
3. Alkaline phosphatase (EC 3.1.3.1)	$M_r$ 127 000–154 000 2 subunits, glc [225–229]	Contains sialic acid [228] and $Zn^{2+}$ [225] Lipid association [214]
4. Leucyl-naphthylamidase (EC 3.4.11.1) and related aminopeptidases	$M_r$ 118 000 glc; [230]	Family of aminopeptidases exists [231]
5. Cytidine monophosphate neuraminic acid hydrolase	ecto [197,232]	
6. Adenylate cyclase (EC 4.6.1.1)	$M_r$ 140 000–120 000 [70,71,74,233–236] endo 183 000–207 000 [344]	Hormonally- and guanyl nucleotide-activated [49,44,234]
7. Guanylate cyclase (EC 4.6.1.2)	$M_r$ 70 000 [237] <sup>c</sup>	Peripheral membrane enzyme [238,239]
8. Nicotinamide adenine dinucleotide glycohydrolase (EC 3.2.2.5)	glc [240]	Present in endoplasmic reticulum [162,240]
9. Monoacylglycerol acyl transferase		Previously called phospholipase A [241–245]. Heparin-sensitive

10.	Adenosine cyclic 3',5'-monophosphate phosphodiesterase		Peripheral membrane enzyme [156,246]
11.	Glutamyl transpeptidase (EC 2.3.2.2)	glc [247,248] $M_r$ 103 000 [344]	Kidney enzyme consists of 2 non identical subunits [443] May control response to hormones [253-256,458]
12.	NADH-oxidising enzymes		[257]
13.	Uridine phosphorylase (EC 2.4.2.3)		Higher mol. wt. subunit phosphorylated [259,261]
14.	( $\text{Na}^+ + \text{K}^+$ )-ATPase (EC 3.6.1.3)	approx. 95 000 and approx. 45 000 subunits <sup>a</sup> ; glc	[262]
15.	UDP-Glucuronate glucuronyl transferase (EC 2.4.1.17)	$M_r$ 160 000. Oligomer of 28 000 subunits [243]	[263]
16.	3'-Nucleotidase (EC 3.1.3.6)		Endonuclease acting in concert with 5'- and 3'-nucleotidases and phosphodiesterases [264]
17.	Endonuclease		[265,266]
18.	Sialidase (EC 3.2.1.18)		Function at cell surface in dispute [250-252,274]. Liver enzyme may be precursor of soluble rat serum enzyme [452]
19.	Glycosyltransferases (EC 2.4.1.22)	$M_r$ 69 000, glc <sup>b</sup>	$\text{Mg}^{2+}$ or $\text{Mn}^{2+}$ activated [454].
20.	Sphingomyelinase (neutral)		

<sup>a</sup> Subunits of renal cortical enzyme [258].

<sup>b</sup> Subunit of mammary gland Golgi membrane galactosyltransferase [249].

<sup>c</sup> Molecular weight of the soluble enzyme.

sue homogenisation maintain the same topographical orientation as in the intact cell [166,168,203]. These ectoenzymes are widely distributed on mammalian cells and have similar kinetic and antigenic properties [204–207]. Many, and probably all plasma membrane ectoenzymes are glycoproteins, but little is known currently of the nature and biological role of the sugar components in hepatocyte plasma membranes [480].

Plasma membranes are particularly rich in enzymes involved in nucleotide metabolism. The hepatic adenylate cyclase has been purified partially [267,344] and it may resemble the enzyme purified from kidney and thyroid membranes in view of the facility with which mammalian adenylate cyclases can be shown by cell fusion studies to be activatable by various hormones and receptors [268,448]. Most (80–90%) of guanylate cyclase is recovered in liver tissue supernatants [239] but a small proportion attached to membranes differs in kinetics and molecular size [78]. The liver plasma membrane enzyme also differs from the soluble form in being activatable by proteolysis [343]. The ( $\text{Na}^+ + \text{K}^+$ )-ATPase of liver plasma membranes has not been purified, possibly owing to its poor stability. Although the enzyme has a baso-lateral location in epithelia [446] the enzyme has been reported to be present at both the sinusoidal and canalicular [270–273] plasma membrane regions but recent cytochemical and biochemical studies indicate a location mainly at the sinusoidal and contiguous regions [269,479]. In view of its postulated participation in mediating the  $\text{Na}^+$  transport-dependent component of bile formation [270] the enzyme activity has been studied in normal and cholestatic liver.

Although possessing specific  $\text{Ca}^{2+}$ -binding properties [76–80] (a  $\text{Ca}^{2+}$ -binding glycoprotein has been isolated from hepatoma cells [482]), controversy surrounds the question of whether there is a  $\text{Ca}^{2+}$ -activated ATPase in the hepatic plasma membrane [77,364]. Complicating the issue is the demonstration in liver plasma membrane fractions of a  $\text{Ca}^{2+}$ -activated ATP pyrophosphohydrolase [453] of broad specificity that hydrolyses a variety of dinucleotides and sugar nucleotides at the pyrophosphate bond [217–222]. Nucleotide pyrophosphatase, after purification to homogeneity, also displayed alkaline phosphodiesterase I activity [217,221], and is a glycoprotein shown by lactoperoxidase-catalysed iodination of isolated hepatocytes to be surface located, and thus optimally positioned for hydrolysis of external sugar nucleotides [222–224,274].

Despite intensive investigation the function(s) in the liver plasma membrane of 5'-nucleotidase is still unclear. The membrane-bound enzyme differs kinetically from a soluble activity [275,277]. Evidence for interaction with phosphatidylinositol [214] (primarily an inner leaflet phospholipid) and with actin in the form of its nuclease complex [215, 216] suggest that it is deeply anchored into the plasma membrane. It is generally thought that 5'-nucleotidase is involved in the transmembrane transport of adenosine and other nucleosides produced by hydrolysis of tri- and monophosphates as demonstrated in heart tissue [276].

Plasma membrane enzymes hydrolysing peptides [230,231] and phospholipids [241–245] have been demonstrated, but their physiological role is unclear. Glycosyltransferases are present in rough and smooth endoplasmic reticulum and the Golgi apparatus [480], but they are also present in lower amounts in liver plasma membrane fractions, especially those enriched in sinusoidal membranes [250–252]. Postulated roles in cell recognition [473,474] and in the uptake of circulating glycoproteins [472] have not been adequately verified. The extensive interaction of intracellular membranes with the sinusoidal plasma membrane that underlies secretion and plasma membrane biogenesis (section IX) may result in spill-over of some enzymes into the sinusoidal region of the hepatocyte's surface membrane. Recently, a plasma membrane location has been shown for NADH-oxidase



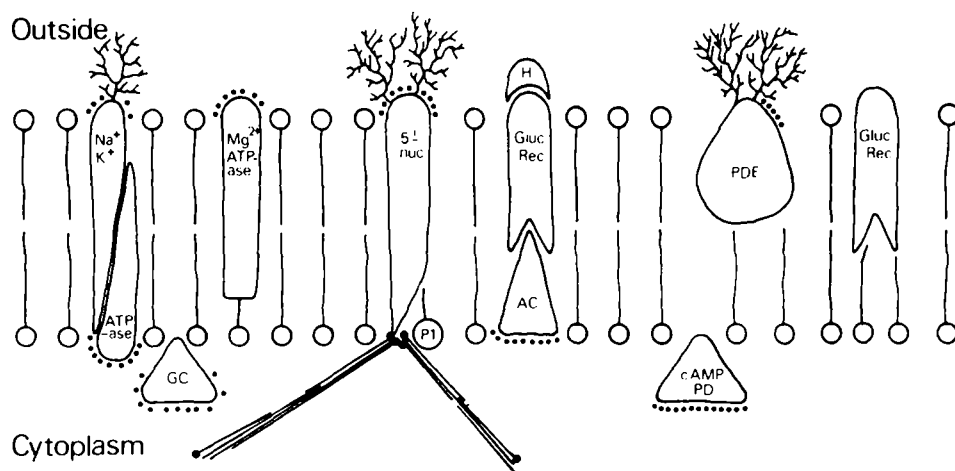


Fig. 4. Schematic diagram showing the disposition of some proteins of the liver plasma (redrawn from Houslay and Palmer [285]). The diagram encompasses a large number of observations on the properties of the enzymes as described in section VIIA and Table III. G.C., guanylate cyclase; 5'-nuc, 5'-nucleotidase; H, hormone interacting with glucagon receptor (gluc. rec.) which, in turn, interacts with adenylate cyclase (A.C.) when the receptor site is occupied. P.D.E., alkaline phosphodiesterase; cAMP PD, cyclic AMP phosphodiesterase; —•, microfilaments interacting in an unknown way with 5'-nucleotidase. P.I., phosphatidylinositol. —•, carbohydrate side chains.

and a role in hormone mediated receptor activation of adenylate cyclase was suggested [253-256].

Analysis of liver plasma membrane proteins using immunological methods has provided important insights into the nature of enzymic and lectin and hormone binding activities. Information on molecular inter-relationships, and especially topographical aspects of membrane enzymes has been obtained [231,278-284]. Current information on the properties of liver plasma membrane proteins, especially their composition and orientation in the lipid bilayer established by lipid melting experiments [285], permits a schematic formulation (Fig. 4).

#### VII B. Chemical composition

Liver plasma membranes, in common with all mammalian plasma membranes contain the highest amounts of glycosylated protein and lipid relative to other membrane systems. The large number of functions occurring at the various plasma membrane regions of the hepatocyte (Table II) is reflected in the complex pattern of polypeptides resolved electrophoretically. The polypeptide patterns differ characteristically from those of other hepatic membrane systems [166,286-290]. Compared to simpler plasma membranes, e.g. erythrocytes, progress is slow in relating polypeptides to enzymic, binding, and transport activities etc. Both actin [291], and myosin [292,293] have been shown to be associated with the liver plasma membrane. Many glycosylated polypeptides [100,287-289], iden-

tified in gels using the Schiff-periodate reagent, lectin-binding [294], and incorporation of radioactive sugars [349] are present, especially in the 70 000–150 000 molecular weight range, and many of these correspond to the enzymes purified from liver membranes (Table III). *b*-Type cytochromes (cytochromes  $b_5$ ,  $P$ -450,  $P$ -420), mainly located in endoplasmic reticulum and outer mitochondrial membranes have also been shown to be associated in low amounts with liver plasma membranes; their functional roles, if any, at the plasma membrane remain to be established [295,296]. Hepatoma cell plasma membranes are being used increasingly to isolate glycoproteins [297] and membrane-associated mucopolysaccharides [298,299,421]. Differences in the polypeptide and glycoprotein patterns of plasma membranes originating mainly from each of the functional domains, are mainly in the staining intensity of polypeptides of similar electrophoretic mobility [160,294]. Lectin-binding studies indicate that a diverse range of glycoproteins are present in sinusoidal plasma membrane fractions (Evans, W.H. and Carey, F., unpublished results). Since many major polypeptides are present in approximately similar amounts in the plasma membrane subfractions, it may be that functional distinctions are more related to differences in glycoprotein (and possibly glycolipid) composition. Gap junction-containing regions have generally a very much simpler protein composition [101–107].

The neutral and phospholipid composition of liver plasma membrane have been analysed and compared with that of intracellular membranes and organelles [136,300–309]. The plasma membrane subfractions are characterised by a high cholesterol/phospholipid ratio and sphingomyelin content [312]. The fatty acid profiles have also been examined

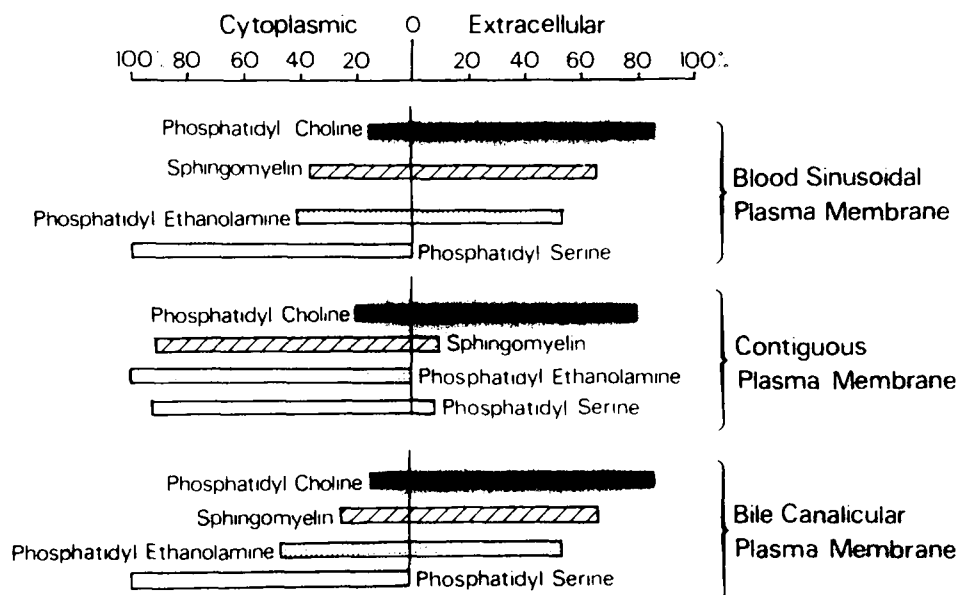


Fig. 5. Tentative transverse distribution of the major phospholipids in the bilayer at the three major hepatocyte plasma membrane regions. The distributions were determined on the basis of phospholipid accessibility to hydrolysis by phospholipase c of *Clostridium welchii*. Phosphatidylinositol was not hydrolysed but is thought to be mainly located in the cytoplasmic half of the bilayer [167,315]. The overall composition range of rat liver plasma membrane phospholipids expressed as mol% is: phosphatidylserine, 3.5–9.0; phosphatidylinositol, 4.2–8.8; sphingomyelin 10.7–23.2; phosphatidylcholine, 30.0–46.2; phosphatidylethanolamine, 18.5–24.7 [300–309].

[310,311]. Analysis of the neutral and phospholipid compositions of plasma membranes derived from the three functional domains indicated that they were less variable than the differences recorded in polypeptide and enzymic composition [312]. The major differences in lipid composition and lipid/protein ratio were found between 'light' and 'heavy' plasma membranes [160,312]. Gangliosides are highly concentrated at the plasma membrane [313]. Analysis of the transverse organisation of the phospholipids indicated a similar asymmetric distribution to that found in erythrocyte membranes, with phosphatidylcholine being the major lipid in the outer bilayer [167]. However, differences were found between the canalicular and sinusoidal plasma membrane fractions and the contiguous fraction (Fig. 5). The arrangement of phospholipids in the bilayer is an important factor when investigating mechanisms for the biogenesis of the plasma membrane, and especially in explaining the resilience of the canalicular plasma membrane region to the action of bile during its transfer to and accumulation in the biliary networks [314,315].

It is generally accepted that glycoproteins, which account for about 4% of the liver plasma membrane dry weight [316], are asymmetrically orientated in the plane of the membrane [317]. Many studies confirm that the hepatocyte plasma membrane (at its three major functional domains) is no exception to this generality [318-322]. No lectin-binding sites are present at the gap junctional region [323], an observation consistent with biochemical analysis of isolated junctional proteins [104,106,324].

## VIII. Plasma membrane pathological modifications

To identify membrane and molecular lesions occurring in hepatic disorders, two major approaches utilising plasma membranes have been used extensively. In the first, the properties of plasma membranes from normal and experimentally manipulated animals have been compared in a number of respects (Table IV), and in the second, agents have been added directly to isolated membranes. Although useful information has emerged from studies that compare 'normal' membranes with those from diseased or poisoned liver, this general approach in a number of instances needs to be evaluated in the context of the following reservations.

The plasma membrane is the biosynthetic product of a series of events involving coordinated activity of a number of intracellular membrane systems and the cytoskeleton. Consequently any lesions affecting their integrity will be reflected sooner if not later in plasma membrane properties, especially in the context of the speed of metabolic turnover and the level of secretory/absorption phenomena (see section IX). This is illustrated by a detailed examination of the effects of phallotoxins and related substances on liver physiology, where wide-ranging effects on membranes and the cytoskeletal components are now reported [372].

A second qualification is that the regional complexity of the hepatocyte plasma membrane opens a Pandora's box with regard to the permutation of deviant fractionation possibilities open to plasma membrane fragments of differing size and density especially when they are prepared from the wide range of hepatoma that have been compared only with respect to a low yield of a single plasma membrane fraction properties. The different methods of cell disruption and fractionation used when comparing normal and abnormal tissues or cells can lead to plasma membrane fragments from overlapping or different surface regions being isolated. Analytical fractionation of hepatoma has shown that in contrast to the bimodal density distribution of many plasma membrane markers of liver tissue, a single modality is obtained, and that 5'-nucleotidase activity may be low or

TABLE IV  
REVIEW OF BIOCHEMICAL APPROACHES TO VARIOUS HEPATO-BILIARY DISORDERS USING LIVER PLASMA MEMBRANE FRACTIONS

	Experimental design	Plasma membrane properties	References
A.	Hepato-biliary pathology. Induced by intra- or extra-hepatic cholestasis or by choleretic infusion	Plasma membrane from treated and control livers compared	Morphology Marker enzymes Membrane lipids
			25,26,325 270-272,326,352 327
B.	Regeneration after hepatectomy (compensating liver growth)	Blood analysis	Plasma membrane vesicles recovered
		Plasma membrane from regenerating and control livers compared	Marker enzymes Response to hormones Ultrastructure Degradation rates of proteins and glycoproteins
			328 329-333,353,444 76,335 336,108 337-339
C.	Malignancy	Plasma membrane from various hepatoma and liver compared	Membrane composition Membrane fluidity Marker enzymes Degradation of proteins Synthesis of glycoproteins
			340-342,154,444,445 449 331,345-347 339,348 349,351

D.	Hormonal imbalance Obesity	Plasma membrane from diabetic, genetically and nutritionally obese animals compared with controls	Polypeptide hormone binding and marker enzymes including nucleotide cyclases:  insulin glucagon others	350,354 360 347,361,362 363
E.	Response to drugs	Plasma membrane from con- trols and drug-treated animals compared: CCl <sub>4</sub>  Sulphonyl urea Phenobarbital  Chlorpromazine Phalloidin N-2-Fluorenylacetamide Colchicin Galactosamine  Plasma membrane from control and fatty-acid deficient animals compared	Marker enzymes Phospholipid metabolism Insulin binding Phospholipid metabolism Marker enzymes Ultrastructure        Marker enzymes Marker enzymes	364 367 367,456 368 369  370 371,372 373 374 481  375,376
F.	Nutritional disorders			

absent [345]. These differences are further accentuated for example, when membranes prepared from different hepatoma are compared [346,377].

A third reservation that applies to many studies using liver plasma membrane also emerges from the biochemically heterogeneous nature of the fractions obtained by using the various procedures. Whereas the standard (Neville-Emmelot) preparation and its many variants yields membranes suitable for investigating modifications induced by choleresis, cholestasis etc., the same fraction, although deficient in sinusoidal plasma membrane fragments is also used for investigating blood-sinusoidal functions, especially membrane-receptor properties and modifications. The fact that hormone-binding studies have yielded functionally meaningful results may be related largely to the expression of these properties at the contiguous region and the retention, especially in methods that minimise the number of steps, of fragments from the microvilli present at the major blood sinusoidal plasma membrane region. Clearly, further studies utilising more clearly defined blood sinusoidal plasma membranes are needed.

A fourth qualification concerns the use of liver tissue plasma membrane fractions to investigate functions that are increasingly attributed to the hepatic non-parenchymal cells (see section IIIA). The resolution of liver tissue into its component cell types, their enzymic analysis [378–380] and analytical fractionation [381], and the availability of methods for preparing plasma membrane subfractions and other membrane fractions from isolated hepatocytes [382] will be expected to feature more in future studies.

## **IX. Plasma membrane biogenesis and turnover – their role in the generation of functional polarity**

The liver plasma membrane is a largely biogenetically inert organelle. Thus, ribosomes are absent and the capability for synthesis of lipids is limited, being confined mainly to smooth endoplasmic reticulum vesicles [383–389]. However, it is the locus of intensive metabolic activity conditioned by intra- and extracellular events. This is especially the case at its blood sinusoidal region where secretory, and absorptive activities are mainly localised. The extent of metabolic involvement of the contiguous (lateral) plasma membrane region is unclear. Undoubtedly some endocytotic processes occur as shown by autoradiographical studies of iodinated insulin uptake [110,111]. Intensive metabolic activity must also underly the discharge of bile across the canalicular membranes into the biliary spaces.

### *IXA. Biogenesis*

Kinetic studies on the incorporation of labelled precursors into liver plasma membrane and intracellular membrane proteins and carbohydrates are often interpreted as indicating a biogenetic route involving a sequence from the rough endoplasmic reticulum to the cell surface via the smooth endoplasmic reticulum and Golgi apparatus [390–399]. The kinetics of membrane protein movement to the cell surface are similar to that of albumin [396] and  $\alpha_2\mu$ -globulin secretion [397]. Approx. 2–4 h elapse before maximal incorporation of precursors into plasma membrane protein is achieved [398,399] but carbohydrates are incorporated into liver plasma membranes at a faster rate, with a maximum at about 1–2 h [400,353]. It is not the intention to discuss here detailed mechanisms for biosynthesis of membrane proteins and lipids but to comment on some possible pathways for transferring them to their final position at the various plasma membrane regions. Sim-

ply, how is the process of plasma membrane synthesis so arranged, such that segregated functional zones are generated and maintained? Although many of the details await direct experimental analysis, two possible mechanisms and three routes for transfer of plasma membrane components to their final position at the hepatocyte surface can be postulated. Transfer of membrane components, for example, phospholipids from their site of synthesis at the cytoplasmic face of the endoplasmic reticulum [402,403] to the plasma membrane may involve exchange proteins similar to those featuring in the exchange of endoplasmic reticulum and mitochondrial phospholipids [404]. This could explain, for example, the 10-fold more rapid transfer of cholesterol and its precursors lanosterol and lathosterol from the endoplasmic reticulum to the hepatocyte's sinusoidal plasma membrane than would be expected if the Golgi apparatus were implicated [450]. Mechanisms for the transfer of membrane proteins via a cytoplasmic rather than a membrane-mediated mechanism have not been described, and routes postulating vesicles [405] or membrane flow [390,395] are currently in vogue.

Three major routes to the hepatocyte surface that generate an unequal distribution of many plasma membrane components can be described (Fig. 6). The first route entails the insertion of components randomly into the plasma membrane, followed by lateral translocation of components in the plane of the membrane that may be aided or controlled, for example, by cytoskeletal elements [406-408]. A second route involves the insertion of components directly into the plasma membrane regions where they carry out their functions. Lateral movement, especially between the major domains, would be restricted by various functional interactions. For example, insulin receptors at the blood sinusoidal plasma membrane region have been shown to aggregate and be interiorised rapidly following interaction with circulating hormone [409], and such receptor-mediated endocytotic process would minimise the extent of lateral redistribution. The inser-

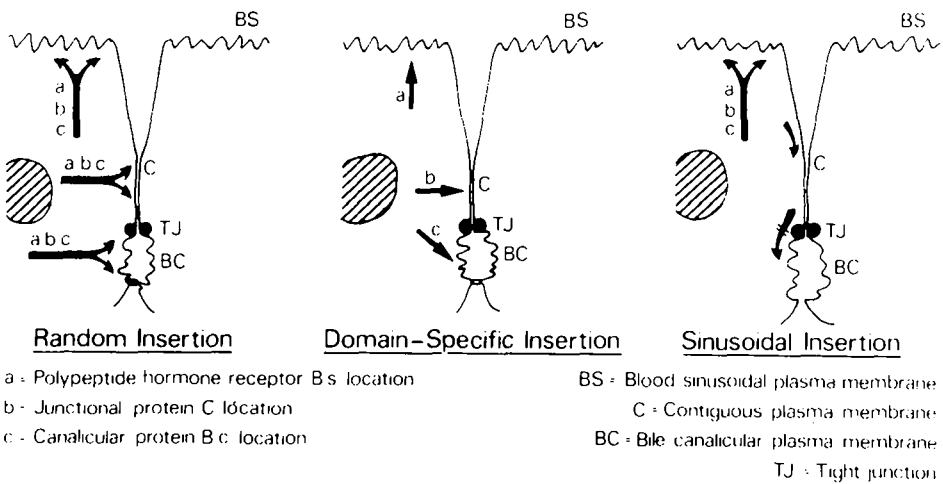


Fig. 6. Diagram showing three routes of insertion of components resulting in the synthesis of a multi-domain plasma membrane. Arrows show routes from intracellular site(s) of synthesis to the hepatocyte's three major plasma membrane domains, and in the route of sinusoidal insertion, the lateral translocation in the plane of the membrane, possibly overcoming the barrier of the tight junction. For other details, see text.

tion of gap junction polypeptides into the contiguous plasma membrane region can lead to junction formation by close packing and intercalation of the subunits with corresponding subunits on neighbouring hepatocytes. Once assembled, the gap junction polypeptides would become locked at regions of closely apposed plasma membrane. Junctions, once formed are difficult to dissociate, and complete junctions are retained on the surfaces of isolated hepatocytes [410]; evidence supporting their breakdown as complete units after internalisation into lysosomal regions has also been presented [411]. Similarly, membrane proteins implicated in discharge of bile would be directly inserted into the bile canalicular plasma membrane region. These pathways of insertion would, of course directly generate the unequal distribution of some components in the plasma membrane. A third route restricts the insertion of plasma membrane components to one plasma membrane region and can be regarded in some aspects as a corollary of secretion. Since Golgi vesicles predominantly interact with the blood sinusoidal plasma membrane region, they provide a vehicle for the insertion of glycoconjugates into the plasma membrane in a selective or gross way [177]. After insertion into the sinusoidal plasma membrane region, some categories of components would become localised around the site of insertion, whereas others could move laterally and into the contiguous plasma membrane regions. Autoradiographical evidence for the insertion of membrane glycoconjugates into the plasma membrane by this pathway has been obtained in liver prelabelled with various radioactive sugars [322, 413]. The constancy in liver of secretion in equilibrium with processes that internalise plasma membrane components (see section IXB), would account for the generation and maintenance of these gradients. Information on the topographical location of 5'-nucleotidase in hepatocytes illustrates the insertion of a specific plasma membrane component by this pathway. 5'-Nucleotidase is synthesised mainly by membrane-bound ribosome [360], a common site for synthesis of intrinsic membrane proteins in liver [414] and the enzyme has been shown to be present in Golgi membranes where histochemical evidence suggests translocation of the active site from the cytoplasmic side in the cisternal elements to the luminal side in secretory vesicles [412]. After insertion into the blood sinusoidal plasma membrane, this ectoenzyme would be in a position to move laterally, thus explaining the lower enzyme levels present at the contiguous plasma membrane region.

However, discussions of mechanisms generating functional domains in liver by invoking lateral mobility between plasma membrane domains must give due regard to the role of the tight junctions in segregating the bile canalicular plasma membrane region from the remainder of the plasma membrane. If the tight junction prevents any lateral spillover into the bile canalicular plasma membrane of glycoproteins initially inserted into the blood sinusoidal and lateral plasma membrane regions, how can one account for the high levels of glycoenzymes shown to be present in this region by biochemical and histochemical techniques? An alternative route postulates direct interaction between the Golgi complex and the canalicular membrane but the evidence is indirect and circumstantial. For example, following bile duct ligation there is a large increase in the Golgi apparatus in the nonobstructed lobules that compensate to maintain bile formation [26]. Also, when increased bile formation is induced by perfusion of liver with taurocholate, morphometric analysis showed an increase in Golgi components and vesicles of diameter more than 1000 Å of unknown origin in the pericanalicular region [461]. On the other hand, it may be argued that if some classes of membrane proteins can move through that part of the plasma membrane comprising the tight junction (this may depend for example on the number and proximity to each other of the sealing strands in the junction), they would then become trapped within the bile canalicular region, thus making it the anatomical correlate



on the hepatocyte's surface of Abercrombie's sink concept [416]. Furthermore, many plasma membrane ectoenzymes are present in bile (although comprising a minor component of the bile proteins [113,116,417,418,420]) and this has led to possible mechanisms being described for entry of plasma membrane proteins and lipids to bile influenced by membrane flow and/or the abrasive action of bile salts [114,115,118-120,314,315,418,420].

The maintenance of polarity in epithelia, and the cellular interactions underlying tissue organisation are being studied by observing changes in surface architecture following tissue dissociation. In various epithelia (urinary bladder, and kidney and intestinal columnar cells) extensive redistribution of cell surface components occurs after enzymic dissociation [415]. Enzyme histochemistry [169] and biochemistry [382] have shown that 5'-nucleotidase and ATPase activities are concentrated at specific sites on the surface of freshly isolated hepatocytes, thus suggesting that the underlying biogenetic mechanisms maintaining polarity, although less effective, remain operational following tissue dissociation [410]. Indeed, the maintenance of surface polarity in isolated hepatocytes, and the proclivity of epithelial cells in general to reform into aggregates leading to reformation of intercellular junctions [108] suggests that polarity of cell surface organisation is the end result of a complex series of intracellular structural and biogenetic events involving the interaction of numerous membrane and organellar networks. This probably involves for example, cytoskeletal elements [407] and the GERL system [422-425] (the GERL system is a specialised part of the smooth endoplasmic reticulum localised near the Golgi apparatus and postulated to be involved in the production of lysosomes).

### *IXB. Turnover*

Liver plasma membrane proteins turnover with a half-life of 41-43 h (determined using  $\text{Na}_2^{14}\text{CO}_3$  or [*guanidino*- $^{14}\text{C}$ ]arginine) [426-428] and these values were obtained with subcellular fractions containing mainly bile-canalicular and lateral plasma membranes. Plasma membrane glycoproteins turnover more rapidly [428,429], e.g. half-lives of between 25 and 37 h have been calculated using various sugar precursors, [ $^{14}\text{C}$ ]glucosamine and [ $^3\text{H}$ ]fucose [337,338,348,430]. Some fucoproteins may turnover more rapidly with a half-life of 4-6 h [431]. In regenerating liver and hepatoma, the half-lives of glycoproteins [337,338] and proteins [339] of plasma membranes are significantly longer, probably reflecting a lower degree of secretory activity and increased cell growth and proliferation. Measurements of turnover and degradation of blood sinusoidal plasma membranes would be heavily influenced by the interplay between exocytotic and endocytotic processes, and these would undermine the assumptions inherent in the techniques used for measuring rates of degradation.

The following routes for interiorised sinusoidal plasma membrane can be described briefly (Fig. 7). In addition to the interaction of interiorised plasma membrane fragments with lysosomes followed by the breakdown of the plasma membrane components by lysosomal hydrolytic enzymes (route D), there is increasing experimental evidence interpreted to suggest that mechanisms are operable that enable the direct return of interiorised membrane to the plasma membrane without undergoing degradation within the lysosomal system (route B). Although such 'shuttle mechanisms' were first postulated to occur in macrophages [432] and fibroblasts [433], experimental evidence for their role in explaining turnover of glycoprotein in isolated hepatocytes [434] and in hepatoma cells [351] and specifically of a hepatic binding protein for asialoglycoproteins [451] have

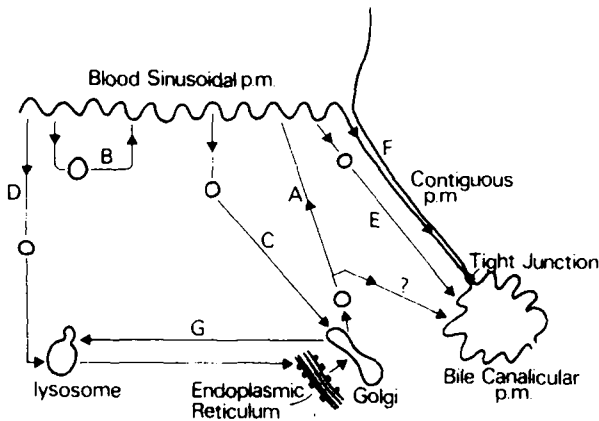


Fig. 7. Diagrammatic representation of the various possible routes of biogenesis, turnover and interplay of the plasma membrane regions of the hepatocyte. A. Route of insertion of glycoproteins from Golgi dictyosomes and secretory vesicles to the blood sinusoidal plasma membrane region. Evidence for any direct interaction of such vesicles with the bile canicular plasma membrane is circumstantial (?). B. Direct vesicle shuttling from and to the blood sinusoidal plasma membrane. C. Direct shuttling of vesicles from the blood sinusoidal plasma membrane to the Golgi apparatus. D. Endocytosis of sinusoidal plasma membrane vesicles and their breakdown after interaction with lysosomes. Membrane components are reutilised for membrane synthesis *de novo*. E. Vesicle shuttling from blood sinusoidal to the bile canicular plasma membrane region. F. Lateral movement of plasma membrane components in the plane of the membrane. G. Translocation between Golgi and lysosomal compartments. For further explanations, see text.

appeared. A further mechanism shown to operate in secretory epithelia including liver features the direct interaction of interiorised plasma membrane components with Golgi dictyosomes (route C) [51,436,437]. Vesicles shuttling to and from the plasma membrane may also interact with Golgi components on their route, especially in view of the apparent role of the Golgi apparatus in directing the biogenesis of cellular components. Finally, an adaptation of such mechanisms for removal and recycling of plasma membrane components may involve translocation of vesicles endocytosed from the blood sinusoidal plasma vesicles and transported across the hepatocyte's interior to the bile canicular membrane (route E), since this membrane-mediated route could explain the rapid appearance in bile of components for which there are receptors at the blood-sinusoidal plasma membrane.

Recent work on the fate of polypeptides bound to hepatocyte surface receptors is now beginning to provide experimental evidence for the intracellular route of internalised polypeptide hormone-receptor complexes and the membrane compartments involved. For example, following the interactions of  $^{125}\text{I}$ -radiolabelled insulin or prolactin with the plasma membrane sinusoidal and lateral regions [110,409,444], radioactivity was detected by autoradiography at 10 min in secretory elements of the Golgi apparatus and lysosome-like vacuoles, and at 20 min the hormone was found mainly in the lysosomal compartment [110,111]. Corroborative evidence using subcellular fractions has also been obtained [51, 438]. Thus, the general concept is emerging that certain polypeptide hormones and their plasma membrane receptors are degraded in specific organelles inside the cell. This leads to the possibility that insulin, prolactin and other polypeptide hormones may produce their biological effects after transfer to the inside of the cells.

A class of endolytic vesicles has been identified that may be involved in facilitating the rapid transport of immunoglobulin A from blood to bile without lysosomal involvement. IgA is a major component of bile [462,463] and autoradiographical [464] and biochemical [465] studies suggest that a direct route (corresponding to E in Fig. 7) may account for its rapid transcellular passage after receptor binding at the sinusoidal plasma membrane leading to release into bile. This route may also cater for transfer of other blood-borne substances also found in high concentrations in bile, e.g. insulin [466].

Coated vesicles, one of the candidates for plasma membrane recycling, and possessing a characteristic composition and lattice-like coat [439-441], have not been isolated from liver, although morphological evidence for their presence has been obtained [422,467].

## X. Concluding remarks

Since the preparation and biochemical analysis of liver plasma membranes were reported some 15 years ago, these membranes have emerged as the loci of an increasing number of enzymic and receptor-implicating hepatic functions. Liver plasma membrane fractions have featured widely in studies directed towards unravelling the molecular lesions underlying diseases of the hepato-biliary system. The present review emphasises the heterogeneous nature of the hepatocyte plasma membrane. By focusing on anatomical and physiological correlates, subcellular fraction heterogeneity is resolved by considering the hepatocyte plasma membrane as a functional mosaic constructed of three major regions or domains. Subfractionation techniques that separate plasma membrane fragments according to an origin from the blood-sinusoidal, contiguous (lateral) and bile canalicular regions are discussed, and information on the chemical and biochemical differences is presented. Although most of the biochemical functions occur predominantly at the blood-sinusoidal plasma membrane region, most membrane fractions used originate mainly from a restricted region of the pericellular membrane and contain mainly contiguous and bile canalicular membranes. It is stressed that methods yielding blood sinusoidal plasma membranes are often more appropriate for use in studies of the ever-increasing blood-sinusoidal plasma membrane functions listed in Table II. The major intercellular junctions present in the contiguous plasma membrane region, viz. desmosomes, tight and gap junctions are also considered as an example of further functional specialisation within a given domain.

The differentiation of the hepatocyte plasma membrane into three functional domains is discussed in the context of biogenetic and degradative processes that constantly synthesise and maintain these functional differences within the framework of a continuous membrane. Various mechanisms and routes for the biogenesis of the plasma membrane domains are evaluated. A major consideration is the intensive metabolic activity confined mainly to the plasma membrane's blood-sinusoidal region. Indeed, such is the intensity of secretory and endocytotic activities localised at the blood sinusoidal plasma membrane that plasma membrane components at a given time are located not only at the cell surface, but are also to be found migrating into the cell where opportunities for interacting with other intracellular membrane components arise. These interactions between derivatives of the sinusoidal plasma membrane and intracellular membrane compartments make measurements of the turnover and degradation rates of cell surface components difficult. The dynamic biogenetic interactions between the surface domains, the nature of the transitory region between sinusoidal and contiguous plasma membrane regions together with the role of the tight junctions in segregating the bile canalicular plasma membrane from the contiguous membrane are further aspects about which much needs to be learnt.

A final aspect of hepatocyte physiology concerns the control of endocytosis and exocytosis and plasma membrane recycling between the various domains of the membrane. Many polypeptides and proteins bind to receptors at the sinusoidal plasma membrane and are rapidly interiorised. Some also are released into bile, and this trans-hepatocyte route, as exemplified by IgA and bile acids, is, of course, a component of their entero-hepatic circulation. These routes raise intriguing questions at the organ level regarding the transporting mechanisms, especially the nature of the putative vesicles that may migrate transcellularly between the sinusoidal and canalicular domains, the identification of intracellular 'ports of call', if any, the propelling mechanisms involved, and the recognition mechanisms controlling trans-cellular movement and the interaction with the appropriate plasma membrane domain interfacing with the bile canaliculi. At the organism level, the biological mechanisms that come into operation after parturition for example, leading to suppression of hepatic secretion of IgA into bile and simultaneously redirecting into milk constitute further modulations of plasma membrane physiology. Clearly, when these mechanism have been further explored at the level of plasma membrane biochemistry, our understanding of hepatic physiology, and epithelial cell functioning in general will have taken a major step forward.

### Acknowledgements

I thank Professor P. Motta (Rome) for Fig. 1, Miss Elaine Brown (Mill Hill) for the filtered image of gap junctions in Fig. 3B, and Dr. D.A. Goodenough for Fig. 3A. I also thank Drs. M.H. Wisher (London), and P. Vischer (Mill Hill) for useful comments on the manuscript.

### References

- 1 Benedetti, E.L. and Emmelot, P. (1968) in *The Membranes* (Dalton, A.J. and Hagenau, P., eds.), Vol. 4, pp. 33–120, Academic Press, New York
- 2 Fawcett, D.W. (1955) *J. Natl. Cancer Inst.* 15 Suppl. 1475–1503
- 3 Bruni, C. and Porter, K.R. (1965) *Am. J. Pathol.* 46, 691–756
- 4 Rouiller, C. and Jezequel, A.M. (1963) in *The Liver* (Rouiller, C., ed.), Vol. 1, pp. 195–264, Academic Press, New York
- 5 Loud, A.V. (1968) *J. Cell Biol.* 37, 27–46
- 6 Motta, P., Muto, M. and Fujita, T. (1978) *The liver; An Atlas of Scanning Electron Microscopy*, Igaku-Shoin Ltd., Tokyo
- 7 Weibel, E.R., Staubli, W., Gnani, H.R. and Hess, F.A. (1969) *J. Cell Biol.* 42, 69–91
- 8 Wisse, E. (1972) *J. Ultrastruct. Res.* 38, 528–562
- 9 Wisse, E. (1974) *J. Ultrastruct. Res.* 46, 393–426
- 10 Wisse, E. and Knook, D.L. (1977) *Kupffer Cells and Other Liver Sinusoidal Cells*, Elsevier/North-Holland Biomedical Press, Amsterdam
- 11 Blouin, A., Bolender, R.P. and Weibel, E.R. (1977) *J. Cell Biol.* 72, 441–455
- 12 Bolender, R.P., Paumgartner, D., Losa, G., Muellener, D. and Weibel, E.R. (1978) *J. Cell Biol.* 77, 565–583
- 13 Gemmell, R.T. and Heath, T. (1972) *Anat. Rec.* 172, 57–70
- 14 Naito, M. and Wisse, E. (1976) *Cell Tiss. Res.* 190, 371–382
- 15 Gale, R.P., Sparkes, R.S. and Golde, D.W. (1978) *Science* 201, 937–938
- 16 Skaaring, P. and Bierring, F. (1976) *Cell Tissue Res.* 171, 141–155
- 17 Elias, H. and Sherrick, J.C. (1969) *Morphology of the liver*, Academic Press, New York
- 18 Grisham, J.W., Nopanitaya, A., Compagno, J. and Nagel, A.E.H. (1975) *Am. J. Anat.* 144, 295–321
- 19 Motta, P.M. and Porter, K.R. (1974) *Cell Tissue Res.* 148, 111–125
- 20 Motta, P. and Fumagalli, G. (1975) *Anat. Rec.* 182, 499–514

- 21 Motta, P.M. (1977) in *International Review of Cytology* (Bourne, G.H. and Danielli, J.F., eds.), Suppl. 6, pp. 347–399, Academic Press, New York
- 22 Pflüger, E. (1869) *Pflüger's Arch. Gesamte Physiol. Menschen Tiere* 2, 459–491
- 23 Kupffer, C. Von (1876) *Arch. Mikrosk. Anat. Entwicklungsmech.* 12, 353–385
- 24 Losa, G.A., Weibel, E.R. and Bolender, R.P. (1978) *J. Cell Biol.* 78, 289–308
- 25 Nemchausky, B.A., Layden, T.J. and Boyer, J.L. (1977) *Lab. Invest.* 36, 259–267
- 26 Layden, T.J. and Boyer, J.L. (1978) *Lab. Invest.* 39, 110–119
- 27 Oda, M., Price, Y.H., Fisher, M.M. and Phillips, M.J. (1974) *Lab. Invest.* 31, 314–323
- 28 Koga, A. and Todo, S. (1978) *Cell Tiss. Res.* 195, 267–276
- 29 Jones, A.L., Smuckler, D.L., Mooney, J.S., Adler, R.D. and Ockner, R.K. (1976) *Gastroenterology* 71, 1050–1060
- 30 Jacobs, S., Hazum, E., Scheckter, Y. and Cuatrecasas, P. (1979) *Proc. Natl. Acad. Sci. U.S.A.* 76, 4918–4921
- 31 Miya, K., Mayr, W.W. and Richardson, A.L. (1975) *Lab. Invest.* 32, 527–535
- 32 Pricer, W.E. and Ashwell, G. (1971) *J. Biol. Chem.* 246, 4825–4833
- 33 Hudgin, R.L. and Ashwell, G. (1974) *J. Biol. Chem.* 249, 7369–7372
- 34 Pricer, W.E. and Ashwell, G. (1976) *J. Biol. Chem.* 251, 7539–7544
- 35 Hubbard, A.L. and Stukenbrok, H. (1979) *J. Cell Biol.* 83, 65–81
- 36 Kawasaki, T. and Ashwell, G. (1976) *J. Biol. Chem.* 251, 1296–1302
- 37 Hudgin, R.L., Pricer, W.E., Ashwell, G., Stockert, R.J. and Morrell, A.G. (1974) *J. Biol. Chem.* 249, 5536–5543
- 38 Tolleshaug, H., Berg, T., Nilsson, M. and Norum, K.R. (1977) *Biochim. Biophys. Acta* 499, 73–84
- 39 Stowell, C.P. and Lee, Y.C. (1978) *J. Biol. Chem.* 253, 6107–6110
- 40 Van Berkel, T.J.C., Van Tol, A. and Koster, J.F. (1978) *Biochim. Biophys. Acta* 529, 138–146
- 41 Bachorik, P.S., Kwitterovich, P.O. and Cooke, J.C. (1978) *Biochemistry* 17, 5287–5299
- 42 Carrella, M. and Cooper, A.D. (1979) *Proc. Natl. Acad. Sci. U.S.A.* 76, 338–342
- 43 Achord, D.T., Brot, F.E., Bell, C.E. and Sly, W.S. (1978) *Cell* 15, 269–278
- 44 Posner, B.I., Raquidan, D., Josefsberg, Z. and Bergeron, J.J.M. (1978) *Proc. Natl. Acad. Sci. U.S.A.* 75, 3302–3306
- 45 Renaud, G., Foliot, A. and Infante, R. (1978) *Biochem. Biophys. Res. Commun.* 80, 327–334
- 46 Accatino, L. and Simon, F.R. (1976) *J. Clin. Invest.* 57, 496–508
- 47 Anwer, M.S., Kroker, R., Hegner, D. and Petter, A. (1977) *Hoppe-Seyler's Z. Physiol. Chem.* 385, 543–553
- 48 Cornelius, C.E., Ben-Ezzer, J. and Arias, I.M. (1967) *Proc. Soc. Exp. Biol. Med.* 124, 665–667
- 49 Salomon, Y. and Rodbell, M. (1975) *J. Biol. Chem.* 250, 7245–7250
- 50 Posner, B.I., Kelly, P.A. and Friesen, H.G. (1978) *Science* 188, 57–59
- 51 Josefsberg, Z., Posner, B.I., Patel, B. and Bergeron, J.J.M. (1979) *J. Biol. Chem.* 254, 209–214
- 52 Silverstein, A.M. and Richards, J.F. (1979) *Biochem. J.* 178, 743–751
- 53 Freychet, P. (1974) *J. Clin. Invest.* 54, 1020–1031
- 54 Freychet, P., Roth, J. and Neville, D.M. (1971) *Proc. Natl. Acad. Sci. U.S.A.* 68, 1833–1837
- 55 Freychet, P., Kahn, C.R., Roth, J. and Neville, D.M. (1972) *J. Biol. Chem.* 247, 3953–3961
- 56 Kahn, C.R., Freychet, P., Neville, D.M. and Roth, J. (1974) *J. Biol. Chem.* 249, 2249–2257
- 57 Evans, W.H., Bergeron, J.J.M., Geschwind, I.I. (1973) *FEBS Lett.* 34, 259–262
- 58 Jacobs, S., Schechter, Y., Bissell, K. and Cuatrecasas, P. (1977) *Biochem. Biophys. Res. Commun.* 77, 981–988
- 59 Matur, J.M. and Hollenberg, M.D. (1978) *Proc. Natl. Acad. Sci. U.S.A.* 75, 3070–3074
- 60 Milson, J.P. and Batey, R.G. (1979) *Biochem. J.* 182, 117–125
- 61 Kemmler, W., Renner, R., Zynamon, A. and Hepp, K.D. (1978) *Biochim. Biophys. Acta* 543, 349–356
- 62 Luly, P. and Shinitzky, M. (1979) *Biochemistry* 18, 445–450
- 63 Megyesi, K., Kahn, C.R., Roth, J., Froesch, E.R., Humbel, R.E., Zapf, J. and Neville, D.M. (1974) *Biochem. Biophys. Res. Commun.* 57, 307–315
- 64 Smigel, M. and Fleischer, S. (1976) *Biochim. Biophys. Acta* 332, 358–373
- 65 Okamura, N. and Terayama, H. (1977) *Biochim. Biophys. Acta* 465, 54–67
- 66 Guellaen, G., Yates-Aggerbeck, M., Vauquelin, G., Strosberg, D. and Hanoune, J. (1978) *J. Biol. Chem.* 253, 1114–1120
- 67 Clarke, W.R., Jones, L.R. and Lefkowitz, R.J. (1978) *J. Biol. Chem.* 253, 5975–5979

- 68 Okamura, N. and Terayama, H. (1978) *Biochim. Biophys. Acta* 544, 113–127
- 69 Hanoune, J., Lacombe, M.L. and Pecker, F. (1975) *J. Biol. Chem.* 250, 4569–4574
- 70 Pohl, S.J., Birnbaumer, L. and Rodbell, M. (1971) *J. Biol. Chem.* 246, 1849–1856
- 71 Salomon, Y., Lin, M.C., Londos, C., Rendell, M. and Rodbell, M. (1975) *J. Biol. Chem.* 250, 4239–4245
- 72 Bregman, M.D. and Levy, D. (1977) *Biochem. Biophys. Res. Commun.* 78, 584–590
- 73 Lafontaine, J.J., Nivez, M.P. and Ardaillou, R. (1979) *Clin. Sci.* 56, 33–40
- 74 Okamura, N. and Terayama, H. (1978) *Biochim. Biophys. Acta* 544, 113–127
- 75 Welton, A.F., Lad, P.M., Newby, A.C., Yamamura, H., Nicosia, S. and Rodbell, M. (1977) *J. Biol. Chem.* 252, 5947–5950
- 76 Kagawa, K., Kurohata, M. and Tomizawa, S. (1978) *Biochem. Biophys. Res. Commun.* 83, 1299–1305
- 77 Chambaut, A.M., Leray-Pecker, F., Feldmann, G. and Hanoune, J. (1974) *J. Gen. Physiol.* 64, 104–126
- 78 Murad, F., Mittal, C.K., Arnold, W.P., Katsuki, S. and Kimura, H. (1978) *Adv. Cyclic Nucleotide Res.* 9, 145–158
- 79 Cheng, S., McQueen, H.M. and Levy, D. (1978) *Arch. Biochem. Biophys.* 189, 336–343
- 80 Hughes, W.A. and Coore, H.G. (1978) *Int. J. Biochem.* 9, 751–755
- 81 Chang, K.J., Bennett, V. and Cuatrecasas, P. (1975) *J. Biol. Chem.* 250, 488–500
- 82 Bowles, D.J. and Kauss, H. (1976) *FEBS Lett.* 66, 16–19
- 83 Keenan, T.W., Franke, W.W. and Kartenbeck, J. (1974) *FEBS Lett.* 44, 274–278
- 84 Riordan, J.R. and Alon, N. (1977) *Biochim. Biophys. Acta* 464, 547–561
- 85 Frimmer, M. and Petzinger, E. (1977) in *Membrane Alterations as Basis of Liver Injury* (Popper, H., Bianchi, L. and Reutter, W., eds.), pp. 293–299, M.T.P. Press, Lancaster
- 86 Lutz, G., Glossmann, H. and Frimmer, M. (1972) *Naunyn-Schmiedeberg's Arch. Pharmacol.* 273, 341–351
- 87 Govindan, V.M., Faulstich, H., Wieland, Th., Agostini, B. and Hasselbach, W. (1972) *Naturwissenschaften* 59, 521–522
- 88 Govindan, V.M., Rohr, G., Wieland, Th. and Agostini, B. (1973) *Hoppe-Seyler's Z. Physiol. Chem.* 354, 1159–1161
- 89 Kroker, R. and Hegner, D. (1973) *Naunyn-Schmiedeberg's Arch. Pharmacol.* 279, 339–346
- 90 Levy, D., Glover, E. and Chang, S. (1977) *Biochim. Biophys. Acta* 469, 194–201
- 91 Evans, W.H. and Bruning, J.W. (1970) *Immunology* 19, 735–741
- 92 Edidin, M. (1976) in *Transplantation Antigens* (Kahan, B.D. and Reisfeld, R.A., eds.), pp. 125–140, Academic Press, New York
- 93 Henriksen, O., Robinson, E.A. and Appella, E. (1978) *Proc. Natl. Sci. U.S.A.* 75, 3322–3326
- 94 Morré, D.J., Schirmacher, V., Robinson, P., Hess, K. and Franke, W.W. (1979) *Exp. Cell Res.* 119, 265–275
- 95 Hopf, U., Mutschall, C., Meyerzumbuschensfelde, K.H. (1979) *Z. Gastroenterol.* 17, 18–27
- 96 Van Amelsvoort, J.M.M., Sips, H.J. and van Dam, K. (1978) *Biochem. J.* 174, 1083–1086
- 97 Bachmann, W. and Challoner, D. (1976) *Biochim. Biophys. Acta* 443, 254–266
- 98 Obrink, B., Kuhlenschmidt, M.S. and Roseman, S. (1977) *Proc. Natl. Acad. Sci. U.S.A.* 74, 1077–1081
- 99 Benedetti, E.L. and Emmelot, P. (1968) *J. Cell Biol.* 38, 15–24
- 100 Evans, W.H. and Gurd, J.W. (1972) *Biochem. J.* 128, 691–700
- 101 Goodenough, D.A. and Stoeckenius, W. (1972) *J. Cell Biol.* 54, 646–656
- 102 Goodenough, D.A. (1974) *J. Cell Biol.* 61, 557–563
- 103 Goodenough, D.A. (1976) *J. Cell Biol.* 68, 220–231
- 104 Culvenor, J.G. and Evans, W.H. (1977) *Biochem. J.* 168, 475–481
- 105 Duguid, J. and Revel, J.P. (1976) *Cold Spring Harbor Symp. Quant. Biol.* 40, 45–47
- 106 Hertzberg, E. and Gilula, N.B. (1979) *J. Biol. Chem.* 254, 2138–2147
- 107 Ehrhart, J.C. and Chauveau, J. (1977) *FEBS Lett.* 78, 295–299
- 108 Wanson, J.C., Drochmans, P., Mosselmans, R. and Ronveaux, M.F. (1977) *J. Cell Biol.* 74, 858–877
- 109 Miettinen, A., Virtanen, I. and Linder, E. (1978) *J. Cell Sci.* 31, 341–353
- 110 Bergeron, J.J.M., Sikstrom, A.R., Hand, A.R. and Posner, B.I. (1979) *J. Cell Biol.* 80, 427–443
- 111 Bergeron, J.J.M., Levine, G., Sikstrom, D., O'Shaughnessy, Kopriwa, B., Nadler, N.J. and Posner, B.I. (1977) *Proc. Natl. Acad. Sci. U.S.A.* 74, 5051–5055

- 112 Song, C.S., Rubin, W., Rifkind, A.B. and Kappas, A. (1969) *J. Cell Biol.* 41, 124-145
- 113 Coleman, R. and Holdsworth, G. (1975) *Biochim. Biophys. Acta* 389, 47-50
- 114 Yousef, I.M., Bloxam, D.L., Phillips, M.J. and Fisher, W.M. (1975) *Can. J. Biochem.* 53, 989-997
- 115 Vyvoda, O.S., Coleman, R. and Holdsworth, G. (1977) *Biochim. Biophys. Acta* 465, 68-76
- 116 Evans, W.H., Kremmer, T. and Culvenor, J.G. (1976) *Biochem. J.* 154, 589-595
- 117 Yousef, I.M., Fisher, M.M., Piekarski, J. and Holub, B.J. (1977) *Lipids* 12, 140-144
- 118 Mullock, B.M. and Hinton, R.H. (1978) *Biochem. Soc. Trans.* 6, 274-276
- 119 Coleman, R., Iqbal, S., Godfrey, P.P. and Billington, D. (1979) *Biochem. J.* 178, 201-208
- 120 Mullock, B.M., Dobrota, M. and Hinton, R.H. (1978) *Biochim. Biophys. Acta* 543, 497-507
- 121 Boyer, J.L. and Renno, D. (1975) *Biochim. Biophys. Acta* 401, 59-72
- 122 Novikoff, A.B. (1959) *J. Histochem. Cytochem.* 7, 240-244
- 123 Menard, D., Penasse, W., Drochmans, P. and Hugon, P. (1974) *Histochemistry* 38, 229-239
- 124 Welsh, F. (1972) *J. Histochem. Cytochem.* 20, 107-111
- 125 Wanson, J.-C., Drochmans, P., May, C., Pennase, W. and Popowski, A. (1975) *J. Cell Biol.* 66, 23-41
- 126 Lin, C. and Chang, J.P. (1975) *Science* 190, 465-467
- 127 Drochmans, P., Wanson, J.-C. and Mosselmans, R. (1975) *J. Cell Biol.* 66, 1-22
- 128 Smuckler, D.L., Mooney, J.S. and Jones, A.L. (1978) *J. Cell Biol.* 78, 319-337
- 129 Yunghans, W.N. and Morre, D.J. (1973) *Prep. Biochem.* 3, 301-312
- 130 Stein, Y., Widnell, C.C. and Stein, O. (1968) *J. Cell Biol.* 39, 185-192
- 131 Touster, O., Aronson, N.N., Dulaney, J.T. and Hendrickson, H. (1970) *J. Cell Biol.* 47, 604-618
- 132 Ray, T.K. (1970) *Biochim. Biophys. Acta* 196, 1-9
- 133 House, P.D.R. and Weidemann, M.J. (1970) *Biochem. Biophys. Res. Commun.* 41, 541-548
- 134 House, P.D.R., Poulis, P. and Weidemann, M.J. (1972) *Eur. J. Biochem.* 24, 429-437
- 135 Coleman, R., Michell, R.H., Finean, J.B. and Hawthorne, J.N. (1967) *Biochim. Biophys. Acta* 135, 573-579
- 136 Dorling, P.R. and LePage, R.N. (1973) *Biochim. Biophys. Acta* 318, 33-44
- 137 Lutz, F. and Frimmer, M. (1970) *Hoppe-Seyler's Z. Physiol. Chem.* 351, 1429-1434
- 138 Nigam, V.N., Morais, R. and Karasaki, S. (1971) *Biochim. Biophys. Acta* 249, 34-40
- 139 Hodson, S. and Brenchley, G. (1976) *J. Cell Sci.* 20, 167-182
- 140 Yousef, I.M. and Murray, R.K. (1978) *Can. J. Biochem.* 56, 713-721
- 141 Brown, A.E., Lok, M.P. and Elovson, J. (1976) *Biochim. Biophys. Acta* 426, 418-432
- 142 Neville, D.M. (1968) *Biochim. Biophys. Acta* 154, 540-552
- 143 Barclay, M., Barclay, R.K., Skipski, V.P., Essner, E.S. and Terebus-Kekish, O. (1972) *Biochim. Biophys. Acta* 255, 931-947
- 144 Wong, N. and Zull, J.E. (1974) *Biochim. Biophys. Acta* 352, 52-63
- 145 Berman, H.M., Gram, W. and Spirtes, M.A. (1969) *Biochim. Biophys. Acta* 183, 10-18
- 146 El-Aaser, A.A., Fitzsimons, J.T.R., Hinton, R.H., Reid, E., Klucis, E. and Alexander, P. (1966) *Biochim. Biophys. Acta* 127, 553-556
- 147 Pflieger, R.C., Anderson, N.G. and Snyder, F. (1968) *Biochemistry* 7, 2826-2833
- 148 Weaver, R.A. and Boyle, W. (1969) *Biochim. Biophys. Acta* 173, 377-388
- 149 Evans, W.H. (1970) *Biochem. J.* 116, 833-842
- 150 Hinton, R.H., Dobrota, M., Fitzsimons, J.T.R. and Reid, E. (1970) *Eur. J. Biochem.* 12, 349-361
- 151 Blyth, C.A., Clark, R.P., Freedman, R.B., Hammond, J., James, D.W., Rabin, B.R., Ridge, D., Vintner, J. and Williams, D. (1973) *Eur. J. Biochem.* 32, 57-62
- 152 Lesco, L., Donlon, M., Marinetti, G.V. and Hare, J.D. (1973) *Biochim. Biophys. Acta* 311, 173-179
- 153 Brivio-Haugland, R.P., Louis, S.L., Musch, K., Waldeck, N. and Williams, M.A. (1976) *Biochim. Biophys. Acta* 433, 150-163
- 154 Leblond-Larouche, L., Morais, R., Nigam, V.N. and Karasaki, S. (1975) *Arch. Biochem. Biophys.* 167, 1-12
- 155 Westwood, S.A., Luzio, J.P., Flockhart, D.A. and Siddle, K. (1978) *Biochem. Soc. Trans.* 6, 278-280
- 156 Westwood, S.A., Luzio, J.P., Flockhart, D.A. and Siddle, K. (1979) *Biochim. Biophys. Acta* 583, 454-466

- 157 Lauter, C.J., Solyom, A. and Trams, E. (1972) *Biochim. Biophys. Acta* 266, 511-523
- 158 Novikoff, A.B. and Noe, E.F. (1955) *J. Morphol.* 96, 189-221
- 159 Thines-Sempoux, D., Amar-Costesec, A., Beaufay, H. and Berthet, J. (1969) *J. Cell Biol.* 43, 189-192
- 160 Wisher, M.H. and Evans, W.H. (1975) *Biochem. J.* 146, 375-388
- 161 Evans, W.H. (1969) *FEBS Lett.* 3, 237-241
- 162 Bock, K.W., Siekevitz, P. and Palade, G.E. (1971) *J. Biol. Chem.* 246, 188-195
- 163 Hardonk, M.J., Dijkhuis, F.W.J., Haarsma, T.J., Koudstaal, J. and Huijbers, W.A.R. (1977) *Histochemistry* 53, 165-181
- 164 Doyle, D., Hou, E. and Warren, R. (1979) *J. Biol. Chem.* 254, 6853-6856
- 165 Thomas, P. and Summers, J.W. (1978) *Biochem. Biophys. Res. Commun.* 80, 335-339
- 166 Evans, W.H. (1978) in *Laboratory Techniques in Biochemistry and Molecular Biology, Preparation and Characterisation of Mammalian Plasma Membranes* (Work, T.S. and Work, E., eds.), Vol. 7, part I, Elsevier/North-Holland Biomedical Press, Amsterdam
- 167 Higgins, J.A. and Evans, W.H. (1978) *Biochem. J.* 174, 563-567
- 168 Losa, G.A., Weibel, E.R. and Bolender, R.P. (1978) *J. Cell Biol.* 78, 289-308
- 169 Essner, E., Novikoff, A.B. and Masek, B. (1958) *J. Biophys. Biochem. Cytol.* 4, 711-716
- 170 Sierakowska, H., Szemplinska, H. and Shugar, D. (1963) *Acta Biochem. Polon.* 104, 399-401
- 171 Vonk, R.J., Jekel, P.A., Meijer, D.K.F. and Hardonk, M.J. (1978) *Biochem. Pharmacol.* 27, 397-405
- 172 Chang, K.J., Bennett, V. and Cuatrecasas, P. (1975) *J. Biol. Chem.* 250, 488-500
- 173 Carey, F. and Evans, W.H. (1977) *Biochem. Soc. Trans.* 5, 103-104
- 174 Fisher, M.M., Bloxam, D.L., Oda, M., Phillips, M.J. and Yousef, I.M. (1975) *Proc. Soc. Exp. Biol. Med.* 150, 177-184
- 175 Blouin, A. (1977) *Can. Vet. J.* 18, 137 (Abstr.)
- 176 Horvat, A., Li, E. and Katsoyannis, P.G. (1975) *Biochim. Biophys. Acta* 382, 609-620
- 177 Bergeron, J.J.M., Evans, W.H. and Geshwind, I. (1973) *J. Cell Biol.* 59, 771-776
- 178 Posner, B.I., Josefsberg, Z. and Bergeron, J.J.M. (1978) *J. Biol. Chem.* 253, 4067-4073
- 179 Bergeron, J.J.M., Posner, B.I., Josefsberg, Z. and Sikstrom, R. (1978) *J. Biol. Chem.* 253, 4058-4066
- 180 Horvat, A. (1978) *J. Cell Physiol.* 97, 37-48
- 181 Claude, P. and Goodenough, D.A. (1973) *J. Cell Biol.* 58, 390-400
- 182 Friend, D.S. and Gilula, N.B. (1972) *J. Cell Biol.* 53, 758-776
- 183 Staehlin, L. (1973) *J. Cell Sci.* 13, 763-786
- 184 Staehlin, L. (1978) *Sci. Am.* 238, 140-153
- 185 Layden, T.J., Elias, E. and Boyer, J.L. (1978) *J. Clin. Invest.* 62, 1375-1385
- 186 Skerrow, C.J. and Matolsty, A.G. (1974) *J. Cell Biol.* 63, 515-523; 524-530
- 187 Drochmans, P., Freudenstein, C., Wanson, J.C., Laurent, L., Keenan, T.W., Stadler, J., Leloup, R., and Franke, W.W. (1978) *J. Cell Biol.* 79, 427-443
- 188 Gilula, N.B. (1978) in *International Cell Biology 1976-1977* (Brinkley, B.R. and Porter, K.R., eds.), pp. 61-69, Rockefeller University Press, New York
- 189 Lowenstein, W.R. (1979) *Biochim. Biophys. Acta* 560, 1-65
- 190 Zampighi, G. and Robertson, J.D. (1977) *Biophys. J.* 17, 31a
- 191 Makowski, L., Caspar, D.L.D., Phillips, W.C. and Goodenough, D.A. (1977) *J. Cell Biol.* 74, 629-645
- 192 Lee, A.G. and Revel, J.P. (1978) *J. Cell Biol.* 78, 554-564
- 193 Lowenstein, W.R. and Penn, R.D. (1967) *J. Cell Biol.* 33, 235-242
- 194 Beaufay, H., Amar-Costesec, A., Thines-Sempoux, D., Wibo, M., Robbi, M. and Berthet, J. (1974) *J. Cell Biol.* 61, 213-231
- 195 Peters, T.J. and Seymor, C.A. (1978) *Biochem. J.* 174, 435-446
- 196 Peters, T.J. and Shio, H. (1976) *Clin. Sci. Mol. Med.* 50, 355-366
- 197 Van Dijk, W., Maier, H. and Van den Eijnden, D.H. (1977) *Biochim. Biophys. Acta* 466, 187-197
- 198 Smith, G.D. and Peters, T.J. (1978) *Biochem. Soc. Trans.* 6, 543-545
- 199 DePierre, J.W. and Karnovsky, M.L. (1974) *J. Biol. Chem.* 249, 7111-7120
- 200 DePierre, J.W. and Karnovsky, M.L. (1974) *J. Biol. Chem.* 249, 7121-7129
- 201 DePierre, J.W. and Dallner, G. (1975) *Biochim. Biophys. Acta* 415, 411-472



- 202 Trams, E.G. and Lauter, C.J. (1974) *Biochim. Biophys. Acta* 345, 180-197
- 203 Schafer, A., Haase, W., Murer, W. and Kinne, R. (1978) *Biochem. J.* 172, 57-62
- 204 Riemer, B.L. and Widnell, C.C. (1975) *Arch. Biochem. Biophys.* 171, 343-347
- 205 Aoyagi, T., Suda, H., Nagai, M., Ogawa, K., Suzuki, J., Takeuchi, T. and Umezawa, H. (1976) *Biochim. Biophys. Acta* 452, 131-143
- 206 Gurd, J.W. and Evans, W.H. (1974) *Arch. Biochem. Biophys.* 164, 305-311
- 207 Abney, E., Evans, W.H. and Parkhouse, R.M.E. (1977) *Biochem. J.* 159, 293-299
- 208 Evans, W.H. and Gurd, J.W. (1973) *Biochem. J.* 133, 189-199
- 209 Widnell, C.C. (1976) *Methods Enzymol.* 32, 368-374
- 210 Nakamura, S. (1976) *Biochim. Biophys. Acta* 426, 339-347
- 211 Slavik, M., Kartner, N. and Riordan, J.R. (1977) *Biochem. Biophys. Res. Commun.* 75, 342-349
- 212 Stanley, K.K. and Luzio, J.P. (1978) *Biochim. Biophys. Acta* 514, 198-205
- 213 Dipple, L., Elliot, K.R.F. and Houslay, M.D. (1978) *FEBS Lett.* 89, 153-156
- 214 Low, M.G. and Finean, J.B. (1978) *Biochim. Biophys. Acta* 508, 565-570
- 215 Mannherz, H.G. and Rohr, G. (1978) *FEBS Lett.* 95, 284-289
- 216 Rohr, G. and Mannherz, H.G. (1979) *FEBS Lett.* 99, 351-356
- 217 Evans, W.H., Hood, D.A. and Gurd, J.W. (1973) *Biochem. J.* 135, 819-826
- 218 Skidmore, J.R. and Trams, E.G. (1970) *Biochim. Biophys. Acta* 219, 93-103
- 219 Sela, B., Lis, H. and Sachs, L. (1972) *J. Biol. Chem.* 247, 7575-7590
- 220 Erecinska, M., Sierakowski, H. and Shugar, D. (1969) *Eur. J. Biochem.* 11, 465-471
- 221 Decker, K. and Bischoff, E. (1972) *FEBS Lett.* 21, 95-98
- 222 Bischoff, E., Tran-thi, T. and Decker, K. (1975) *Eur. J. Biochem.* 51, 353-361
- 223 Bischoff, E., Wilkening, J., Tran-thi, T. and Decker, K. (1976) *Eur. J. Biochem.* 62, 279-283
- 224 Evans, W.H. (1974) *Nature* 250, 391-394
- 225 Ohkubo, A., Langerman, N. and Kaplan, M.M. (1974) *J. Biol. Chem.* 249, 7174-7180
- 226 Ikehara, Y., Takahashi, K., Mansho, K., Eto, S. and Kato, K. (1977) *Biochim. Biophys. Acta* 470, 202-211
- 227 Komoda, T. and Sakagishi, Y. (1976) *Biochim. Biophys. Acta* 438, 138-152
- 228 Badger, K.S. and Sussman, H. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 73, 2201-2205
- 229 Ikehara, Y., Mansho, K., Takahashi, K. and Kato, K. (1978) *J. Biochem.* 83, 1471-1483
- 230 Starnes, W.L. and Behal, F.J. (1974) *Biochemistry* 13, 3221-3227
- 231 Berzins, K., Lando, P., Raftell, M. and Blomberg, F. (1977) *Biochim. Biophys. Acta* 497, 337-348
- 232 Kean, F.L. and Bighouse, K.J. (1974) *J. Biol. Chem.* 249, 7813-7824
- 233 Swislocki, N.A., Johnson, C.B. and Tierney, J. (1977) *Arch. Biochem. Biophys.* 179, 157-165
- 234 Houslay, M.D., Ellory, J.C., Smith, G.A., Hesketh, T.R., Stein, J.M., Warren, G.B. and Metcalfe, J.C. (1977) *Biochim. Biophys. Acta* 467, 208-219
- 235 Swislocki, N.A. and Tierney, J. (1975) *Arch. Biochem. Biophys.* 168, 455-462
- 236 Houslay, M.D. and Palmer, R.W. (1978) *Biochem. J.* 174, 909-919
- 237 Tsai, S.C., Manganiello, V.C. and Vaughan, M. (1978) *J. Biol. Chem.* 253, 8452-8457
- 238 Kimura, H. and Murad, F. (1975) *J. Biol. Chem.* 250, 4810-4817
- 239 Sulakhe, P.V., Sulakhe, S.J., Leung, N.L., St. Louis, P.J. and Hickie, R.A. (1976) *Biochem. J.* 157, 705-712
- 240 Diaugustine, R.P., Abe, T. and Voytek, P. (1978) *Biochim. Biophys. Acta* 526, 518-530
- 241 Newkirk, J.D. and Waite, M. (1973) *Biochim. Biophys. Acta* 298, 562-576
- 242 Waite, M., Sisson, P., Maghrabi, R.E., Yousef, I.M. and Fisher, M.M. (1977) *Biochim. Biophys. Acta* 470, 134-139
- 243 Chowdhury, J.R., Chowdhury, W.R., Bargava, M.M. and Arias, I.M. (1979) *J. Biol. Chem.* 254, 8336-8339
- 244 Waite, M. and Sisson, P. (1973) *J. Biol. Chem.* 248, 7985-7992
- 245 Waite, M. and Sisson, P. (1973) *J. Biol. Chem.* 248, 7201-7206
- 246 Thompson, W.J. and Appleman, M.M. (1971) *Biochemistry* 10, 311-316
- 247 Tate, S.S. and Meister, A. (1978) *Proc. Natl. Acad. Sci. U.S.A.* 75, 4806-4809
- 248 Kottgen, E., Reutter, W. and Gerok, W. (1978) *Eur. J. Biochem.* 82, 279-284
- 249 Smith, C.A. and Brew, K. (1977) *J. Biol. Chem.* 252, 7294-7299
- 250 Keenan, T.W. and Morré, D.J. (1975) *FEBS Lett.* 55, 8-13
- 251 Munro, J.R., Narasimhan, S., Wetmore, S., Riordan, J.R. and Schachter, H. (1975) *Arch. Biochem. Biophys.* 169, 269-277

- 252 Merritt, W.D., Morre, D.J., Franke, W.W. and Keenan, T.W. (1977) *Biochim. Biophys. Acta* 497, 820-824
- 253 Crane, F.L. and Low, H. (1976) *FEBS Lett.* 68, 153-156
- 254 Low, H. and Crane, F.L. (1976) *FEBS Lett.* 68, 157-159
- 255 Low, H. and Crane, F.L. (1978) *Biochim. Biophys. Acta* 515, 141-162
- 256 Low, H., Crane, F.L., Grebing, C., Tally, M. and Hall, K. (1978) *FEBS Lett.* 91, 166-168
- 257 Bose, R. and Yamada, E.W. (1977) *Can. J. Biochem.* 55, 528-533
- 258 Kyte, J. (1972) *J. Biol. Chem.* 247, 7642-7649
- 259 Lo, C.S. and Edelman, I.S. (1976) *J. Biol. Chem.* 251, 7834-7840
- 260 Yancey, S.B., Easter, D. and Revel, J.-P. (1979) *J. Ultrastruct. Res.* 67, 229-242
- 261 Schwartz, A., Lindenmayer, G.E. and Allen, J.C. (1975) *Pharmacol. Rev.* 27, 4-134
- 262 Jansen, P.L.M., Chowdhury, J.R., Fischberg, E.B. and Arias, I.M. (1977) *J. Biol. Chem.* 252, 2710-2716
- 263 Franklin, J.E. and Trams, E.G. (1971) *Biochim. Biophys. Acta* 230, 105-116
- 264 Yannarell, A. and Aronson, N.N. (1973) *Biochim. Biophys. Acta* 311, 191-204
- 265 Schengrund, C.L., Jensen, D.S. and Rosenberg, A. (1972) *J. Biol. Chem.* 247, 2742-2746
- 266 Visser, A. and Emmelot, P. (1973) *J. Membrane Biol.* 14, 73-84
- 267 Newby, A.C. and Chrambach, A. (1979) *Biochem. J.* 177, 623-630
- 268 Schulster, D., Orly, J., Seidel, G. and Schramm, M. (1978) *J. Biol. Chem.* 253, 1201-1206
- 269 Blitzer, B.L. and Boyer, J.L. (1978) *J. Clin. Invest.* 62, 1104-1108
- 270 Erlinger, S. (1977) in *Liver and Bile* (Bianchi, L., Gerok, W. and Sickinger, K., eds.), pp. 55-62, M.T.P. Press, Lancaster
- 271 Layden, T.J. and Boyer, J.L. (1976) *J. Clin. Invest.* 57, 1009-1018
- 272 Simon, F.R., Sutherland, E. and Accatino, L. (1977) *J. Clin. Invest.* 59, 849-861
- 273 Reichen, J. and Paumgartner, G. (1977) *J. Clin. Invest.* 60, 429-434
- 274 Deppert, W., Werchau, H. and Walter, H. (1974) *Proc. Natl. Acad. Sci. U.S.A.* 71, 3068-3072
- 275 Fritzson, P. and Smith, I. (1971) *Biochim. Biophys. Acta* 235, 128-141
- 276 Frick, G.P. and Lowenstein, J.M. (1977) *J. Biol. Chem.* 253, 1240-1244
- 277 Van Den Berghe, G., Van Pottelsberghe, C. and Hers, H.G. (1977) *Biochem. J.* 162, 611-616
- 278 Blomberg, F. and Perlmann, P. (1971) *Biochim. Biophys. Acta* 233, 53-60
- 279 Gurd, J.W., Evans, W.H. and Perkins, H.R. (1973) *Biochem. J.* 135, 827-832
- 280 Raftell, M. and Blomberg, F. (1974) *Eur. J. Biochem.* 49, 31-39
- 281 Blomberg, F. and Raftell, M. (1974) *Eur. J. Biochem.* 49, 21-30
- 282 Blomberg, F. and Berzins, K. (1975) *Eur. J. Biochem.* 56, 319-326
- 283 Berzins, K. and Blomberg, F. (1975) *FEBS Lett.* 54, 139-143
- 284 Bjerrum, O.J. (1977) *Biochim. Biophys. Acta* 472, 135-195
- 285 Houslay, M.D. and Palmer, R.W. (1978) *Biochem. J.* 174, 909-919
- 286 Evans, W.H. (1970) *Biochim. Biophys. Acta* 211, 578-581
- 287 Elder, J.H., Morré, D.J. and Keenan, T.W. (1976) *Cytobiologie* 13, 279-284
- 288 Neville, D.M. and Glossmann, H. (1971) *J. Biol. Chem.* 246, 6335-6338
- 289 Glossmann, H. and Neville, D.M. (1971) *J. Biol. Chem.* 246, 6339-6346
- 290 Yousef, I.M. and Murray, R.K. (1978) *Can. J. Biochem.* 56, 713-721
- 291 Covindan, V.M. and Wieland, T. (1975) *FEBS Lett.* 59, 117-119
- 292 Brandon, D.L. (1975) *FEBS Lett.* 58, 349-352
- 293 Brandon, D.L. (1976) *Eur. J. Biochem.* 65, 139-146
- 294 Gurd, J.W. and Evans, W.H. (1976) *Can. J. Biochem.* 54, 477-480
- 295 Remacle, J. (1978) *J. Cell Biol.* 79, 291-313
- 296 Jarasch, E.D., Kartenbeck, J., Bruder, G., Fink, A., Morré, D.J. and Franke, W.W. (1979) *J. Cell Biol.* 80, 37-52
- 297 Mutoh, S., Funakoshi, I. and Yamashina, I. (1978) *J. Biochem.* 84, 483-489
- 298 Funakoshi, I. and Yamashina, I. (1976) *J. Biochem.* 80, 1185-1193
- 299 Nakajo, S., Nakaya, K. and Nakamura, Y. (1979) *Biochim. Biophys. Acta* 579, 88-94
- 300 Dod, B.J. and Gray, G.M. (1968) *Biochim. Biophys. Acta* 150, 397-404
- 301 Ray, T.K., Skipski, V.P., Barclay, M., Essner, I. and Archibald, F.M. (1969) *J. Biol. Chem.* 244, 5528-5536
- 302 Wood, R. (1970) *Arch. Biochem. Biophys.* 141, 174-182
- 303 Colbeau, A., Nachbaur, J. and Vignais, P.M. (1971) *Biochim. Biophys. Acta* 249, 462-492

- 304 Keenan, T.W. and Morré, D.J. (1970) *J. Lipid Res.* 9, 19–25
- 305 Van Hoeven, R.P. and Emmelot, P. (1972) *J. Membrane Biol.* 9, 105–126
- 306 Bergelson, L.D., Dyatlovskaya, T.V., Torkhovskaya, T.I., Sorokina, I.B. and Gorkova, N.P. (1970) *Biochim. Biophys. Acta* 210, 287–298
- 307 Zambrano, F., Fleischer, S. and Fleischer, B. (1975) *Biochim. Biophys. Acta* 380, 357–369
- 308 Franke, W.W., Keenan, T.W., Stadler, J., Genz, R., Jarasch, E.D. and Kartenbeck, J. (1976) *Cytobiologie* 13, 28–56
- 309 Yousef, I.M. and Fisher, M.M. (1976) *Can. J. Biochem.* 54, 1040–1046
- 310 Van Hoeven, R.P., Emmelot, P., Krol, J.H. and Oomen-Meulemans, E.P.M. (1975) *Biochim. Biophys. Acta* 380, 1–11
- 311 Emmelot, P. and Van Hoeven, R.P. (1975) *Chem. Phys. Lipids* 14, 236–246
- 312 Kremmer, T., Wisher, M.H. and Evans, W.H. (1976) *Biochim. Biophys. Acta* 455, 655–664
- 313 Keenan, T.W., Huang, C.M. and Morré, D.J. (1972) *FEBS Lett.* 24, 204–208
- 314 Coleman, R., Holdsworth, G. and Uyvoda, O.S. (1977) in *Membrane Alterations as Basis of Liver Injury* (Popper, H., Bianchi, L. and Reutter, W., eds.), pp. 143–156, M.T.P. Press, Lancaster, U.K.
- 315 Evans, W.H. and Higgins, J.M. (1979) in *Processing and Turnover of Proteins and Organelles in the Cell* (Rapoport, S. and Schewe, T., eds.), Vol. 53, *FEBS Proc.*, pp. 169–178, Pergamon Press, Oxford
- 316 Franke, W.W. and Kartenbeck, J. (1976) in *Progress in Differentiation Research* (Muller-Berat, N., ed.), pp. 213–243, Elsevier/North-Holland, Amsterdam
- 317 Singer, S.J. and Nicolson, G. (1972) *Science* 175, 720–731
- 318 Guillouzo, A. and Feldmann, G. (1977) *J. Histochem. Cytochem.* 25, 1303–1310
- 319 Virtanen, I., Miettinen, A. and Wartiovaara, J. (1978) *J. Cell Sci.* 29, 287–296
- 320 Cook, G.M.W. (1977) in *The Synthesis, Assembly and Turnover of Cell Surface Components* (Poste, G. and Nicolson, G.L., eds.), pp. 85–136, Elsevier/North-Holland, Amsterdam
- 321 Rambourg, A. (1971) *Int. Rev. Cytol.* 31, 57–114
- 322 Bennett, G., Leblond, C.P. and Haddad, A. (1974) *J. Cell Biol.* 60, 258–284
- 323 Benedetti, E.L., Dunia, I. and Diawara, A. (1973) *Eur. J. Cancer* 9, 263–272
- 324 Carreira, J. and Evans, W.H. (1978) *Biochem. Soc. Trans.* 6, 276–278
- 325 Phillips, M.J., Oda, M., Mak, E., Fisher, M.M. and Jeejeebhoy, K.N. (1975) *Gastroenterology* 69, 48–58
- 326 Toda, G., Oka, H., Oda, T. and Ikeda, Y. (1975) *Biochim. Biophys. Acta* 413, 52–64
- 327 Davis, R.A., Kern, F., Showalter, R., Sutherland, E., Sinensky, M. and Simon, F.R. (1978) *Proc. Natl. Acad. Sci. U.S.A.* 75, 4130–4134
- 328 DeBroe, M.E., Borgers, M. and Wieme, R.J. (1975) *Clin. Chim. Acta* 59, 369–372
- 329 Goridis, C. and Reutter, W. (1976) *Nature* 257, 698–700
- 330 Wright, G.A. (1977) *Biochim. Biophys. Acta* 470, 368–381
- 331 Koizumi, K., Ito, Y., Kojima, K. and Fujii, T. (1976) *J. Biochem.* 79, 739–748
- 332 Masuda, Y., Nishimura, T., Nojiri, T. and Murano, T. (1976) *Biochim. Biophys. Acta* 426, 335–338
- 333 Pekarthy, J.M., Short, J., Lansing, A.I. and Lieberman, I. (1972) *J. Biol. Chem.* 242, 1767–1774
- 334 Henriksen, O., Robinson, E.A. and Appella, E. (1979) *J. Biol. Chem.* 256, 7651–7658
- 335 Leoni, S., Luly, P., Mangiantini, M.T., Spagnuolo, S., Trentalance, A. and Verna, R. (1975) *Biochim. Biophys. Acta* 394, 317–322
- 336 Grisham, J.W., Tillman, R.L., Nagel, A.E.H. and Compagno, J. (1975) in *Liver Regeneration after Experimental Injury* (Lesch, R. and Reutter, W., eds.), pp. 6–23, Stratton Intercontinental Medical Book Corp., New York
- 337 Sirica, A.E., Goldblatt, P.J. and McKelvy, J.F. (1977) *J. Biol. Chem.* 252, 5895–5899
- 338 Tauber, R. and Reutter, W. (1978) *FEBS Lett.* 87, 135–138
- 339 Tauber, R. and Reutter, W. (1978) *Eur. J. Biochem.* 83, 37–45
- 340 Emmelot, P. and Bos, C.J. (1969) *Int. J. Cancer* 4, 705–722
- 341 Emmelot, P. and Bos, C.J. (1969) *Int. J. Cancer* 4, 723–734
- 342 Emmelot, P. and Bos, C.J. (1972) *J. Membrane Biol.* 9, 83–104
- 343 Lacombe, M.L. and Hanoune, J. (1979) *J. Biol. Chem.* 254, 3697–3699
- 344 Stengel, D. and Hanoune, J. (1979) *Eur. J. Biochem.* 102, 21–34

- 345 Barclay, M. and Terebus-Kekish, O. (1973) *J. Natl. Cancer Inst.* 51, 1709–1710
- 346 Dau, P.C. (1978) *J. Natl. Cancer Inst.* 61, 935–937
- 347 Emmelot, P. and Bos, C.J. (1971) *Biochim. Biophys. Acta* 249, 285–292
- 348 Harms, E. and Reutter, W. (1974) *Cancer Res.* 34, 3165–3172
- 349 Vischer, P. and Reutter, W. (1978) *Eur. J. Biochem.* 84, 363–368
- 350 Kahn, C.R., Neville, D.M. and Roth, J. (1973) *J. Biol. Chem.* 248, 244–250
- 351 Doyle, D., Baumann, H., England, B., Friedman, B., Hou, E. and Tweto, J. (1978) *J. Biol. Chem.* 253, 965–973
- 352 Simon, F.R. and Arias, I.M. (1973) *J. Clin. Invest.* 52, 765–775
- 353 Sirica, A.E., Goldblatt, P.J. and McKelvy, J.F. (1975) *J. Biol. Chem.* 250, 6464–6468
- 354 Kahn, C.R., Neville, D.M., Gorden, P., Freychet, P. and Roth, J. (1972) *Biochem. Biophys. Res. Commun.* 48, 135–142
- 355 Soll, A.H., Kahn, C.R. and Neville, D.M. (1975) *J. Biol. Chem.* 250, 4702–4707
- 356 Soll, A.H., Kahn, C.R., Neville, D.M. and Roth, J. (1975) *J. Clin. Invest.* 56, 769–780
- 357 Davidson, M.B. and Kaplan, S.A. (1977) *J. Clin. Invest.* 59, 22–33
- 358 Vigneri, R., Pliam, N.B., Cohen, D.C., Pezzino, V., Wong, K.F. and Goldfine, I.D. (1978) *J. Biol. Chem.* 253, 8192–8197
- 359 Caron, M., Picard, J. and Kern, P. (1978) *Biochim. Biophys. Acta* 512, 29–40
- 360 Bergeron, J.J.M., Berridge, M.V. and Evans, W.H. (1975) *Biochim. Biophys. Acta* 407, 325–337
- 361 Pilgis, S.J., Exton, J.H., Johnson, R.A. and Park, C.R. (1974) *Biochim. Biophys. Acta* 343, 250–267
- 362 Soman, V. and Felig, P. (1978) *J. Clin. Invest.* 61, 552–560
- 363 Chandramouli, V., Williams, S., Marshall, J.S. and Carter, J.R. (1977) *Biochim. Biophys. Acta* 465, 19–33
- 364 Isutsu, K.T. and Smuckler, E.A. (1978) *Am. J. Pathol.* 90, 145–158
- 365 Le Page, R.N. and Dorling, P.R. (1971) *Aust. J. Exp. Biol. Med. Sci.* 49, 345–350
- 366 Dorling, P.R. and Le Page, R.N. (1972) *Biochem. Pharmacol.* 21, 2139–2141
- 367 Kamath, S.A. and Rubin, E. (1974) *Lab. Inv.* 30, 494–499
- 368 Feinglos, M.N. and Lebovitz, H.E. (1978) *Nature* 276, 184–185
- 369 Kamath, S.A. and Rubin, E. (1974) *Lab. Invest.* 30, 494–499
- 370 Farber, J.L., Martin, J.T. and Chien, K.R. (1978) *Am. J. Pathol.* 92, 713–732
- 371 Hegner, D., Lutz, F., Eckermann, V., Gries, J. and Schnorr, B. (1970) *Biochem. Pharmacol.* 19, 487–493
- 372 Wieland, T. and Faulstich, H. (1978) *CRC Crit. Rev. Biochem.* 5, 185–260
- 373 Chandrasekhara, N. and Narayan, K.A. (1970) *Cancer Res.* 30, 2876–2880
- 374 Low, I., Lengsfeld, A.M. and Wieland, T. (1974) *Histochemistry* 38, 253–258
- 375 Chandrasekhara, N. and Narayan, K.A. (1970) *J. Nutr.* 100, 477–480
- 376 Brivio-Haugland, R.P., Louis, S.L., Musch, K., Waldeck, N. and Williams, M.A. (1976) *Biochim. Biophys. Acta* 433, 150–163
- 377 Lopez-Saura, P., Trouet, A. and Tulkens, P. (1978) *Biochim. Biophys. Acta* 543, 430–449
- 378 Lentz, P.E. and DiLuzio, N.R. (1971) *Exp. Cell Res.* 67, 17–26
- 379 Solyom, A. and Trams, E.G. (1972) *Enzyme* 13, 329–372
- 380 Wootton, A.M., Neale, G. and Moss, D.W. (1977) *Clin. Sci. Mol. Med.* 52, 585–590
- 381 Selden, C., Wootton, A.M., Moss, D.W. and Peters, T.J. (1978) *Clin. Sci. Mol. Med.* 55, 423–427
- 382 Wisher, M.H. and Evans, W.H. (1977) *Biochem. J.* 164, 415–422
- 383 Victoria, E.J., van Golde, L.M.G., Hostetler, K.Y., Scherphof, G.L. and Van Deenan, L.L.M. (1971) *Biochim. Biophys. Acta* 239, 443–457
- 384 Van den Bosch, H. (1974) *Annu. Rev. Biochem.* 43, 243–277
- 385 Higgins, J.A. (1974) *J. Cell Biol.* 62, 635–646
- 386 Van Golde, L.M.G., Raben, J., Batenburg, J.J., Fleischer, B., Zambrano, F. and Fleischer, S. (1974) *Biochim. Biophys. Acta* 360, 179–192
- 387 Williamson, F.A. and Morré, D.J. (1976) *Biochem. Biophys. Res. Commun.* 68, 1201–1205
- 388 Polokoff, M.A., Coleman, R.A. and Bell, R.M. (1979) *J. Lipid Res.* 20, 17–21
- 389 Jelsema, C.L. and Morré, D.J. (1978) *J. Biol. Chem.* 253, 7960–7971
- 390 Franke, W.W., Morré, D.J., Deumling, B., Cheetham, R.D., Kartenbeck, J., Jarasch, E.D. and Zentgraf, H.W. (1971c) *Z. Naturforsch.* 26B, 1031–1039

- 391 Whaley, W.G. (1975) *The Golgi Apparatus*, Cell Biol. Monogr. 2, 1-190
- 392 Morré, D.J. (1977) in *Membrane Alterations as Basis of Liver Injury* (Popper, H., Bianchi, L. and Reutter, W., eds.), pp. 15-28, M.T.P. Press, Lancaster
- 393 Parry, G. (1978) *Subcell. Biochem.* 5, 261-326
- 394 Meldolesi, J., Borgese, N., De Camilli, P. and Ceccarelli, B. (1978) in *Cell Surface Reviews* (Poste, G. and Nicolson, G.L., eds.), Vol. 5, pp. 509-627, Elsevier/North-Holland, Amsterdam
- 395 Morré, D.J. (1977) in *Cell Surface Reviews* (Poste, G. and Nicolson, G.L., eds.), Vol. 4, pp. 1-83, Elsevier/North-Holland, Amsterdam
- 396 Peters, T., Fleischer, B. and Fleischer, S. (1971) *J. Biol. Chem.* 246, 240-244
- 397 Chan, K.M. and Neuhaus, O.W. (1978) *Biochim. Biophys. Acta* 521, 333-341
- 398 Ray, T.K., Lieberman, I. and Lansing, I. (1968) *Biochem. Biophys. Res. Commun.* 31, 54-59
- 399 Barancik, L.C. and Lieberman, I. (1971) *Biochem. Biophys. Res. Commun.* 44, 1084-1088
- 400 Evans, W.H. and Gurd, J.W. (1971) *Biochem. J.* 125, 615-624
- 401 Kawasaki, T. and Yamashina, I. (1971) *Biochim. Biophys. Acta* 225, 234-238
- 402 Coleman, R. and Bell, R.M. (1978) *J. Cell Biol.* 76, 245-253
- 403 Brophy, P.J., Burbach, P., Al Nelemens, S., Westerman, J., Wirtz, K.W.A. and Van Deenen, L.L.M. (1978) *Biochem. J.* 174, 413-420
- 404 Zilversmit, D.B. and Hughes, M.E. (1977) *Biochim. Biophys. Acta* 469, 99-110
- 405 Palade, G.F. (1978) in *Molecular Specialization and Symmetry in Membrane Function* (Solomon, A.K. and Karnovsky, M., eds.), pp. 3-30, Harvard University Press, Cambridge, MA
- 406 Wallach, D.F.H. (1977) in *Structural and Kinetic Approaches to Plasma Membrane Functions* (Nicolau, C. and Paraf, A., eds.), pp. 1-27, Springer-Verlag, Heidelberg
- 407 Nicolson, G.M. (1976) *Biochim. Biophys. Acta* 457, 57-108
- 408 Edidin, M. (1974) *Annu. Rev. Biophys. Bioeng.* 3, 179-201
- 409 Gordon, P., Carpenter, J.-L., Freychet, P., LeCam, A. and Orci, L. (1978) *Science* 200, 782-785
- 410 Evans, W.H. and Wisher, M.H. (1979) in *Cell Populations* (Reid, E., ed.), pp. 7-13, Ellis Horwood, Chichester
- 411 Larsen, W.J. and Tung, H.N. (1978) *Tissue Cell* 10, 585-598
- 412 Little, J.S. and Widnell, C.C. (1975) *Proc. Natl. Acad. Sci. U.S.A.* 72, 4013-4017
- 413 Bennett, G. and Leblond, C.P. (1977) *Histochem. J.* 9, 393-418
- 414 Elder, J.H. and Morré, D.J. (1976) *J. Biol. Chem.* 251, 5054-5068
- 415 Pisam, M. and Ripoche, P. (1976) *J. Cell Biol.* 71, 907-920
- 416 Abercrombie, M. (1961) *Exp. Cell Res. Suppl.* 8, 188-198
- 417 Kakis, G. and Yousef, I.M. (1978) *Can. J. Biochem.* 56, 287-290
- 418 Hinton, R.H. and Mullock, B.M. (1977) *Clin. Chim. Acta* 78, 159-162
- 419 Unwin, P.N.T. and Zampighi, G. (1980) *Nature* 283, 545-549
- 420 Holdsworth, G. and Coleman, R. (1975) *Biochem. Soc. Trans.* 3, 746-747
- 421 Olberg, A., Kjellen, L. and Hook, M. (1979) *J. Biol. Chem.* 254, 8505-8510
- 422 Novikoff, A.B. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 73, 2781-2787
- 423 Novikoff, P.M. and Yam, A. (1978) *J. Histochem. Cytochem.* 26, 1-13
- 424 Jaeken, L., Thines-Sempoux, D. and Verheyen, F. (1978) *Cell Biol. Int. Reports* 2, 501-515
- 425 Novikoff, A.B. and Shin, W.Y. (1978) *Proc. Natl. Acad. Sci. U.S.A.* 75, 5039-5042
- 426 Arias, I.M., Doyle, D. and Schimke, R.T. (1969) *J. Biol. Chem.* 244, 3303-3315
- 427 Reutter, W., Bauer, C., Bachmann, W. and Lesch, R. (1975) in *Liver Regeneration after Experimental Injury* (Lesch, R. and Reutter, W., eds.), pp. 259-272, Stratton Medical Book Corp., New York
- 428 Landry, J. and Marceau, N. (1975) *Biochim. Biophys. Acta* 389, 154-161
- 429 Gurd, J.W. and Evans, W.H. (1973) *Eur. J. Biochem.* 36, 273-279
- 430 Kawasaki, T. and Yamashina, I. (1971) *Biochim. Biophys. Acta* 225, 234-238
- 431 Reutter, W., Tauber, R., Vischer, P., Harms, E., Grunholz, H.-J. and Bauer, Ch. (1978) in *Protein Turnover and Lysosome Function* (Segal, H. and Doyle, D., eds.), pp. 779-790, Academic Press, New York
- 432 Steinman, R.M., Brodie, S.E. and Cohn, Z.A. (1976) *J. Cell Biol.* 68, 665-687
- 433 Tulkens, P., Schneider, Y.-J. and Trouet, A. (1977) *Biochem. Soc. Trans.* 5, 1809-1815
- 434 Stanley, K.K., Edwards, M.R. and Luzio, J.P. (1980) *Biochem. J.* 186, 59-69
- 435 Gorgio, N.A., Johnson, C.B. and Blecher, M. (1974) *J. Biol. Chem.* 249, 428-437
- 436 Farquhar, M.G. (1975) *J. Cell Biol.* 77, R35-R42

- 437 Herzog, V. and Farquhar, M. (1977) *Proc. Natl. Acad. Sci. U.S.A.* 74, 5073-5077
- 438 Desbuquois, B., Willeput, J. and Huet de Froberville, A. (1979) *FEBS Lett.* 106, 338-344
- 439 Pearce, B.M.F. (1975) *J. Mol. Biol.* 97, 93-98
- 440 Crowther, R.A., Finch, J.T. and Pearce, B.M.F. (1976) *J. Mol. Biol.* 103, 785-798
- 441 Woods, J.W., Woodward, M.P. and Roth, T.F. (1978) *J. Cell Sci.* 30, 87-97
- 442 Jacken, L. and Thines-Sempoux, D. (1978) *Cell Biol. Int. Rep.* 2, 515-524
- 443 Tate, S. and Meister, A. (1977) *Proc. Natl. Acad. Sci. U.S.A.* 74, 931-935
- 444 Carpentier, J.-L., Gorden, P., Barazzone, P., Freychet, P., LeCam, A. and Orci, L. (1979) *Proc. Natl. Acad. Sci. U.S.A.* 76, 2803-2807
- 445 Dnistrian, A.M., Skipski, V.P., Barclay, M. and Stock, C.C. (1977) *Cancer Res.* 37, 2182-2187
- 446 Dibona, D.R. and Mills, J.W. (1979) *Fed. Proc.* 38, 134-143
- 447 Postel-Vinay, M.C. (1976) *FEBS Lett.* 69, 137-140
- 448 Schramm, M. (1979) *Proc. Natl. Acad. Sci. U.S.A.* 76, 1174-1178
- 449 Van Hoeven, R.P., Van Blitterswijk, W.J. and Emmelot, P. (1979) *Biochim. Biophys. Acta* 551, 44-54
- 450 Pascaud, A., Auliac, P. and Pascaud, P. (1980) *Biochimie* 61, 1065-1071
- 451 Tanabe, T., Pricer, W.E. and Ashwell, G. (1979) *J. Biol. Chem.* 254, 1038-1043
- 452 Fraser, I.H. and Mookerjee, S. (1976) *Biochem. J.* 156, 347-355
- 453 Flodgaard, H. and Torp-Pederson, C. (1978) *Biochem. J.* 171, 817-820
- 454 Hostetler, K.Y. and Yazaki, P.J. (1979) *J. Lipid Res.* 20, 456-471
- 455 Gharbi, J. and Torresani, J. (1979) *Biochem. Biophys. Res. Commun.* 88, 170-177
- 456 Mourelle, M. and Rubalcava, B. (1979) *Biochim. Biophys. Res. Commun.* 88, 189-198
- 457 Wood, C.L., Caron, M.G. and Lefkowitz, R.J. (1979) *Biochem. Biophys. Res. Commun.* 88, 1-8
- 458 Goldenberg, H., Crane, F.L. and Morre, D.L. (1979) *J. Biol. Chem.* 254, 2491-2498
- 459 Kimura, N. and Nagata, N. (1979) *J. Biol. Chem.* 254, 3451-3457
- 460 Schlesinger, P.H., Doebber, T.W., Mandell, B.F., White, R., De Schryver, C., Rodman, J.S., Miller, M.J. and Stahl, P. (1979) *Biochem. J.* 176, 103-109
- 461 Jones, A.L., Smuckler, D.L., Mooney, J.S., Ockner, R.K. and Adler, R.D. (1979) *Lab. Invest.* 40, 512-517
- 462 Orlans, E., Peppard, J., Reynolds, J. and Hall, J. (1978) *J. Exp. Med.* 147, 588-592
- 463 Lemaitre-Coelho, I., Jackson, G.D.F. and Vaerman, J.P. (1977) *Eur. J. Immunol.* 8, 588-590
- 464 Birbeck, M.S.C., Cartwright, P., Hall, G., Orlans, E. and Peppard, J. (1980) *Immunology* 37, 477-484
- 465 Mullock, B.M., Hinton, R.H., Dobrota, M., Peppard, J. and Orlans, E. (1979) *Biochim. Biophys. Acta* 587, 381-391
- 466 Henderson, J.R. (1974) *Physiol. Rev.* 54, 1-22
- 467 Goldstein, J.L., Anderson, R.G.W. and Brown, M.S. (1979) *Nature* 279, 679-685
- 468 Wolkoff, A.W. and Chung, C. (1978) *Gastroenterology* 75, 995
- 469 Craig, S. and Pardo, J.V. (1979) *J. Cell Biol.* 80, 203-210
- 470 Bernaert, D., Wanson, J.C., Mosselmans, R., de Paermentier, F. and Drochmans, P. (1979) *Biol. Cell.* 34, 159-174
- 471 Martin, B.R., Houslay, M.D. and Kennedy, E.L. (1977) *Biochem. J.* 161, 639-642
- 472 Aronson, N.N., Tan, L.Y. and Peters, B.P. (1973) *Biochem. Biophys. Res. Commun.* 53, 112-118
- 473 Roseman, S. (1970) *Chem. Phys. Lipids* 5, 270-297
- 474 Roth, S. (1973) *Q. Rev. Rev. Biol.* 48, 541-563
- 475 Tiribelli, C., Lunazzi, G., Luciani, M., Panfili, E., Gazzin, B., Liut, G., Sandri, G. and Sottocasa, G. (1978) *Biochim. Biophys. Acta* 532, 105-112
- 476 Pietras, R.J., Hutchens, T.W. and Szego, C.M. (1978) *Endocrinology (Suppl.)* 102, 76
- 477 Gonzalez, M.C., Sutherland, E. and Simon, F.R. (1979) *J. Clin. Invest.* 63, 684-694
- 478 Silverstein, M. and Richards, J.F. (1979) *Biochem. J.* 178, 743-751
- 479 Poupon, R. and Evans, W.H. (1979) *FEBS Lett.* 108, 374-378
- 480 Schachter, H. (1978) in *The Glycoconjugates* (Horowitz, M.J. and Pigman, W., eds.), pp. 87-181, Academic Press, New York
- 481 Bachmann, W. and Reutter, W. (1979) *Hoppe-Seyler's Z. Physiol. Chem.* 360, 81-87
- 482 Baumann, H. and Doyle, D. (1979) *J. Biol. Chem.* 254, 3935-3946
- 483 Obrink, B. and Ocklind, C. (1978) *Biochem. Biophys. Res. Commun.* 85, 837-843
- 484 Pilam, N.B. and Goldfine, I.D. (1977) *Biochem. Biophys. Res. Commun.* 79, 166-172
- 485 Torp-Pederson, C., Flodgaard, H. and Saermark, T. (1979) *Biochim. Biophys. Acta* 571, 94-104