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STUDIES ON PLASMA MEMBRANES

XII. FRACTIONATION OF THE ATPase OF DEOXYCHOLATE-SOLUBILIZED RAT LIVER AND HEPATOMA PLASMA MEMBRANES AND THE MORPHOLOGICAL APPEARANCE OF THE PREPARATIONS

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SUMMARY

1. Plasma membranes, isolated from rat liver and rat hepatoma-484, were solubilized with sodium deoxycholate, fractionated by differential and density gradient centrifugation and subjected to enzyme assay and electron microscopy.

2. A liver membrane fraction was obtained of buoyant density *d* 1.10–1.12, showing a specific ATPase (EC 3.6.1.3) activity of about 1 mmole P_i released per mg protein per h. This fraction contained 1 % of the protein, 7 % of the phospholipid-P and 15 % of the total ATPase activity of the liver plasma membranes.

3. On account of the electron microscopic observations, the conclusion was drawn that the solubilized liver membrane enzyme was present in a non-preexistent particle of myelin-like appearance, rich in phospholipids.

4. Tight junctions (*zonulae occludentes*) were present in the most readily sedimenting fraction of the solubilized liver membranes following differential centrifugation and in a *d* 1.20 bottom pellet following gradient centrifugation.

5. Results on the hepatoma plasma membranes differed markedly from those obtained with normal liver membranes, attesting to basic differences in organization between these normal and homologous neoplastic membranes.

INTRODUCTION

Solubilization of rat liver plasma membranes using sodium deoxycholate (1 %) and by centrifugation at 105000 × *g* yielded a pellet in which tight junctions (*zonulae occludentes*) were present next to other unidentified material[1]. Since the pellet has been found[2] to contain appreciable ATPase activity, whereas the (Na^+-K^+)-ATPase was virtually lost by this procedure[3], an attempt has been made to fractionate the sedimentable material by differential and density gradient centrifugation. The present paper reports the results of these experiments on rat liver and hepatoma-484 plasma membranes and seeks to correlate the ATPase activity of the liver preparations with a particle of distinct electron microscopic appearance present in these preparations. Preliminary results have been communicated[4].

MATERIALS AND METHODS

Plasma membranes were isolated[5,6] from livers of rats of the inbred strain R-Amsterdam and transplants of the hepatocellular rat hepatoma-484 (ref. 6) induced and maintained in the same strain, using 1 mM $NaHCO_3$ (and 2 mM $CaCl_2$ in the case of the hepatoma) as homogenization medium.

Membranes were solubilized by being kept for 15 min at 22° in a final concentration of 1 % sodium deoxycholate to yield 1.5–2.3 mg membrane protein per ml. The solubilized membranes were chilled and subjected to 3 successive centrifugations in a Spinco SW 39 or 50 rotor varying speed and/or duration as indicated in the text. The resulting three pellets were washed with 1 mM $NaHCO_3$, resedimented and used for the analysis of protein[7], phospholipid-P[8] and enzyme assay[5]. The final supernatant was dialyzed against 3 × 500 ml 1 mM $NaHCO_3$ for 28–40 h at 5°.

The pellet (containing about 1 mg membrane protein), obtained from the deoxycholate-solubilized membranes by centrifugation for 2 h at 105000 × *g*, was washed with 1 mM $NaHCO_3$, resedimented and suspended in a total volume of 1.4 ml of sucrose–water of *d* 1.18. This material was included in a discontinuous sucrose gradient of *d* 1.10, 1.12, 1.14, 1.16 (all consisting of 0.8 ml) and 1.20 (0.4 ml). Centrifugation was carried out for 60 min at 35000 rev./min in a Spinco SW 50 rotor; prolonged centrifugation did not improve the resolution of ATPase activity. Most of the material gathered at the interfaces. The layers were separated at half their height starting with the *d* 1.12 layer in the case of liver. In the case of hepatoma the material floating at the *d* 1.10 surface and the greater part of the *d* 1.18 layer were obtained separately. The various fractions were diluted with 13 ml 1 mM $NaHCO_3$, washed and resedimented in the diluted bicarbonate (60 min, 105000 × *g*).

Electron microscopy

Pellets of the fractions were fixed overnight with 3 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.3), rinsed with the same buffer and postfixed during 1 h in a 1 % phosphate buffered OsO_4 solution. After dehydration in a graded concentration of acetone, the pellets were embedded in Vestopal W. Only a few embeddings could be made per pellet due to the small size of the pellets. Sections of at least two blocks per pellet were observed. The thin sections were stained during 2 h with uranyl acetate. For negative staining the pellets were suspended and diluted in a 0.1 % ammonium acetate solution and stained on the grid with 1 % sodium phosphotungstate (pH 7.2).

The observations were made with a Philips EM-200 electron microscope operating at 80 kV using the double-condensor system (spot size about 15 μ) and the anticontamination device.

RESULTS

Liver plasma membranes

Membranes were solubilized with 1 % sodium deoxycholate and subjected to three successive differential centrifugations varying speed and/or duration. Three pellets were thus obtained: Fraction 1 spun down at 25000 × *g* for 15 min, followed by Fraction 2 at 105000 × *g* for 15 min and Fraction 3 at 105000 × *g* for 2 h. The final supernatant was dialyzed and also studied.

Protein and phospholipid-P contents of membrane fractions obtained by differential centrifugation

Table I lists the percentages of protein and phospholipid-P in Fractions 1–3 and the supernatant fraction. The sedimentable material present in Fractions 1–3 contained 28.3 ± 2.5 % of the protein and 23.0 ± 3.1 % of the phospholipid-P of the membranes. However, the protein/phospholipid-P ratios of the various fractions differed markedly, the relative phospholipid content decreasing in order: Fraction 2 > supernatant > Fraction 3 > Fraction 1. As compared with the unit composition of intact membranes, Fraction 1 was very much enriched in protein, and Fraction 2 was moderately enriched in phospholipid.

TABLE I

PROTEIN AND PHOSPHOLIPID-P CONTENT OF FRACTIONS OBTAINED BY DIFFERENTIAL CENTRIFUGATION FROM DEOXYCHOLATE-SOLUBILIZED LIVER AND HEPATOMA PLASMA MEMBRANES

Centrifugations were carried out as described in the text. Mean with standard deviation of 4 experiments.

Fractions	*Protein*		*Phospholipid-P*	
Liver				
Fraction 1	12.1 ± 2.0	28.3 ± 2.5	5.0 ± 1.2	23.0 ± 3.1
Fraction 2	8.4 ± 1.0		10.5 ± 1.0	
Fraction 3	7.8 ± 0.3		7.5 ± 0.3	
Supernatant	71.7 ± 2.4		77.0 ± 3.1	
Hepatoma				
Fraction 1	33.8 ± 6.0	49.1 ± 3.0	10.2 ± 2.2	26.7 ± 3.7
Fraction 2	7.6 ± 1.9		12.3 ± 2.8	
Fraction 3	7.7 ± 1.1		4.2 ± 1.1	
Supernatant	50.9 ± 3.3		73.3 ± 3.6	

ATPase, (Na^+-K^+)-ATPase and 5'-nucleotidase activities

The specific enzyme activities of the various fractions and the percentage recovery of the enzyme activities are illustrated in Table II for typical experiments. Under the present conditions only some 10–15 % of the (Na^+-K^+)-ATPase survived, mostly in Fraction 2 which exhibited also the highest specific activity of this enzyme. By contrast, more than 100% of the 5'-nucleotidase activity (EC 3.1.3.5, measured on 5'-AMP) was recovered (*cf.* ref. 9), the bulk of the latter activity residing in the supernatant fraction, whereas the highest specific nucleotidase activity was demonstrated by Fraction 2. Some 55 % of the ATPase activity of intact membranes was recovered in the four fractions obtained by differential centrifugation. Fraction 2 contained 30 % of the membrane ATPase and about 9 % of the membrane protein. The specific ATPase activity of the latter fraction was 3–4-fold that of intact membranes. Inspection of Tables I and II shows that among the four fractions Fraction 2 contained the highest amount of phospholipid per unit weight of protein and the highest specific activity and amount of ATPase.

Density gradient centrifugation

Membranes were solubilized with deoxycholate and centrifuged for 2 h at 105000 × *g*, the resulting pellet thus containing the material of Fractions 1–3. This

TABLE II

SPECIFIC ACTIVITY AND RECOVERY OF ATPase, (Na^+-K^+)-ATPase AND 5′-NUCLEOTIDASE IN FRACTIONS OBTAINED FROM DEOXYCHOLATE-SOLUBILIZED LIVER PLASMA MEMBRANES BY DIFFERENTIAL CENTRIFUGATION

Enzyme source	*μmoles P_i/mg protein per h*				*% Recovery*					
	ATPase		(Na^+-K^+)-*ATPase*	*Nucleotidase*	*Protein*		*ATPase*		(Na^+-K^+)-*ATPase*	*Nucleotidase*
	Expt. 1	*Expt. 2*	*Expt. 1*	*Expt. 2*	*Expt. 1*	*Expt. 2*	*Expt. 1*	*Expt. 2*	*Expt. 1*	*Expt. 2*
Intact membranes	67.1	47.3	19.3	53.4	(100)		(100)		(100)	(100)
Fraction 1	53.7	53.6	0.3	70.2	13.9	8.9	11.2	9.9	0.2	11.3
Fraction 2	197.1	176.1	22.7	158.2	10.0	8.1	29.4	30.0	11.8	23.8
Fraction 3	41.9	42.7	1.0	104.3	8.1	7.9	5.0	7.1	0.4	15.4
Supernatant	7.1	5.9	0.0	51.6	68.0	75.1	7.2	9.3	0.0	72.2
Sum					(100)		52.8	56.3	12.4	122.7

TABLE III

ATPase, PROTEIN AND PHOSPHOLIPID-P IN FRACTIONS OBTAINED BY DENSITY GRADIENT CENTRIFUGATION OF THE SEDIMENTABLE MATERIAL FROM DEOXYCHOLATE-SOLUBILIZED LIVER PLASMA MEMBRANES

Material gathered at the surface of the gradient (*d* 1.10 ↑) combined with that at *d* 1.10/1.12. Bottom pellet designated as *d* 1.20 ↓.

Fraction (d)	*ATPase*			*Protein*	*Phospho-lipid-P*	*Protein/phospho-lipid-P*
	μmoles P_i/mg protein per h	% *	*μmoles P_i/μmole phospho-lipid-P per h*	(%) *	(%) *	*(mg/μmole)*
1.10 ↑ / 1.10/1.12	920.1	31.5	285.2	4.1	30.3	0.31
1.12/1.14	312.2	22.6	424.6	8.7	15.1	1.36
1.14/1.16	149.9	21.8	289.3	17.6	20.9	1.93
1.16/1.18	85.6	14.1	191.8	20.0	20.1	2.24
1.18/1.20	31.8	5.4	330.7	12.4	2.7	10.4
1.20 ↓	13.2	4.6	104.4	37.2	10.9	7.91

* Sum fractions = 100.

material was subjected to centrifugation in a discontinuous sucrose gradient of *d* 1.10–*d* 1.20, as described under MATERIALS AND METHODS. The separate layers were assayed for protein, phospholipid-P and ATPase activity (Table III). The ATPase activity of the unfractionated pellet amounted to 104.5 μmoles P_i released per mg protein per h, and the recovery of the ATPase activity, calculated from the sum contents of the six fractions obtained by the gradient centrifugation, amounted to 114 %. The protein contents and the protein to phospholipid-P ratios increased regularly from top (*d* 1.10 fraction) to bottom (*d* 1.20 pellet fraction) of the gradient, whereas the ATPase activity per mg protein decreased in the same order, showing a 70-fold difference between the fractions of highest and lowest buoyant density. The latter fraction gathering on the surface of the *d* 1.10 layer and at the *d* 1.10/1.12 interface (taken together to obtain sufficient material for analysis), contained about 4 % of the protein and 30 % of the phospholipid-P of the six fractions. This top fraction showed the highest specific ATPase activity, *i.e.* on the order of 1 mmole P_i released per mg protein per h, and contained the greatest amount of ATPase activity (31.5 %) among the six fractions. On account of the data of Tables I–III, it was calculated that the low-density top fraction contained about 1 % of the protein, 7 % of the phospholipid-P and 15 % of the total ATPase activity of intact liver membranes.

Rat hepatoma plasma membranes

Protein and phospholipid-P content of fractions obtained by differential centrifugation

Similar experiments were carried out with plasma membranes isolated from the transplanted rat hepatoma-484. The percentages of protein and phospholipid-P in Fractions 1–3 and the supernatant fraction following the three successive differential centrifugations of the deoxycholate-solubilized hepatoma membranes are listed in Table IV. As compared with liver, one striking feature emerges, namely, the much greater amount of sedimentable protein (some 20 % more, limited to Fraction 1) of the hepatoma membrane preparations. A similar high increase of sedimentable phospholipid-P was not observed.

When hepatoma-484 membranes are solubilized by 1 % sodium deoxycholate, the resulting preparation shows a cloudy appearance, which can be cleared by ex-

TABLE IV

SPECIFIC ACTIVITY AND RECOVERY OF ATPase, $(Na^+\text{-}K^+)$-ATPase AND 5′-NUCLEOTIDASE IN FRACTIONS OBTAINED FROM DEOXYCHOLATE-SOLUBILIZED HEPATOMA PLASMA MEMBRANES

Enzyme source	*μmoles P_i/mg protein per h*			*% Recovery*			
	ATPase	*$(Na^+\text{-}K^+)$-ATPase*	*Nucleotidase*	*Protein*	*ATPase*	*$(Na^+\text{-}K^+)$-ATPase*	*Nucleotidase*
Intact membranes	8.1	21.5	40.2	(100)	(100)	(100)	(100)
Fraction 1	1.1	0.0	52.0	38.5	5.2	0.0	49.8
Fraction 2	0.5	0.0	149.6	6.4	0.4	0.0	23.8
Fraction 3	0.0	0.0	70.4	5.0	0.0	0.0	8.8
Supernatant	6.1	0.0	19.1	50.1	37.7	0.0	23.8
Sum				(100)	43.3	0.0	106.2

traction with light petroleum (b.p. 40–60°). The extracted material contains triglycerides as shown by thin-layer chromatography (our unpublished observations). Also Fraction 1 contained these lipids, which had to be removed before the protein content could be measured properly. What appear to be lipid droplets have also been detected in this fraction by electron microscopy (compare below). These findings suggest that the triglycerides are in some way associated with the hepatoma plasma membranes and might not merely contaminate these preparations. No difference in the sialic acid content[5] of Fraction 1 and the supernatant fraction (concentrated by ultradialysis) could be demonstrated, both fractions containing 31 ± 0.5 nmoles sialic acid per mg protein.

TABLE V

ATPase, PROTEIN AND PHOSPHOLIPID-P IN FRACTIONS OBTAINED BY DENSITY GRADIENT CENTRIFUGATION OF THE SEDIMENTABLE MATERIAL FROM DEOXYCHOLATE-SOLUBILIZED HEPATOMA-484 PLASMA MEMBRANES

Fraction (*d*)	*ATPase*			*Protein* (%)*	*Phospholipid-P* (%)*	*Protein/phospholipid-P* (*mg/μmole*)
	μmoles P_i/mg protein per h	%*	*μmoles P_i/μmole phospholipid-P per h*			
1.10 ↑	0.0	0.0	0.0	3.5	17.6	0.36
1.10/1.12	0.0	0.0	0.0	3.6	13.2	0.50
1.12/1.14	0.0	0.0	0.0	11.0	21.6	0.93
1.14/1.16	0.0	0.0	0.0	8.6	15.0	1.04
1.16/1.18	5.3	42.3	13.7	23.1	16.4	2.58
1.18	3.9	14.9	20.2	10.9	3.6	5.17
1.18/1.20	3.3	42.8	18.2	37.2	12.6	5.50
1.20 ↓	0.0	0.0	0.0	2.1	0.0	

* Sum fractions = 100.

Enzyme activities

As shown in Table IV, the results of the nucleotidase measurements of the hepatoma membrane fractions obtained by differential centrifugation resembled those of liver with respect to total recovery and the occurrence of the highest specific activity in Fraction 2. However, differences were apparent in that (a) the specific nucleotidase activity of the hepatoma supernatant fraction (19.1–21.3 μmoles P_i released) was much lower than that of the corresponding liver fraction (45–55.1 μmoles) and (b) the greatest amount of the nucleotidase activity was recovered in hepatoma Fraction 1 and not in the supernatant fraction as was the case with liver. No (Na^+-K^+)-ATPase activity could be demonstrated in any of the hepatoma fractions.

The hepatoma membranes have previously been shown[6,10] to exhibit a much lower specific ATPase activity than the liver membranes. In the present experiments, the pattern of ATPase activities among the four hepatoma fractions was completely different from that of liver. The hepatoma ATPase activity appeared to reside mainly in the supernatant fraction, whereas Fractions 1 and 2 exhibited only minute activity and Fraction 3 was devoid of ATPase activity. Thus, whereas the overall loss of ATPase activity resulting from detergent treatment was but little more pronounced for the hepatoma and for the liver membranes, the bulk of the surviving ATPase

activity of the solubilized hepatoma membranes was shifted to nonsedimentable material as compared with liver. By contrast, the small amount of sedimentable hepatoma ATPase was almost exclusively present in Fraction 1 and thus appeared to be more readily sedimentable than was the corresponding liver enzyme activity which was concentrated in Fraction 2. The latter shift was also apparent from the results following gradient centrifugation of the sedimentable hepatoma material (Table V). The sedimentable hepatoma ATPase now concentrated in fractions of higher buoyant densities (*d* 1.16–*d* 1.20) than in the case of liver.

In the typical experiment illustrated in Table V, the untreated hepatoma membranes released 11 μmoles P_i from ATP per mg protein per h, whereas the sedimented material (2 h at 105000 $\times$ *g*), obtained from these deoxycholate-solubilized membranes and used for gradient centrifugation, released only 2.4 μmoles. After centrifugation 119% of the sedimentable ATPase activity was recovered in fractions *d* 1.16–*d* 1.20. From the very small specific ATPase activities exhibited by these fractions it follows that the present method did not fractionate the hepatoma membrane ATPase which otherwise was converted into a soluble form by the detergent treatment. This situation was markedly different from that encountered with the liver membrane enzyme.

Electron microscopic observations

Liver membrane fractions obtained by differential centrifugation

Fractions 1–3 were morphologically distinguishable by the predominance in each fraction of a certain characteristic structure (Figs. 1–5).

Fraction 1, containing the most readily sedimentable material, was composed of a few membrane remnants, some irregular vesicles (thin and thick walled), tight junctions and, as background, a rather structureless flocculent and sometimes filamentous material (Fig. 1). Only the tight junctions, which characterize this fraction, had preserved a fine structure which allowed their identification[11]. The penta-layered arrangement was clearly discernable, and in negatively stained preparations (Fig. 1,

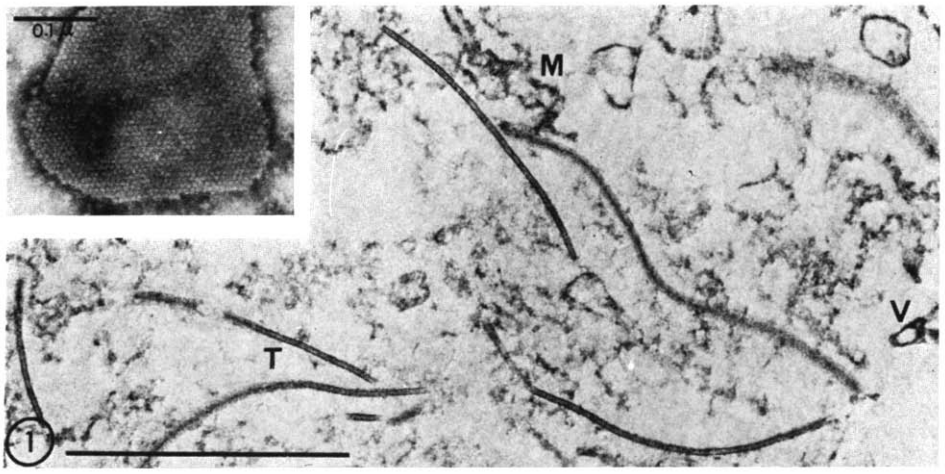


Fig. 1. Fraction 1 contains many tight junctions (T), some membrane remnants (M), irregular vesicles (V) and a flocculent material. $\times$ 37500. Insert: negatively stained membrane sheet with a hexagonal pattern. $\times$ 110000. (Unless otherwise indicated, the bars represent 1 μ.)

insert) membrane sheets with a hexagonal array of subunits, typical of tight junctions[1], were present. The edges of these sheets were not smooth but gave the impression that rows of subunits were in the process of detachment. In thin sections tight junctions of very short extension and occasionally desmosomal fragments in the process of breakdown have also been observed.

In Fraction 2 no membrane fragments or tight junctions were present. The characteristic component (Fig. 2) consisted of irregular vesicles, some thin walled and many thick walled, against a background of the flocculent material. The vesicles had a mean diameter of about 150 nm. In sections at high magnification (Fig. 4) the thick-walled vesicles appeared to be built up with concentric lamellae with a center-to-center distance of about 50 Å. This arrangement was particularly obvious in negatively stained preparations (Fig. 3); here the center-to-center distance of the lamellae amounted to 75 Å. Most of these vesicles were locally very sodium phosphotungstate negative attesting to their hydrophobic nature.

Fraction 3 (Fig. 5) consisted nearly exclusively of the flocculent material encountered in the former fractions. Only some vesicles, generally smaller than those predominant in Fraction 2, were observed.

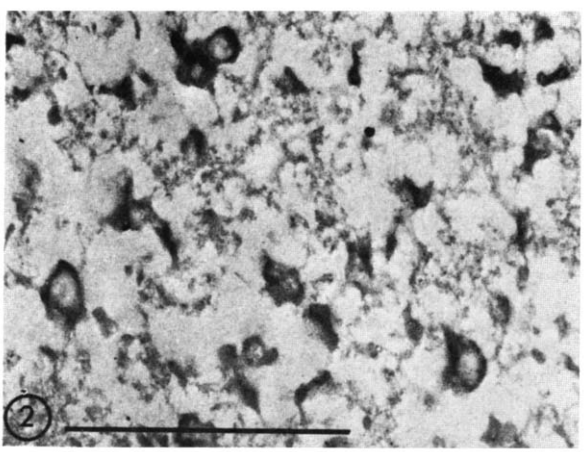
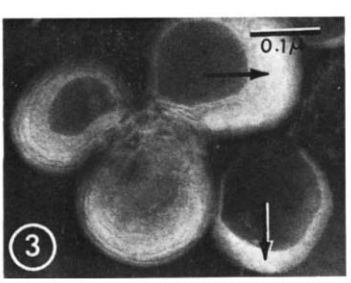
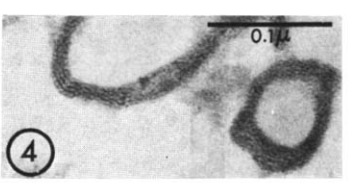


Fig. 2. Fraction 2 is characterized by a predominance of irregular vesicles; the flocculent material is also present. × 37500.





Figs. 3 and 4. After negative staining (Fig. 3, × 91500) and in thin sections (Fig. 4, × 165000), the vesicles of Fraction 2 appear to be built up with concentric lamellae. Locally, the negatively stained material is very sodium phosphotungstate negative (Fig. 3, arrows).

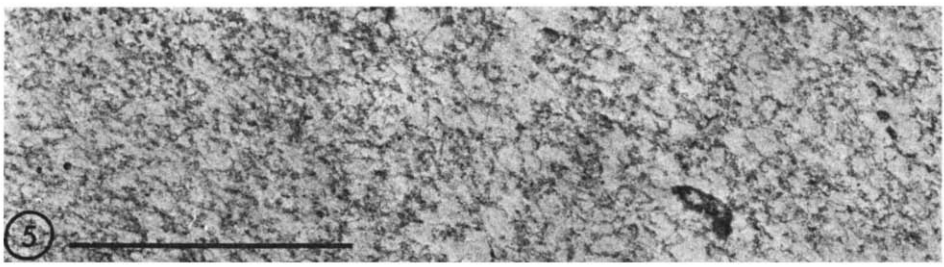


Fig. 5. The flocculent material is the main component of Fraction 3. × 37500.

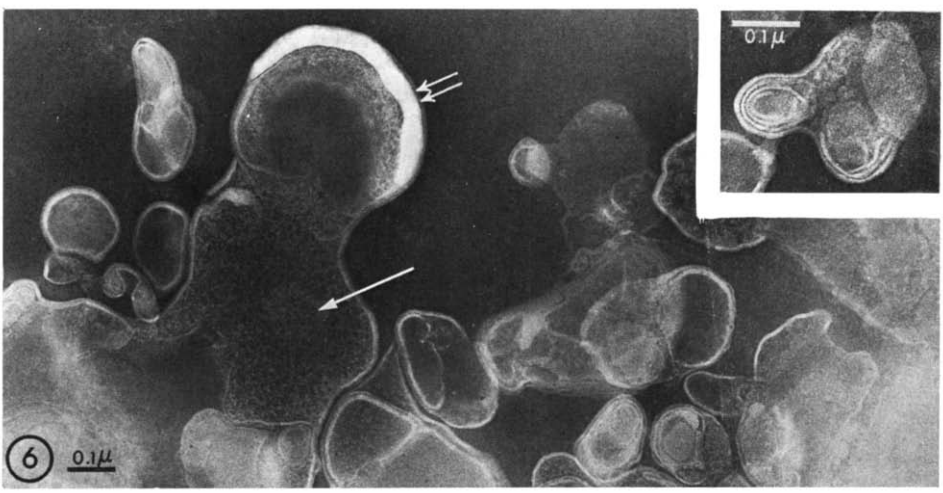


Fig. 6. Surface layer of *d* 1.10 negatively stained. Spherical and sheet-like bodies have a smooth surface and are built up with several parallel or concentric lamellae (see insert, × 91500). Locally, the structures seemed to be filled with the flocculent material (arrow). Highly sodium phosphotungstate-negative areas are present (double arrow). × 61000.

Fractions obtained by gradient centrifugation of the combined liver membrane Fractions 1–3

Of the fractions isolated in this manner, the surface layer of *d* 1.10 was used for negative staining, whereas the material gathered at the *d* 1.12/1.14 interface and the *d* 1.20 bottom pellet were embedded and studied in thin sections (Figs. 6–8).

The low-density top layer consisted exclusively of sheet-like and spherical bodies with a smooth surface, being locally strongly sodium phosphotungstate negative (Fig. 6). Both the sheets and vesicles were built up with several parallel or concentric lamellae with a center-to-center distance of 70–75 Å, resembling the vesicles encountered mostly in Fraction 2 obtained by differential centrifugation. Part(s) of these structures seemed to be filled with the flocculent material observed previously in Fractions 1–3. The material of the *d* 1.12/1.14 interface (Fig. 7) also contained the flocculent material, mixed with intact or broken vesicles of a mean diameter of 130 nm, exhibiting sometimes (Fig. 7, insert) a double- or multi-lamellar structure (center-to-center: 55–60 Å). Only very occasionally was a structure resembling a tight junction observed.

Finally, in the *d* 1.20 bottom pellet densely packed groups of large amounts of

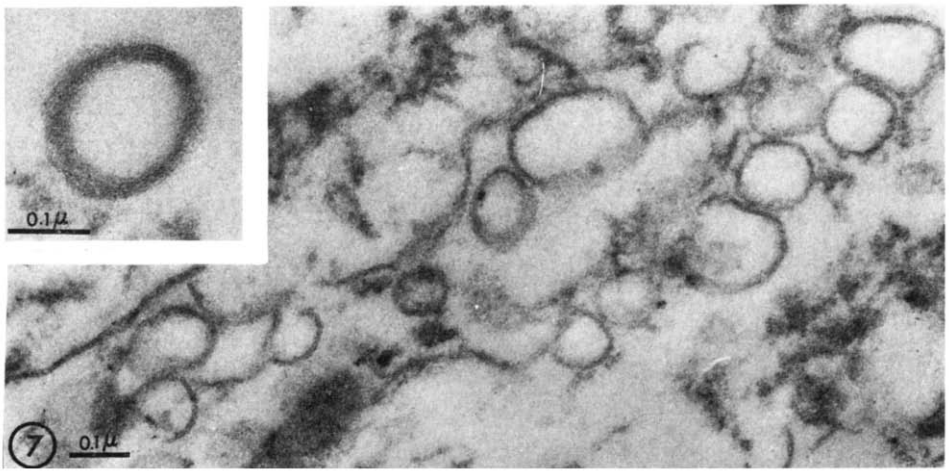


Fig. 7. Thin section of *d* 1.12/1.14 interface containing double- or multi-layered vesicles (see insert) together with flocculent material. × 76250. Insert, × 106750.

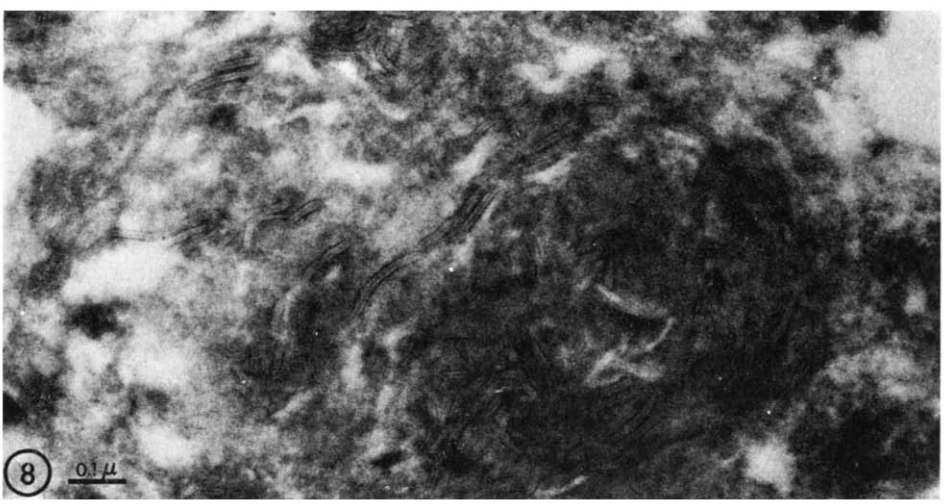


Fig. 8. A group of densely packed tight junctions in the *d* 1.20 bottom pellet, surrounded by rather amorphous material. × 76250.

tight junctions were present (Fig. 8). These aggregates were lying in a background of clumped amorphous material, which might represent the flocculent material, which also contained a few vesicles of the type described.

Hepatoma membrane fractions obtained by differential centrifugation

Fraction 1 (Fig. 9) contained definitely more membrane remnants than in the case of liver, some irregular vesicles and the flocculent background material. Some very electron-dense bodies, lacking inner structure and very probably representing fat droplets, were also present (not illustrated). Since the hepatoma membranes are markedly deficient in tight junctions[12], it was not surprising to find that Fraction 1 contained only very few (Fig. 10) if any tight junctions (*cf.* ref. 1). Characteristic of

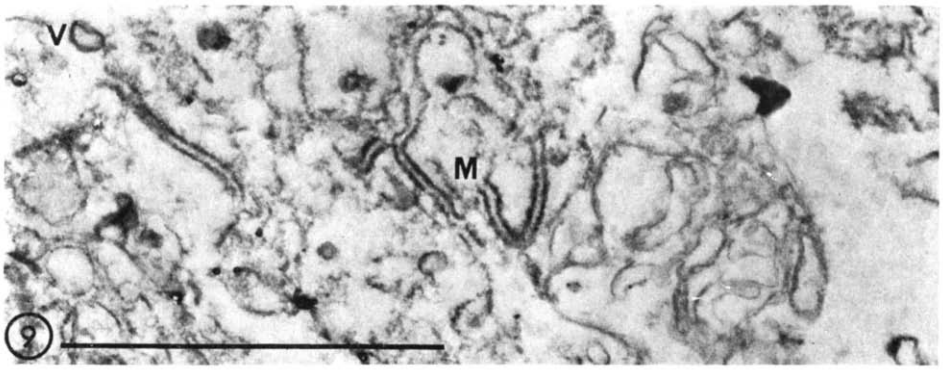


Fig. 9. Thin section of Fraction 1 shows the presence of many membrane remnants often in parallel arrangements (M), some vesicles (V) and filamentous material. × 51000.

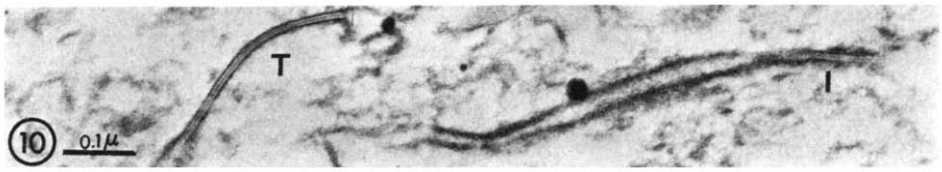


Fig. 10. One of the few tight junctions (T) in Fraction 1, and membranes forming an intermediate junction-like structure (I). × 91500.

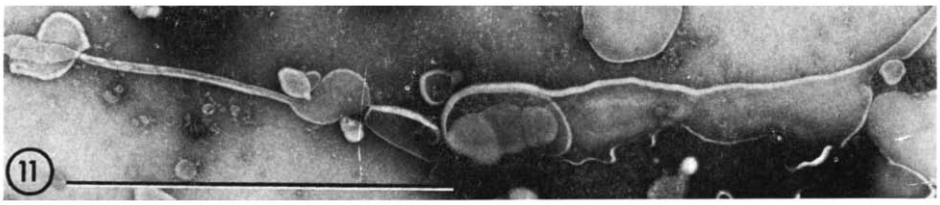


Fig. 11. Negatively stained preparation of Fraction 1 containing smooth sheets interconnected by narrow tubular structures. × 51000.

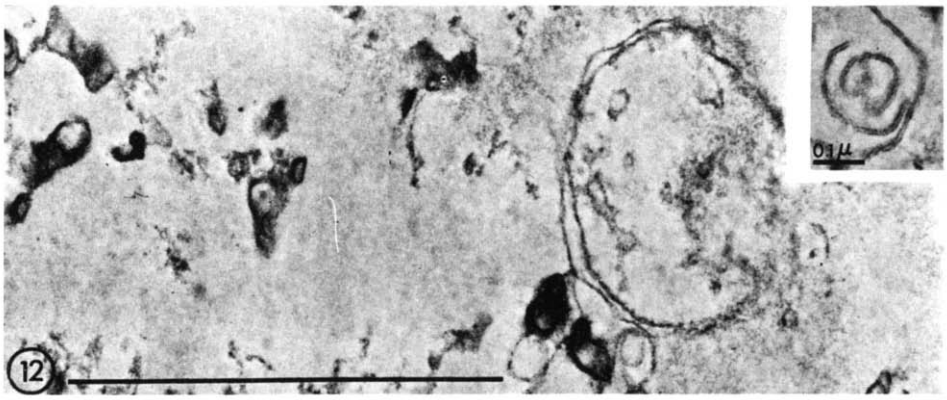


Fig. 12. In Fraction 2 membrane remnants, vesicles and flocculent material are present. × 57500 Insert (× 69000) shows triple-layered structure.

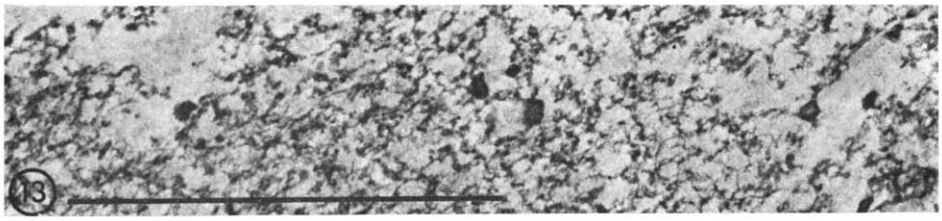


Fig. 13. Fraction 3 contains predominantly flocculent material fully comparable to that of Fraction 3 of normal liver. × 57500.

hepatoma was the presence in Fraction 1 of apposed membrane fragments, *i.e.* short membrane segments arranged in pairs, parallel to each other. Their surfaces were not unfrequently covered by some electron-dense material. Locally, a thin electron-dense inner layer (Fig. 10) suggested that some of these membrane pairs represented remnants of an intermediate junction-like structure[11]. Negative staining demonstrated (Fig. 11) smooth sheets with tubular-like interconnections (not seen in the case of liver). Only once did a membraneous sheet exhibit a hexagonal array of subunits (tight junction).

Fraction 2 differed from that of normal liver in the greater amount of membrane remnants and the presence of some fat droplets (Fig. 12). As in the previous fraction, the membranes were not infrequently oriented in parallel pairs, but now they were mostly shorter. Also here could the triple-layered membrane structure be observed (Fig. 12, insert). The few vesicles and background material had the same appearance as in Fraction 2 of normal liver.

Fraction 3 (Fig. 13) closely resembled the corresponding one of normal liver in appearance.

DISCUSSION

Table VI schematically summarizes the distribution of the three main morphological components encountered in the liver plasma membrane fractions and the latter's ATPase activities. The ATPase activity appears to correlate with the multilamellar vesicles or bodies preponderant in Fraction 2 obtained by differential cen-

TABLE VI

SCHEMATIC SUMMARY OF THE MORPHOLOGY AND ATPase ACTIVITIES OF FRACTIONS OBTAINED FROM DEOXYCHOLATE-SOLUBILIZED LIVER PLASMA MEMBRANES BY DIFFERENTIAL AND GRADIENT CENTRIFUGATION

Fraction	*Morphological entities*			*ATPase*
	Flocculent material	*Tight junctions*	*Vesicles*	
1	+	+	±	+
2	±	−	+++	+++
3	±	−	+	+
d 1.10 top	−	−	+++	+++
d 1.12/1.14	+	−	+	+
d 1.20 pellet	+	+	(±)	(±)

trifugation and in the low-density top fraction following gradient centrifugation. Both fractions are enriched in phospholipids, and the myelin-like structure of the vesicles resembles that exhibited by phospholipid (spherulites)[13,14] and phospholipid–protein complexes[15]. The multilamellar vesicles are apparently breakdown products of the membranes resulting from the action of deoxycholate. Not being preexistent structures, they must have been formed by rearrangement of membrane components. From the various results it is tentatively concluded that the ATPase activity is associated with the phospholipid-rich vesicles. Since it may appear that the various fractions differ in the amount of the "ATPase-vesicles", it is of interest that the differences in ATPase activity among the various fractions are much less pronounced when the specific enzyme activities are expressed on a phospholipid-P, instead of a protein, basis (Table III).

The tight junctions are present in the most readily sedimenting fraction following differential centrifugation and in the fraction of highest buoyant density following gradient centrifugation. In the latter fraction the tight junctions are freed from the membrane remains and vesicles, present in a previous preparation[1], but are still contaminated with flocculent material. Hopefully, media of higher buoyant density will allow the further purification of these interesting membrane junctions.

Finally, the differences observed in this investigation between normal and neoplastic liver plasma membranes are worth attention. Most striking in the present context is the different association of the ATPase activity with the various membrane-derived fractions, reflecting a divergent organization of the hepatoma plasma membranes. Other instances of differences in properties between isolated plasma membranes of the rat hepatoma-484 and rat liver have been reported previously[6,10,12,16–18].

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