ISOLATION OF TWO CLASSES OF ROUGH VESICLES FROM RAT LIVER MICROSOMES

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In a previous paper [1] we described a method for fractionating total rat liver microsomes by sucrose density gradient centrifugation. The biochemical composition and enzyme activities of the three fractions obtained were characteristic of the three well known microsomal structures: smooth vesicles, rough vesicles and free ribosomes. We then observed that after density gradient recentrifugation the rough vesicles could be separated into two density zones of different composition, and we suggested that there might be more than one class of rough vesicles. The present paper confirms this result and shows that with a suitable sucrose gradient it is possible to obtain two classes of rough vesicles besides the smooth vesicles and free ribosomes.

Male rats (Wistar Commentry strain) of about 300 g were fed a standard diet and starved 16 hours before sacrifice by decapitation. The liver was quickly removed, washed with cold water and homogenized in 0.88 M sucrose. All the subsequent operations were performed at 4°C. The nuclei and mitochondria were spun down and the microsomal fraction was obtained by a 2 hour centrifugation at 225,000 × *g* max. The microsomes were then resuspended in 2.5 M sucrose and 2 ml of this suspension corresponding to the microsomes of 1 g of liver were layered at the bottom of the tube under the gradient. The latter was previously formed by a lower phase of 11 ml of sucrose $d = 1.28$ overlayered by a linear density gradient ranging from 1.26 to 1.21. The total gradient volume was 27 ml. This was then centrifuged for 40 hours at 25,000 rpm in a Spinco SW 25 rotor, and automatically recovered in an ISCO density gradient fractionator which simultaneously recorded the optical density at 254 mμ.

After centrifugation, the material was distributed throughout the gradient in 4 zones: zone 1 corresponding to the material of density lower than 1.21 appeared as a pellicle at the top of the tube and was removed with a spatula; the three other zones were revealed by the sedimentation profile (fig. 1).

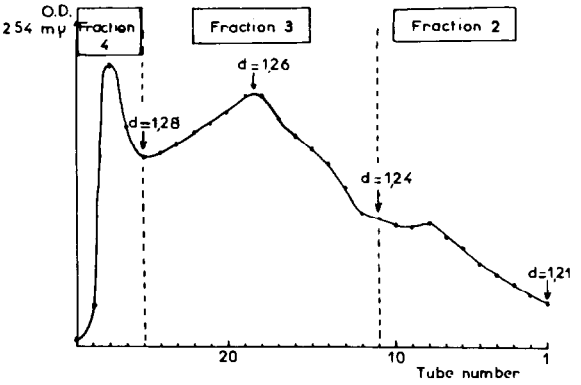

Fig. 1. Sedimentation profile of microsomal subfractions in sucrose density gradient.

The contents of each zone were analyzed for RNA (RNA.P), phospholipids (PL.P), total protein + nucleic nitrogen (N) and for the following enzyme activities: 5′-nucleotidase (5′-AMPase), glucose-6-phosphatase (G-6-Pase) and dimethylaminoazobenzene reductase (DAB red.). Details of the techniques were given in an earlier publication [1].

Table 1 shows the amount of RNA, phospholipids

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Table 1
Distribution of RNA, phospholipids and N in microsomal subfractions obtained by density gradient centrifugation.

	RNA.P (μg/g liver)	PL.P (μg/g liver)	N (μg/g liver)	RNA.P / N	PL.P / N	RNA.P / PL.P
Fraction 1 $d \leqslant 1.21$	6.0 ± 0.83 (9)	35.0 ± 4.28 (10)	216 ± 38 (10)	0.028	0.162	0.17
Fraction 2 $1.21 < d < 1.24$	38.1 ± 2.07 (10)	86.7 ± 5.04 (10)	595 ± 77 (9)	0.064	0.146	0.44
Fraction 3 $1.24 < d < 1.28$	132.0 ± 7.78 (10)	200.0 ± 7.56 (10)	1270 ± 83 (9)	0.104	0.158	0.66
Fraction 4 $d \geqslant 1.28$	54.2 ± 4.68 (10)	19.2 ± 2.90 (10)	492 ± 33 (10)	0.110	0.039	2.82
Microsomes	258.0 ± 19.5 (8)	369.0 ± 19.4 (8)	3189 ± 320 (8)	0.081	0.116	0.70
Recovery	89.4%	92.5%	83.8%			

Table 2
Distribution of enzymic activities in microsomal subfractions obtained by density gradient centrifugation.

	Activities/g liver *			Specific activities **		
	5'AMPase	G6Pase	DAB red.	5'AMPase / N	G6Pase / N	DAB red. / N
Fraction 1 $d \leqslant 1.21$	1.13 ± 0.353 (3)	0.15 ± 0.062 (4)	0.002	5.23	0.70	0.009
Fraction 2 $1.21 < d < 1.24$	0.76 ± 0.272 (3)	2.32 ± 0.428 (4)	0.030 ± 0.0022 (3)	1.28	3.90	0.051
Fraction 3 $1.24 < d < 1.28$	0.41 ± 0.356 (3)	4.57 ± 0.431 (4)	0.043 ± 0.0049 (4)	0.32	3.60	0.034
Fraction 4 $d \geqslant 1.28$	0	0.38 ± 0.138 (3)	0.004 ± 0.0011 (4)	0	0.77	0.007
Microsomes	2.51 ± 0.490 (3)	9.88 ± 0.685 (3)	0.100 ± 0.009 (6)	0.79	3.10	0.031
Recovery	91.6%	77%	79%			

* 5'AMPase = μmoles of P released/min/g liver
G6Pase = μmoles of P released/min/g liver
DAB red. = μmoles of reduced DAB/min/g liver
** Specific activities per mg of N.
The number of experiments is given in parentheses. The values are the means ± standard deviation of the mean.

and protein nitrogen present in each fraction: fraction I was very rich in phospholipids but poor in RNA; fractions 2 and 3 presented equal concentration of phospholipids but varied greatly in their RNA.P/N ratios: 0.064 and 0.104, respectively; so, the RNA.P/PL.P ratios ranged from 0.44 for fraction 2 to 0.66 for fraction 3. Fraction 4 contained primarily RNA and nitrogen and was poor in phospholipids. The distribution of enzyme activities is shown in table 2. The 5'-nucleotidase was found chiefly in fraction 1, while glucose-6-phosphatase and DAB-reductase were localized in fractions 2 and 3 with specific activities slightly higher in fraction 2. Fraction 4 was almost free from these enzyme activities.

Assuming that the phospholipid content can be considered as a marker for membranes and that the RNA was supplied essentially by the ribosomes, we concluded that fraction 1 contained the smooth vesicles, that fractions 2 and 3 were formed by the rough vesicles and that the free ribosomes were concentrated in fraction 4. These conclusions were confirmed by electron microscope examination.

The properties of zone 1, i.e., richness in phospholipids, low content in RNA, lack of glucose-6-phosphatase and presence of 5′-nucleotidase, the latter supplied exclusively by the plasma membranes [2,3], were characteristic of the smooth vesicles as it was previously shown [1,4–7].

The RNA of zones 2 and 3 was mainly attributed to the bound ribosomes: recentrifugation of each of the two zones showed that the RNA.P/PL.P ratios were not appreciably modified; thus the possibility of the presence of free ribosomes and smooth vesicles was excluded and the powerful binding of the ribosomes to the membranes was confirmed [8]. Furthermore, since experiments carried out in the presence of Mg^{++} ions also revealed the existence of different forms of rough vesicles more or less rich in RNA [9], our results cannot be attributed to the absence of Mg^{++} ions. The different RNA contents must therefore point out differences in the number of ribosomes bound to the vesicles, leading to variations in vesicle density. Morphological studies which will appear elsewhere confirmed that the number of ribosomes per vesicle may largely vary, and this agreed with the findings of other authors [9,10]. The smooth and rough forms present in the endoplasmic reticulum show a continuity in the cell and are probably in constant evolution: zone 2 of the gradient might therefore represent a transition stage between these two classes of structures.

Zone 4 on the base of its high RNA.P/N ratio was ascribed to the ribosomes and free polysomes; a small amount of phospholipids revealed the presence of some membranes, but the lack of enzyme activity in this fraction shows that this contamination is certainly slight.

Our results showed that with a suitable sucrose density gradient it was possible to obtain simultaneously the smooth vesicles, the rough vesicles, the free polysomes and furthermore demonstrated the presence of two classes of rough vesicles in rat liver.

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